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Effect of Administering Serially Diluted Suprecur and Motilium (Dopamine Blocker) on the Breeding Performance of *Clarias gariepinus*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study was designed to determine the effect of using serially diluted Buserelin acetate (Suprecur), which is a luteinizing hormone-releasing hormone analogue (LHRHa) with dopamine antagonist (Motilium**)** on the latency period, fecundity, percentage fertilization and percentage hatching of *Clarias gariepinus*. Treatments administered include 50ug/l, 40ug/l, 20ug/l and 10ug/l of Suprecur in tandem with 5mg/kg of Motilium. Metrics obtained include egg numbers, latency period, fertilization rate, hatching rates, and survival to first feeding. The results obtained demonstrated that the use of Suprecur (LHRHa) together with dopamine antagonist (Motilium) successfully induced

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ovulation in the experimental *Clarias gariepinus* broodfish. The highest fecundity was from the treatment T40 There was no significant difference in egg weights stripped from each treated group. The application of 50 µg/kg of Suprecur with 5mg/kg of Motilium resulted in earlier synchronization of ovulation (Latency period; 12 hours). Results of the fertilization percentage indicated that an increase in the dose of LHRHa did not significantly affect the fertilization rate in treated groups of broodfish. Overall superiority of 50 µg/kg of Suprecur plus 5mg/kg of Motilium in spawning induction was proved by significantly high hatchability, 83.56%. Broodstock used for the 40µg/kg dose had the highest fecundity. There was no significant correlation ($p>0.05$) between the water quality parameters and the breeding parameters. In conclusion, the result obtained clearly indicates an overall superiority of using 50 µg/kg of Suprecur together with 5mg/kg of Motilium to induce spawning with regard to the recorded high hatchability percentage.

Keywords: Hormone; ovulation; fertilization; hatchability; hypophysation; synthesis.

1. INTRODUCTION

"Wild fry collection has become outdated for *Clarias gariepinus* species since artificial hatchery technology exists, and the availability of seed is the bedrock of commercial aquaculture" [1]. Accordingly, "induced spawning of captive African catfish becomes popular with merits such as improved fertilization and hatching rates, higher survival rates, as it is with all year production of fry. However, one major concern of commercial farming of the African catfish is the availability of seed (fingerlings) since *Clarias gariepinus* does not freely breed in captivity" [2], added to the need to constantly sustain improved seed quality for commercial availability all year round. Increasing population and market demand for fish in Nigeria has led to increased demand for seeds of the fish species for grow-out production [1]. "The insufficiency of quality seeds can be linked to the absence of environmental cues, required for gonadal maturation and spawning" [3], just as well as stress induces ovarian atresia [4]. "Some farmers obtain their seeds from the wild but this procedure is unreliable and does not guarantee quality (uniform size, and parasite or disease-free) seeds, and the process requires a long period of waiting, time consuming and unprofitable for commercial production of the fish" [5].

Successful attempts have been made to copy or imitate the environmental cues (temperature and water depth) [6] critical to stimulating gonadal maturation and spontaneous spawning in the African catfish. "Studies have indicated that *Clarias gariepinus* can be induced to spawn under controlled conditions (such as water depth, water volume, and temperature at appropriate stocking densities), However, it did not prove that this technique can be reliably used in the commercial production of fingerlings, moreover, this technique is seasonal" [7].

Over time, the most effective approach to overcome the problems of breeding catfish in captivity is through hypophysation to induce the final oocyte maturation, ovulation, and spawning of fish through hormonal inducement used to breed fish species that do not possess the ability to spawn under confinement.

"In the case of the African catfish, hypophysation methods have been employed in semi-artificial or semi-natural propagation methods (where the female African catfish is injected with natural or synthetic hormone and placed together with the males in ponds or tanks to spawn) and artificial propagation method (where the female African catfish is injected with natural or synthetic hormone and eggs stripped into a container to be fertilized with milt collected from the gonads of sacrificed males)" [8].

