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Research Article
Effect of Phthalates from Plastic Culture Materials on the Growth and Survival of African Catfish

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Abstract

Background and Objective: The study was carried out in the hatchery section of Fisheries and Aquaculture Department of Chukwuemeka Odumegwu Ojukwu University, Igbariam. The aim of the study is to determine the effect of phthalates that leach from plastic culture tanks like plastic basins and tarpaulin tanks on the growth and survival of African catfish. **Materials and Methods:** Randomized Complete Block Design was used and the three treatments were replicated three times. The fish were fed on a 5% body weight ration level, twice a day. **Results:** The phthalates leached from these plastic materials into the water and also get into the fish they did not affect the growth of the fish and their survival. The mean percentage weight gain recorded for the three treatments (concrete tank, plastic basin and tarpaulin tank) was 664±3.05, 658±18.26 and 653±16.70, respectively and the significance test (0.798) $p>0.05$ indicating no significant difference suggesting that phthalates that leached from the plastic tanks are not enough to be growth inhibitors. The same trend was same when the specific growth rate, food conversion ratio and protein efficiency ratio were calculated. All of them resulted in no significant difference. Also, the rate of mortality was nearly the same for all the treatments with no significant difference suggesting that the low levels of phthalates that leached from the plastic culture materials were not enough to affect the survival of the fish negatively. **Conclusion:** The concentrations of phthalates that leach from plastic culture tanks do not affect the growth and survival of the African catfish.

Key words: Phthalates, growth, percentage weight gain, specific growth rate, African catfish, plastic tanks

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish farming and aquaculture are increasingly popular in emerging nations, particularly in Asia, South America and Africa[1,2]. The main reason for this is that fish, which is a more affordable source of high-quality protein, may be used to replace beef, milk and eggs, which are likewise high-quality proteins but are more expensive. As fish is less expensive and in high demand in these nations, many people have entered the industry and more are doing so today[3]. Fish pond construction is one of the limitations of the fish farming industry. This discouraged people from entering the fish farming industry in the past, but today's use of plastic basins and tarpaulin in fish culture has made it easier for others who are less buoyant to enter the industry[4,5]. Also, more plastic is now being used to hold and package food products for human consumption, which some experts are seriously criticizing because their study indicates that doing so could be harmful to human health[6]. Some claim that since the widespread usage of plastics for food processing and packaging, certain illnesses, like cancer, have also increased[7,8] and this has grown to be a significant source of worry for many people around the world. Due to many reasons, many people in Nigeria do not care where their fish comes from, instead, they are more concerned with finding cheap fish to buy, eat and satisfy their hunger. It is almost impossible to live in the current world without using plastic products for regular tasks[5,6,9]. These days, a variety of agents, including softeners, plasticizers, fillers, stabilizers and pigments, are added to the manufacturing of plastic materials to improve their quality. The most popular plasticizers for a while now have been phthalic acid esters, or phthalates, with Diethylhexyl phthalate (DEHP) making up 50% of this usage[10]. The global output of phthalates has increased to 3.5 million tonnes annually[10]. Depending on the kind and function of the product, phthalates may make up to 45% of the mass of a plastic substance[5,9,11,12]. Plastic garbage is already making up 1% of all solid waste annually due to the steady rise in the usage of plastic containers in all spheres of human activity. Both professional circles and the general public are now interested in the phthalate issue as a result of recent studies indicating adverse effects of phthalates in experimental animals and a difference in sensitivity of human and animal hepatocytes to the activity of phthalates[7,8]. These studies showed a wide range of unfavorable phthalate effects in experimental animals, from the potentially less harmful to the extremely unfavorable, including spontaneous abortion, stillbirth and low birth weight of the offspring, along with toxic, carcinogenic, mutagenic and teratogenic effects of phthalates[13,14] to the extremely unfavorable, like spontaneous abortion, stillbirth and low birth mass of the offspring, along with toxic, carcinogenic, mutagenic and teratogenic effects of phthalates[8,15-17]. Furthermore, it has been found that environmental phthalates mimic estrogens and harm test animals' male genitalia[18,19]. There are two different views on the potential harm that phthalates may do to human health. The opposing camp maintains that there is little risk to human health from exposure to phthalates. It was pointed out that even at the highest levels of exposure in humans, this exposure is thousands or even millions of times lower than that experienced by laboratory animals and is typically only intermittent and gradual, occurring over years or even decades[18,19].

