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PROOF
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Gerontological Impact on Embryonic Development of Common Carp *Cyprinus Carpio* Var. *Communis*

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Abstract

The present study deals with the gerontological impact on embryonic development of hatchery-based broodstock of *Cyprinus carpio* var. *communis* (Linnaeus, 1758) in two different age groups (0 and +1 year). The average size of fertilized eggs (adhesive, round and yellowish) of the experimental fish was recorded as 1.00±0.04 and 1.15±0.06 mm for 0 (t_0) and +1year (t_1) age groups, respectively. About eight hours post-fertilization (hpf), the gastrula disc began to protrude gradually and formed the germinal ring in fertilized eggs of the +1-year brooder group while the development of eggs of 0-year group was comparatively slow. The head and tail region development became visible at 16 hpf in the +1-year group eggs. Head region became visible in the 0-year group embryos at 30 hrs post-fertilization while in the +1-year group optic primordium and somites developed prominently at this stage. Hatching took place 52 hpf in +1 year while in the other group, it occurred at 54 hpf. The yolk sac was absorbed rapidly in the hatchlings of +1-year group compared to 0-year group while after the yolk sac absorption, development was observed almost to be the same in both groups' embryos. This embryological study is advantageous in choosing better broodstock for aquaculture production.

Introduction

Understanding the fish reproductive biology is crucial and fundamental for effectively designing conservation and management strategies to enhance fishery resources (Brewer et al., 2008; Grandcourt et al., 2009; Griffeth, 2024). The evaluation of fundamental life-history information and the analysis of fish population dynamics, along with assessing the effects of environmental fluctuations on them, are essential components (Schlosser, 1990). Furthermore, reproductive traits play a vital role in facilitating the advancement of commercial aquaculture for specific aquatic species (Muchlisin et al., 2014). Reproduction in most teleosts follows a cyclical pattern and is limited to a relatively short time span (Meijide et al., 2013). Variation in early life history traits, including egg quantity or quality, is often related to maternal effects (Lauer *et al*., 2005). Key factors in this activity include the size of fish eggs and the level of fecundity exhibited by female broodstock. In males, it is sperm motility, duration and spermatocrit (Rana, 1995; Runangwa et al., 2004). The intraspecific disparity of egg size is associated with fish age, size, physiological condition of the female, spawning times and variations in environmental conditions (Baynes & Howell, 1996)**.** The variation in diameter seems to be one of the most important criteria for the determination of egg quality for fish (Kamler, 2005). A correlation between age and size of female fish with egg quality shows that larger and older females produce larger eggs, which show higher fertilization and hatching rates (Quintero et al., 2011). Similarly, semen characteristics also correlate with male brooder's age (Rahbra et al., 2012).

The *Cyprinus carpio* (common carp) is the fourth most widely cultivated and commercially important freshwater fish species in the world (FAO, 2022). It generally inhabits freshwater environments, especially ponds, lakes and rivers (Barus et al., 2001). It is widely distributed worldwide, especially in Asia and some European countries (Barus et al., 2001; Weber & Brown, 2011). It was introduced in India during 1959 (Parkos & Wahl, 2014). It is the most preferred fish for pond culture due to its fast growing and omnivorous feeding habit (Parkos et al., 2003; Rahman et al., 2009). It was included as an exotic species but with time, it has become an integral constituent of three, four and six species of composite fish farming in India (Thamizhselvi & Thirumathal, 2016). The ecological spectrum of this carp is broad as it is a hardy fish, which easily thrives in a wide variety of aquatic habitats (Froese & Pauly, 2002). At present, the contribution of common carp to global aquaculture production remains at about 8.6% (FAO, 2022).

Broodstock management is a key factor determining gamete quality and hence the success of artificial reproduction (Alavi et al., 2008; Weber and Brown, 2009). Due to undesirable ecosystem effects, common carp is intensively managed in areas of introduction or invasion to control abundance (Weber & Brown, 2009). However, control of adult populations of common carp is difficult due in part to their early maturation, high fecundity and rapid growth rates (Weber & Brown, 2011). Knowledge of the factors regulating broodstock productivity is therefore of great importance for the further development of fish production (Coward & Bromage, 2000). Raising seed productivity in hatcheries is the ultimate aim of broodstock management. Such information will assist commercial facilities in organizing and, if possible, optimizing egg and fry production. The gonadosomatic index (GSI) is one of the important parameters used for fish reproduction studies. The GSI is used to detect hydrated ovaries and therefore it detects the reproductive period from weight increase.