"Inducement is done through injection of one of the various hormones including; fish pituitary extracts, HCG hormone, gonadotropin hormone (GTH), luteinizing hormone-releasing hormone (LHRH), and LHRH agonists (LHRHa) (i.e. gonadotropin-releasing hormone (GnRH) and GnRHa) in commercial synthetic or processed forms such as Ovatide, Ovaprim, Ovopel, Ovupin-L, Ovulin, Aquaspawn, and many others" [9-12].

Hormone-induced hatching of fish has been used for about 60 years in fish hatcheries for the production of fry or fingerlings which contributes positively to the over all aquaculture production [13]. It has opened the door of a new era world wide for high quality and high quantity of fish production [14]. In Africa, induced breeding started after the Second World War, with the first successful attempt of fingerlings being that of *Clarias garepinus* in Egypt [15]. Amazingly, "the same procedures, with only minor contextual modifications, have been used to spawn an entire range of fishes from the ancient sturgeon

and paddle fish to carp, catfish, salmon, sea bass, sea bream, and mullet. In addition to breeding other desirable fish species, hypophisation using hormones provides direct control over the final stages of the reproduction cycle in teleosts" [16].

Induced spawning of *Clarias gariepinus using* GnRH has remained a method of producing the species for research [17-21]; recently in Egypt [22-24]. On the other hand, the use of HCG is the popular protocol to induce spawning in many common fish species such as Sea bream, *Sparusaurata* [25]; the Japanese eel, *Anguilla japonica* [26]; Benni, *Barbuss sharpeyi* [27]; Pigfish, *Orthopristis chrysoptera* [28]. Research works done and presented by authors [22,29,23,30] shows that the catalysing of final oocyte maturation time, ovulation, and spawning, of African catfish can be done by using of human chorionic gonadotropin (HCG).

Buserelin acetate (Suprefact) is a Gonadotropin releasing hormone analog (GnRHa) that is co-administered with domperidone (DOM), an antagonist of dopamine that is produced when fish are stressed. This cocktail has been frequently used (Peter et al. 1993). Buserelin is an analogue of GnRHa, which regulates gonadotropin hormone (GtH). GtH comprises luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which can affect the development of the ovary and testis. The essential hormones for ovulation, GnRH together with the GnRH receptor, which is located on the gonadotrope membrane in the pituitary gland, can stimulate gonadotropin production. Gonadotropin then will be released into the blood by G protein-coupled receptor systems [31,32].

Induced breeding using hormones also contributes to the cost of fingerling production. This is true considering Suprefact (Buserelin acetate, an LHRHa) used in aquaculture. Efforts at increasing the number of fish induced using one vial of the hormone will greatly optimize production and reduce cost.

This will attempt to induce fish using diluted suprecur, a brand of Buserelin acetate. Therefore, the objectives of this study are to determine the latency period and fecundity of ***Clarias gariepinus*** induced with serially diluted suprecur with a dopamine- antagonist, and to determine the fertilization and hatching rates of ***Clarias gariepinus*** hatched from broodstock induced with serially diluted suprecur.

2. MATERIALS AND METHODS

This study was done in the fishery Hatchery of the Department of fisheries and Aquaculture, Joseph Sarwuan Tarka University Makurdi (University of Agriculture Makurdi).

The broodstocks were obtained from Obedience Fish Farm Makurdi, Benue State. A total number of Twelve fish, eight females, and four males were purchased. All broodstocks were selected by external morphological characteristics using the method of [33]. The broodstocks were acclimatized for two days before use.

Suprecur, a brand of Buserelin acetate meant for females was obtained from www.drugstore.ng #29 Ayangbure road, Ikorodu, Lagos State, and Motilium was acquired from Wino pharmacy Makurdi, Benue State.