One of the most crucial elements for preserving society and the way we live today is plastic[20]. Sadly, they are also linked to serious environmental problems because they are generally made of non-renewable basic materials (such as oil), are frequently used in transient items (such as food packaging) and are primarily landfilled or burned after being used[21]. Several scientific investigations have demonstrated links between exposure to particular chemicals and harmful health effects in both people and animals. Typically, some of the compounds have impacts on the hormonal system and are therefore suspected of causing a variety of diseases like cancer, genital abnormalities, or fertility issues. They may also be able to cause other negative health effects like obesity, insulin resistance and diabetes. Due to their disruption of the endocrine systems of both humans and animals, these substances are considered to be or are suspected to be, so-called endocrine-disrupting compounds. Concern over the research on the effects of man-made chemicals on wildlife and humans has grown over the past 20 years. Human urine and wastewater (such as water used to remove cosmetics, facial cream, lotion and shampoo) contain phthalates and their metabolites, which are expelled by humans[22]. According to Shamker *et al.*[22] untreated sewage discharged into streams, rivers, lakes, seas and other bodies of water can contain phthalates.

The aim of this study was to determine the effect of the phthalates that leach from plastic culture materials which include big plastic basins and tarpaulin tanks on the growth and survival rate of the African catfish.

MATERIALS AND METHODS

Study area: The study was carried out in the hatchery section of the Fisheries and Aquaculture Department of Chukwuemeka Odumegwu Ojukwu University, Igbariam. The research lasted for 12 weeks from February to April, 2021.

Study sample: To make sure that errors due to growth and age were eliminated, artificial breeding/spawning was carried out to get the juveniles needed for this study. A reputable fish farmer was engaged and the spawning was done in his facility at Ogbunike in Idemili North Local Government Area of Anambra State. This helped to make sure that the fish were of the same age and growing at the same rate because those selected were those of similar size. Out of the spawned juveniles, 120 juveniles of similar size were selected for the research. The whole population for the research grew at the study site after a month they were hatched at Ogbunike.

Inducement of the female brood-stock: One male of similar size to the female was sacrificed or killed, the skull was opened and the pituitary gland was extracted and ground using laboratory mortar. About 3 mL of physiological saline solution was added into the mortar and the mixture of the milt and physiological saline solution was drawn into a syringe with a needle and injected into the female brood-stock on the dorsal part of the fish just after the head[23]. After injecting the female brood stock, it was placed in a separate basin (100 L basin) filled with water halfway. The female was induced by 7 am in the morning. At 6:30 pm the female was ready for striping.

Extracting the milt: Before the female catfish was stripped of her eggs, the milt from the male was removed from him just after the pituitary gland was removed. It was then diluted with a physiological saline solution (9 g of table salt dissolved in 1 L of boiling water)[24,25]. The benefit of this is that eggs from several females can be fertilized because one male testis can readily fertilize the eggs of 10-15 females. This solution is afterward mixed with the stripped eggs[24,25].

The staff of the university in charge of ethical consideration examined the animals and the study conditions and gave his approval before the commencement of the study.

Striping of eggs: The female broodstock was gently picked from the waiting plastic basin and placed on a wooden table. The head of the fish was covered using a clean towel and held firmly. Holding the head covered with a towel firmly the fish was turned with the ventral side facing upwards and then the ventral side turned sideways, the genital pore over a small plastic bowl, then the stomach was gently pressed under the head but firmly down towards the genital pore[24]. As the eggs were pressed to flow into the bowl until the eggs come out very little with little blood stains on them. Immediately after striping the eggs, the milt was poured into the bowl with the eggs and stirred with chicken feathers. This stirring helps to free the eggs from sticking together so that they can be separated from each other for fertilization by the sperm calls of the milt.