2.1 Experimental Design

2.1.1 Preparation of hormone

Suprecur obtained was manufactured with a concentration of 1mg/ml of solution. A stock solution containing 2ml (2000µg) of the original Suprecur made up to 20ml using normal saline (18ml) was made to obtain a concentration of 100µg/ml of solution. All ten Motilium tablets (100mg) were also removed from the pack and pounded using a porcelain mortar and pestle. The volume of the powdered product was determined to be 4ml. This was made up to 10ml by adding 6ml of normal saline. The dosage of Suprecur for catfish as recommended by [34] is 10-30µg/kg body weight in combination with 5-10mg/kg body weight for Motilium. The current trial utilized four different dosages of Suprecur: 50 µg/kg, 40µg/kg, 20µg/kg, and 10µg/kg. The dose of Motilium was fixed at 5mg/kg of bodyweight. Hence, the following volumes were used in all cases:

Table 1. Dose of Hormones (Suprecur and Motilium) administered to female *Clarias gariepinus*

Treatment	Doses of hormones	
	Suprecur (µg/kg)	**Motilium (mg/kg)**
T50	50 (0.5ml/kg)	5.0 (0.5ml/kg)
T40	40 (0.4ml/kg)	5.0 (0.5ml/kg)
T20	20 (0.2ml/kg)	5.0 (0.5ml/kg)
T10	10 (0.1ml/kg)	5.0 (0.5ml/kg)

2.1.2 Hormone administration

The female broodstock was obtained from the holding tanks by using a scoop net, so that the weight of the fish could be taken using a digital weighing scale. The weighed fish was then covered with a clean towel and injected intramuscularly above the lateral line towards the dorsal section and pointed towards the ventral side. After the withdrawal of the needle, the fish was finger rubbed mildly, to avoid the out flow of the injected fluid. The injected females were returned separately to their respective plastic bowls.

2.2 Stripping and Fertilisation

Injected female broodstock were carefully removed from the plastic bowl after 12-13 hours and stripped in a dry bowl by holding the fish at the head and tail by an assistant. The ovulated eggs oozed out on slight pressure by thumb into the dry plastic bowl and 10g of eggs were collected from each sample, put into a petri-dish for counting to know the total number of eggs produced from each of the female broodstock. The male broodstock was removed after dissecting them and the milt was collected by laceration of the testes with a clean razor blade. The sperm was then used to fertilize each treatment by mixing both eggs collected and sperm with a plastic spoon before adding distilled water. The bowl was vigorously shaken for a few seconds to improve fertilization.

2.3 Incubation

Incubation of the fertilized eggs was done in 60 liters plastic bowl containing about 45 liters of clean water which was equipped with water aerators. Nylon mesh size (1mm) was suspended above the floor in the plastic bowl for spreading of fertilized eggs. The fertilized eggs were spread evenly in a single layer on the suspended nylon meshed net for incubation. Upon hatching (about 24 hours after incubation), the nylon meshed net was removed with the eggshells while the hatched larvae clustered in active movement at the bottom of the incubation tank.

2.4 Determination of Fertilization Rate

The fertilization rate was determined using 750 eggs from each cross. The eggs were covered in the dry, labeled Petri dish and were kept with labels. The number of eggs was estimated using the gravimetric method (number of eggs/g). The translucent eggs containing embryonic eyes at the time of polar cap formation 10 - 20 minutes after fertilization were considered fertilized and counted to estimate the fertilization rate by percentage [35].

2.5 Hatchability

Eggs were incubated in plastic aquaria with a water volume of 40L and mosquito mesh as substrate. Percentage hatchability was estimated 24 hours after hatching was completed. This was estimated using the volumetric method. To do this, the incubation bowl was stirred gently to disperse the larvae evenly in the water. A beaker (100ml) was used to collect water from the bowl with the dispersed larvae swimming freely inside. The number of larvae in the volume of water was counted. This was repeated three times and the average number was taken. The value was then estimated to cover 40 liters of water volume using a mathematical relationship to determine hatching rate using a modified version of the formula provided by [36] thus:

$$HatchingRate = \frac{Total\ Number\ of\ Hatched\ Eggs}{Total\ Number\ of\ Incubated\ Eggs} \times 100$$