Incubating the eggs: A mosquito net was used as kakaban and placed at the bottom of the hatchery (a small concrete tank of 1 m^2 and held to a particular point in the hatchery using two pieces of small stones thoroughly washed and sterilized. The height of the water at the hatchery is just above 30 cm and a flow-through system was maintained. This helped to eliminate infection of disease and to supply enough oxygen for incubation and hatching. Immediately after the fertilization of the eggs, they were poured on the kakaban making sure that they are well spread over the kakaban. A flow-through system was maintained in the hatchery with very clean water rich in dissolved oxygen this fall in line with the method stated by de Graaf and Janssen[25]. The level of dissolved oxygen in the water (from the borehole) was tested using a Dissolved oxygen meter, Ys8060 Digital D.O. Sensor, Shanghai Jui Zhuang Instruments Co. Ltd, Shanghai, China.

Study design: Randomized Complete Block Design (RCBD) was used where the experiment had three treatments (fish cultured in the concrete tank which is control, some cultured in a plastic basin and others cultured in tarpaulin tanks). The treatments were replicated three times and placed in the same place to make sure the environment is the same. Each replicate had 10 fish. After every 2 weeks the fish were weighed and recorded and a new quantity of feed of 5% body weight was given to them for the next 2 weeks. The study lasted for 12 weeks. Four millimeters of vital feed which is a product of United Africa Company of Nigeria a Public Liability company. The reason for this feed is that it is an extruded feed that floats and will not contaminate the water like the one pelletized.

Weighing of the fish: The fish were weighed every 2 weeks and recordings were made. During the initial weighing, a digital electronic sensitive compact scale from Labtech (Telnice, Czech Republic) model BL 7501 was used.

The growth parameters considered in the course of this study were:

- Weight increase
- Percentage weight gain
- Specific growth rate
- Feed conversion ratio
- Protein efficiency ratio

Statistical analysis: These growth parameters were analyzed using ANOVA statistics at a 0.05 significant level.

RESULTS

Concentrations of phthalates: Tests for plasticizers (phthalates) were conducted using the Gas Chromatography method to determine the concentrations of different plasticizers in the different treatments of the study. The result shows that most of the time culture water from the second treatment (water from the plastic basin) contains the highest concentration of plasticizers, followed by culture water from the tarpaulin tank. The water from the concrete tank contains the least value of plasticizers. The reasons for the presence of phthalates in the water from the concrete tank: (i) During the collection of the rainwater it must have gone through a plastic pipe to the underground concrete tank, (ii) Plasticizers released during the burning of plastic materials settle on the water surface, (iii) Feed introduced some quantity of plasticizers into the water from all the treatments which came from the feed bag made of plastic and (iv) Some laboratory equipment used for the analysis was made up of plastics and can also introduce phthalates into the analytical results.

Bi-weekly weight gains: The bi-weekly weight gains of fish was shown in Table 2. The result showed that all the weekly weight gains were similar. The weight of the fish was similar every 2 weeks (all the p-values were greater than 0.05) as shown in Table 2.

Percentage weight gain: The fishes which were cultured in the concrete tank had the highest percentage weight gain (664), followed by those cultured in the plastic basin (658) and finally those cultured in tarpaulin (653) as shown in Table 3.

The test for significance (Table 4) shows there was a significant difference among the treatments indicating that phthalates were not growth inhibitors.

Specific growth rate of fish: The specific growth rate of all the treatments was similar but those cultured in the plastic basin had the highest value (2.45) and those cultured in a tarpaulin tank recorded the least value (2.41) as shown in Table 5.

The ANOVA result (Table 6) showed no significant difference among the treatments in terms of growth rate.

Food conversion ratio: The food conversion ratio of the three treatments was similar, indicating they converted their food to flesh at the same rate. Those cultured in concrete tank recorded 2.14 while those cultured in tarpaulin tank recorded 2.21 as shown in Table 7.