2.6 Survival

The survival rate of larvae was estimated at day 4 after hatching i.e. post yolk sac absorption. The volumetric method was employed in determining survival rate. Here water in the holding tanks was stirred to ensure even dispersion of fry using a glass rod. After this, a representative sample of the water (100ml) was taken in a beaker and fry within the water volume were counted. This was repeated three times and the average was taken. The population was then estimated to cover the entire water volume (40,000ml). Therefore the following equations were used:

$$A_{100} = \frac{\sum No.\ of\ fry\ in\ three\ samples}{3}$$

$$Survial\ rate = \frac{A_{100} \times 40000ml / 100ml}{No.hatched} \times 100$$

2.7 Water Quality Parameters

Water quality parameters such as pH, Electrical Conductivity, Total Dissolved Solids (TDS), and Dissolved Oxygen of the water were monitored using Hanna Multiparameter Water Quality Probe

Model HI-98129. Mercury in a glass thermometer was used to take temperature readings.

2.8 Statistical Analysis

Data were analyzed using R version 4.0.0 [37] Descriptive statistics for hatching success were obtained using Rmisc package in R [38] and reshape2 [39]. Differences in the hatching rates across the treatments were determined using one-way ANOVA in R [37] via agricolae and emmeans packages [40,41]. Mean separation was done using the Tukey HSD method implemented in multcomp package [42] and viewed using multcompView [43]. Graphs were drawn using the ggplot2 package in R [44].

3. RESULTS

3.1 Fecundity

Fecundity of the female broodstock to be induced with a combination of Suprecur and Motilium (Fig. 1) shows that broodstock used for the 40µg/kg dose had the highest fecundity followed closely by broodstock allotted to the 20µg/kg dose while broodstock selected for the 50µg/kg dose had the least fecundity. The fecundity of the species was independent of treatments to be administered and is, therefore, a random effect in the current experiment.

3.2 Breeding Performance

The effect of each dose administered on respective breeding parameters (Table 2) shows that the weight of eggs stripped from each female for each treatment was not significantly different ($p>0.05$) and also reflective of the fecundity (Fig. 1). Fertilization rates did not differ across the treatments ($p>0.05$). Latency period differed significantly ($p<0.05$) among the treatments with the least period of 12 hours being recorded for fish treated with 50µg/kg of Suprecur and 5mg/kg of Motilium. Hatchability differed significantly across the treatments ($p>0.05$) with the highest hatchability (83.56%) being observed for fish administered 50µg/kg of Suprecur and 5mg/kg of Motilium. Survival at yolk sac absorption was not significantly different among the treatments ($p>0.05$).

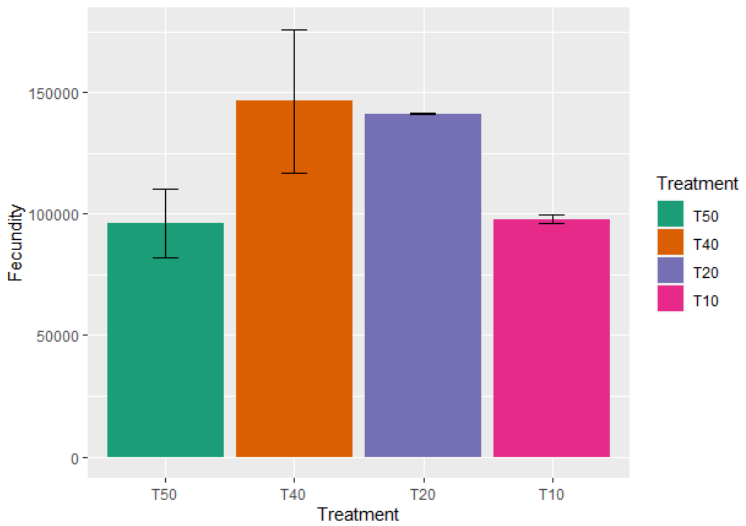

Fig. 1. Fecundity of Female Broodstock of *Clarias gariepinus* stripped under each treatment

Table 2. Egg and breeding parameters of *Clarias gariepinus* induced using serially diluted suprefact and motilium