Table 1: Concentration of phthalates in the culture water during the study in ppm

Phthalates	Treatment	Week 4	Week 8	Week 12	Total	Mean
Diethyl pH	T1	0.5961	0.4625	0.2562	3.9640	1.3213
	T2	1.6375	1.3224	1.0041	3.9640	1.3213
	T3	1.2341	1.0451	0.8591	3.1383	1.0461
Dibutyl pH	T1	0.0034	0.0026	0.0001	0.0061	0.0020
	T2	0.9341	0.7561	0.5751	2.2653	0.7551
	T3	1.1761	0.8751	0.6442	2.6954	0.8985
di-isobutyl pH	T1	0.0641	0.0315	0.0211	0.1167	0.0389
	T2	0.5631	0.4552	0.3421	1.3604	0.4535
	T3	0.4561	0.3611	0.2652	1.0824	0.3608
di(2-ethyloxy) pH	T1	0.0016	0.0003	-	0.0019	0.0006
	T2	0.7615	0.7741	0.5320	2.3076	0.6863
	T3	0.8752	0.7862	0.6462	2.3076	0.7692
di-n-octy pH	T1	0.0002	-	-	0.0002	0.0001
	T2	0.3561	0.3441	0.2861	0.9863	0.3288
	T3	0.0761	0.0872	0.0642	0.2275	0.0758
Benzyl butyl pH	T1	0.2115	0.2004	0.0117	0.4236	0.1412
	T2	0.4117	0.4052	0.3761	1.1779	0.3926
	T3	0.5622	0.4965	0.2452	1.3039	0.4346

Concentrations are in parts per million

Table 2: Mean bi-weekly weight gain in grams

Treatment	Initial	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
T1	410.00	640.00	1120.00	1566.67	2000.00	2602.33	3133.33
T2	436.67	633.33	1161.67	1811.33	2208.33	2717.33	3282.67
T3	426.33	664.33	1284.33	1672.33	2194.33	2653.67	3321.33
p-value	0.535	0.777	0.288	0.167	0.244	0.377	0.095

Level of significance = 0.05

Table 3: Percentage weight gain of the fish during the study

	Replicates					
Treatment	1	11	111	Total	Mean	SE
T1	760	660	662	1992	664	3.05
T2	644	686	624	1974	658	18.26
T3	663	629	675	1958	653	16.70

Test for significance = 0.05 and SE: Standard error

Table 4: ANOVA table of percentage weight gain of fish during study

		Weight gain (%)					
			Sum of squares	Degree of freedom	Mean square	F statistics	Level of significance
Between groups	Combined		290.667	2	145.333	0.234	0.798
	Linear term	Contrast	192.667	1	192.667	0.310	0.598
		Deviation	98.000	1	98.000	0.158	0.705
Within groups			3731.333	6	621.889		
Total			4022.000	8			

Table 5: Specific growth rate of the fish during the study

	Replicates					
Treatment	1	11	111	Total	Mean	SE
T1	2.43	2.42	2.42	7.27	2.42	0.003
T2	2.39	2.60	2.36	7.35	2.45	0.076
T3	2.43	2.36	2.44	7.23	2.41	0.025

Test for significance = 0.05 and SE: Standard error

Table 6: ANOVA table for specific growth rate of fish during the study

		SGR					
			Sum of squares	Degree of freedom	Mean square	F statistics	Level of significance
Between groups	Combined		0.002	2	0.001	0.196	0.827
	Linear term	Contrast	0.000	1	0.000	0.042	0.844
		Deviation	0.002	1	0.002	0.350	0.576
Within groups			0.038	6	0.006		
Total			0.041	8			

Test for significance is at 0.05

Table 7: Feed conversion ratio of the fish during the study

	Replicates					
Treatment	1	11	111	Total	Mean	SE
T1	2.22	2.12	2.09	6.43	2.14	0.04
T2	2.13	2.13	2.19	6.45	2.15	0.02
T3	2.25	2.38	2.00	6.63	2.21	0.11

Test for significance = 0.05 and SE: Standard error

The ANOVA table for food conversion ratio Table 8 showed no significant difference among the three treatments under study. Indicating there is no significant difference among the treatments.

Protein efficiency ratio of the fish: The protein efficiency ratio in which those cultured in concrete tank and plastic basin recorded the same value and the least value was recorded by those cultured in tarpaulin tank as shown in Table 9.

Once again the ANOVA table (Table 10) showed there was no significant difference in the protein efficiency ratio of the three treatments. Indicating that phthalates are not growth inhibitors.

Mortality among the treatment growth: During the study the mortality of the fish among the treatments was recorded and the data was shown in Table 11 below. The rate of mortality looked similar.

The ANOVA table (Table 12) showed there was no significant difference among the three treatments. The mortality among the three treatments was low. Indicating that the level of concentration of phthalates leached out from the culture materials was not up to the lethal level.

Table 8: ANOVA table of food conversion ratio of the fish during the study

			FCR				
			Sum of squares	Degree of freedom	Mean square	F statistics	Level of significance
Between groups	Combined		0.008	2	0.004	0.281	0.764
	Linear term	Contrast	0.007	1	0.007	0.464	0.521
		Deviation	0.001	1	0.001	0.099	0.764
Within groups			0.086	6	0.014		
Total			0.094	8			

Test for significance is at 0.05

Table 9: Protein efficiency ratio of the fish during the study

	Replicates					
Treatment	1	11	111	Total	Mean	SE
T1	1.07	1.12	1.14	3.33	1.11	0.02
T2	1.12	1.12	1.08	3.32	1.11	0.01
T3	1.06	1.00	1.19	3.25	1.08	0.06

Test for significance = 0.05 and SE: Standard error

Table 10: ANOVA table of protein efficiency ratio of the fish during the study

			PER				
			Sum of squares	Degree of freedom	Mean square	F statistics	Level of significance
Between groups	Combined		0.001	2	0.001	0.169	0.849
	Linear term	Contrast	0.001	1	0.001	0.284	0.613
		Deviation	0.000	1	0.000	0.053	0.825
Within groups			0.023	6	0.004		
Total			0.024	8			

Test for significance is at 0.05

Table 11: Mortality among the treatments during study

	Replicates		
Treatment	I	II	III
T1	3	2	4
T2	4	3	3
T3	3	2	4

Table 12: ANOVA table of the mortality among the treatments during the study

			Replicate				
			Sum of squares	Degree of freedom	Mean square	F statistics	Level of significance
Between groups	Combined		0.667	2	0.333	0.600	0.579
	Linear term	Contrast	0.667	1	0.667	1.200	0.315
		Deviation	0.000	1	0.000	0.000	1.000
Within groups			3.333	6	0.556		
Total			4.000	8			

Test for significance is at 0.05

DISCUSSION

The analysis for the presence of phthalates in the culture tanks during the study indicated that higher concentrations were recorded in the plastic basin and tarpaulin tank indicating leaching of these phthalates from plastic materials into the culture water. Dickson-Spillmann *et al.*[26] and Huang *et al.*[27] stated that phthalate contamination in foods occurs via migration from contact materials. Phthalates that migrate from plastic materials contaminate substances or food materials in contact with them. The presence of phthalates in the culture water of a concrete tank came from the contact between water and plastic pipe used in channeling the water into the concrete tank and laboratory equipments used in

analyzing the water. Some studies have demonstrated that blood bags and tubings are in addition to food ingestion an important source of di-2-ethyl hexyl phthalates DEHP exposure[28,29]. This result also showed that with time the level of concentration of phthalates continues to reduce, indicating a reduction in the phthalate load of the plastic basin and tarpaulin tank and also a reduction in the level of leaching. When the concentration of the migrating substance decreases with time, the migration rate decreases[30]. This suggests that after a while the concentration of phthalates in the culture water must have reduced significantly and can be very suitable for fish culture without fear of the phthalates.