Treatment	Egg_Wt	Latency	Fertilization	Hatchability	Survival
T10	199.85 ± 23.70	13.00 ± 0.00^{b}	72.10 ± 8.80	11.44 ± 0.76^{a}	45.93 ± 14.90
T20	178.70 ± 6.40	13.00 ± 0.00^{b}	88.98 ± 0.56	56.32 ± 1.36^{b}	77.92 ± 14.00
T40	205.75 ± 42.30	13.00 ± 0.00^{b}	85.03 ± 1.59	64.12 ± 2.89^{b}	63.52 ± 20.30
T50	165.95 ± 29.60	12.00 ± 0.00^{a}	85.66 ± 2.94	83.56 ± 2.91^{c}	56.16 ± 25.60
p-value	0.749	$<2.0\times10^{-16}$	0.199	8.79×10^{-5}	0.709

Means in the same column followed by different superscripts differ significantly ($p<0.05$)

Table 3. Water quality parameters in aquaria used for incubation of *Clarias gariepinus* eggs

Treatment	pH	EC µS/cm	TDS (mg.l^{-1})	Temp	DO (mg.l^{-1})
T10	7.55 ± 0.03	138.00 ± 4.00	75.0 ± 1.0^{b}	26.00 ± 0.30	4.2 ± 0.1
T20	7.49 ± 0.06	132.00 ± 4.00	57.0 ± 1.0^{a}	26.15 ± 0.15	4.6 ± 0.2
T40	7.58 ± 0.07	126.50 ± 5.50	70.0 ± 3.0^{b}	26.50 ± 0.30	4.8 ± 0.1
T50	7.52 ± 0.04	139.00 ± 3.00	48.0 ± 2.0^{a}	26.70 ± 0.20	4.3 ± 0.2
p-value	0.723	0.272	0.002	0.306	0.156

Means in the same column followed by different superscripts differ significantly (p<0.05)

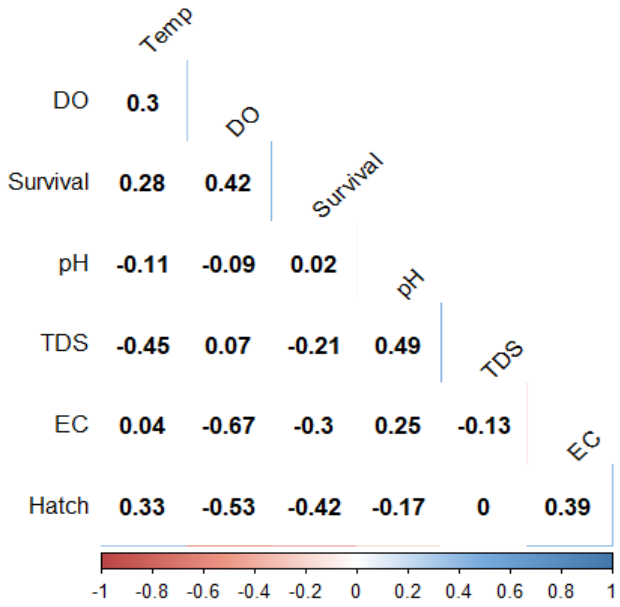

Fig. 2. Correlation plot for water quality parameters and hatchability/survival of *Clarias gariepinus* fry (Increasing color intensity signifies increasing p-values and correlations without color are not significant (p>0.05); Blue color = positive correlation and Red color = Negative correlation)

3.3 Water Quality

Water quality in the incubation tanks (Table 3) reveals that the pH, temperature, electrical conductivity (EC), and Dissolved Oxygen (DO) were not significantly different among the treatments (p>0.05). Total Dissolved Solids (TDS) was highest in incubation tanks used for 10µg/kg dose of Suprecur and least in the tanks used to incubate eggs derived from 50µg/kg dose of Suprecur.

3.4 Relationship between Water Quality and Breeding

Correlations between water quality parameters and breeding parameters: hatchability and survival (Fig. 2) show that there was no significant correlation (p>0.05) between the water quality parameters themselves and the breeding parameters as well.