The recorded weekly weight gains of the study showed that the fish cultured in a tarpaulin tank (T3) had the best mean weight gain (3321.33 g) when compared to those cultured in the concrete tank (T1) (3133.33 g) and plastic basin (T2) (3282.67 g). The three results show no significant difference ($p>0.05$) among them and this indicated that the culture material did not have a clear or significant impact on their growth and did not create any significant difference in the growth of the fish. This indicated that the culture of fish with any of these three materials will give you nearly the same result in terms of growth. These findings, which have generally demonstrated no negative effects when fish are exposed to lower levels of phthalates, corroborated this study. For instance, in a multi-generational study (14 days' post fertilization (dpf) to 140 dpf of the F1 generation), Japanese medaka exposed to 21.9 and 19.3 g, g1 DINP and DIDP, respectively, via the diet, with a daily feeding regime of 5% of the body weight, failed to detect any effects on reproduction (gonad somatic index, egg production, embryo survival and sex ratios), growth, or survival[31]. The development of germ cells was unaffected when medaka was exposed to doses of up to 5 mg DEHP l1 for 90 days after hatching[32]. The percentage weight gain shows that fish cultured in the concrete tank had the highest value (664) followed by those cultured in the plastic basin (658) and finally those cultured in tarpaulin (653). This is an indication that phthalates do not cause hindrance in the growth of African catfish (*Clarias gariepinus*). Staples and co-researchers stated that low levels of phthalates may affect the endocrine system of the fish and not the growth[33]. That is why they are called endocrine disruptors[33]. The results were close and the test for significance using ANOVA statistics shows no significant difference.

Specific growth rate also falls in line with previous tables showing that those fish cultured in the plastic basins had the highest value (2.45), followed by those cultured in the concrete tank (2.42) and the least value by those cultured in the tarpaulin tank (2.41). Here the results were very similar and the test of significance shows no tangible difference among the treatments (Table 6), indicating once more that phthalates do not hinder the growth of African catfish.

The food conversion ratio table shows that those cultured in a tarpaulin tank recorded the highest value (2.21), followed by those cultured in the plastic basin (2.15) and the least value recorded by those cultured in the concrete tank (2.14). The results were closely related once again showing no significant difference from the ANOVA statistics, indicating no growth hindrance from the phthalates. Obiezue *et al.*[34] stated that apart from the test for acute toxicity, mild concentrations of phthalates in water do not inhibit the growth of fish.

The protein efficiency ratio shows that those cultured in concrete tank and plastic basin recorded the highest value (1.11 each) and least value recorded by those cultured in tarpaulin tank (1.08). These results are close and show no significant difference indicating no impact on growth by the phthalates.

The mortality rate of all the treatments was low indicating that the level of concentrations of phthalates that migrated or leached from the plastic materials (plastic basin and tarpaulin tank) into the culture water was low and well below the lethal concentration level. The test for significance among them showed no difference. Patyna *et al.*[31] stated that low concentrations of phthalates in water do not affect the growth and survival of fish.

This study encourages many who are not buoyant enough in constructing earthen or concrete ponds before going into fish farming that using plastic tanks of various types which are far cheaper can be very helpful in starting a fish farming business. Further study will be very necessary to check if the phthalates do have an impact on the organs and well-being of the fish and if the concentrations in the fish can be harmful to man.

CONCLUSION

This study has shown that culturing African catfish in a culture material made of plastics (which are more cost-effective) does not affect the growth and development of the fish negatively (they are not growth inhibitors). Though, phthalates leach from the plastic tank materials (big plastic basin and tarpaulin tank) into the culture water, it did not cause stunted growth in the fish. And also when the mortality rates of the three treatments were compared, there was no significant difference.

SIGNIFICANCE STATEMENT

There has been mixed feelings on the issue of toxicity of plastic materials in aquaculture practices. Some believed that phthalates from plastics are toxic and can affect the growth and development of fish and should not be used in aquaculture or fish farming while others believed that it is not bad to use plastics in culturing of fish. This study has been able to prove that phthalates from plastics are not enough to cause stunted growth in fish but the study did not go further to check if the phthalates can be disruptive to the internal organs of fish. This can be a topic for further study.

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