4. DISCUSSION

4.1 Fecundity

The fecundity obtained in the current study is quite higher, compared with the range of 9918.83 to 11544.13 eggs obtained from *Heterobranchus bidorsalis* induced to spawn with homoplastic hormone ovaprim by [45]. The egg number recorded in the current study was higher in fish treated with 40 µg/kg of Suprecur followed by those treated with 20 µg/kg. This result is in line with a previous report by [46], where a number of ovulated eggs using 50 µg/kg of Buserelin acetate (LHRHa) with 10mg/kg of dopamine antagonist was 33856 compared to 2541 eggs with LHRHa alone and even more than all other hormone cocktails used.

4.2 Latency Period

The latency period observed in the current study was least in the highest dose of Suprecur (50 µg/kg) with a value of 12 hours. According to Sharaf [47], the latency period of *Clarias gariepinus* induced using GnRHa and the dopamine blocker pimozide ranged from 9.5-12 hours. In an experiment using LHRHa and pimozide, a dopamine antagonist, [48] reported a latency period of 12.3 hours for all the females of African catfish (*Clarias gariepinus*). However, in another trial using the same combination of hormones, [49] reported a latency period of 16 hours. The time between injection and stripping of eggs from female broodstock in the hatchery is actually indicative of the physiological response of the females to inducement by artificial hormones [50]. Results from previous breeding trials using various hormonal treatments did show that the effect of hormonal treatment on spawning performance and larval quality can be very inconsistent with a tilt of performance being observed in a particular spawning agent over the others considering the spawning success and survival of larvae produced [23]. This notwithstanding, the plethora of factors that can be used to determine an appropriate inducing hormone, its ability to synchronize ovulation is critical. Synchronization of spawning is a critical factor in hatchery management with the benefit of maximizing time spent on breeding with equal disposition on the percentage hatchability.

4.3 Fertilization Rate

Percentage fertilization in each treatment group of this study show that co-administration of Suprecur and Motilium (Dopamine antagonist) successfully increased the fertilization rate across all treatments regardless of the decreasing levels of Suprecur. Studies that utilized a hormone in tandem with a dopamine blocker in clariid fish species including *Clarias gariepinus* [18] and Vundu catfish (*H. bidorsalis*) [44] had concluded that the combination of a hormone with a dopamine antagonist showed much more effective compared to the LHRHa or GnRHa hormone alone. A confirmation is in the report by [51] where simultaneous injection of pimozide (10 mg kg−1) and a higher dose of LHRHa (100 µg kg−1) elicited >75% *fertilization* rate.

4.4 Hatchability

The hatchability rates recorded in this study differed significantly ($p<0.05$) among the differently treated broodstock. The range of hatchability observed in this study is wider than the range observed by [23] Similarly, [52] reported a significantly lower hatching rate in GnRHa treated females than those injected with ovatide or GnRHa and a dopamine antagonist (Domperidone) with their results being attributed to the poor quality of eggs.

4.5 Survival Rate

From this study, the percentage survival rate of *Clarias gariepinus* larvae across treatment groups was generally above 50% except in the group obtained from 10 µg/kg of Suprecur which had about 45% survival to first feeding. Again, despite the large number of eggs produced in the treatment with 10 µg/kg of Suprecur, the lower survival rate recorded in this treatment might be attributed to the poor quality of eggs stripped. The survival rates recorded in this study are similar to those obtained by [53] who recorded a 50–60% percentage survival rate for hatchlings of African catfish (HCG injected) and (10–30%) for those injected with "Ovaprim".

5. CONCLUSION

The use of Suprecur (LHRHa) together with dopamine antagonist (Motilium) successfully induced ovulation in *Clarias gariepinus* broodfish. The addition of dopamine antagonists successfully increased fertilization rates. However, the obtained results clearly indicated an overall superiority of using 50 µg/kg of Suprecur together with 5mg/kg of Motilium to induce spawning with regard to the recorded high hatchability percentage. With effective management, the survival rate can be increased.

6. RECOMMENDATION

Suprecur can be used to induce *Clarias gariepinus* at a dose between 40 µg/kg and 50 µg/kg with co-administration of a dopamine antagonist: Motilium at 5mg/kg body weight. Reduction of dosage below 40 µg/kg is not advisable in order to optimize fry or fry production.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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