Spawning occurs during a specific phase of the reproductive cycle. Some fishes spawn once a year while others spawn at regular intervals throughout the year (Jackson et al., 2006; Tubert et al., 2012). Fish spawning is affected by many factors such as species, origin of population, fish condition, size and age as well as environmental factors including photo-thermal conditions (Bromage et al., 2001). The environmental factors affect the natural spawning of *Cyprinus carpio.* In tropical and subtropical regions it usually matures during its first year and may spawn several times (Sivakumaran et al., 2003; Muller et al., 2024). It may spawn throughout the year in tropical areas of India, with peaks during January-March and July-August.

Understanding the quality of gametes with reference to the age of broodstock is a prerequisite for aquaculture practices and hatchery management (Aliniya et al., 2013). The present study embodies the assessment of the reproductive performance of *Cyprinus carpio* var. *communis* of different age groups.

Materials and Methods

Collection of mature broodstock

Mature broodstock (male and female) of *Cyprinus carpio* var. *communis* having different age groups (0 and +1 years) with an average weight of 580±51.76 g (virgin male), 734±54.62 g (+1 year male), 790±49.26 g (virgin female), 1100.5±49.26 g (+1 year female) were collected from Instructional Fish Farm, College of Fisheries, G.B. Pant University of Agriculture & Tech., Pantnagar for the process of fertilization.

Assessment of fertilization and hatching rates

Selected brooders were placed in breeding hapa in the tank, where a layer of grass was spread as the substratum for adhesive eggs. After fertilization, the eggs were collected. The fertilization rate was assessed by random counting of approximately 300 eggs adopting the standard formula given by Muir & Robert (1985).

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Number of total eggs}} \times 100$$

After fertilization, the developing eggs were carefully collected from the breeding tank by the use of a dropper. The growing eggs were sampled every two hours interval until hatching and every four hours up to yolk sac absorption (Mojer, 2015).

After completing the incubation period (about 72 hrs), the hatching percentage was calculated for both age groups adopting the standard formula (Rashid et al., 2014).

$$\text{Hatching rate (\%)} = \frac{\text{Total no. of hatchlings}}{\text{Total no. of fertilized eggs}} \times 100$$

Study of larval developmental stages

In the period of larval development after hatching, until the end of the yolk sac absorption (pre-larvae) and subsequently, until the end of metamorphosis (post-larval), all the stages were observed through over mounted compound digital microscope camera (Make: Motic, Model: BA 410) for both age groups and the developmental stages. The duration of yolk absorption was determined by the change with time in the yolk sac and its dissolution.

The weight and length of 10 fries from each group were recorded using an electronic weighing balance and centimetre scale, respectively:

$$\text{Average weight} = \text{Sum of the weight of all fry}/10$$

$$\text{Average length} = \text{Sum of the length of all fry}/10$$

The data were analysed using one-way ANOVA at a 5% level of significance using SPSS.

Results

The embryonic development begins immediately after fertilization. Fertilized eggs of *Cyprinus carpio* var. *communis* were adhesive, round and yellowish with an average diameter of 1.00±0.04 and 1.15±0.06 mm of 0 and +1-year groups respectively. The mean fertilisation rate (%) in the 0-year group and +1-year class was 70.00±1.73 and 74.33±1.15, respectively (Table 1). A positive correlation between egg diameter and fertilization rate was recorded in both groups which was significantly higher (r=0.838) at 5% level of significance in the 1-year group as compared to the 0-year group (r=0.771).

Hatching occurred at 54.33±3.05 and 52.66±2.08 hours of post-fertilization in the 0-year and +1-year groups and the average hatching rate was recorded as 60.33±3.21 and 62.66±2.52%, respectively. The significantly higher average survival rate ($p<0.05$) of larvae in the 0-year group (97.00±1.00%) was recorded compared to the +1-year group (91.66±2.08%).

In the present study, the embryonic development of common carp is divided into two stages *viz.* embryonic stage and larval stage (Figure 1). At eight hours of post-fertilization, the gastrula disc began to protrude gradually inwards and form germinal ring. After 16 hours of fertilization, the formation of germinal layer started while blastoderm occupied most of the yolk. The progress was somewhat similar in both groups.

Closure of the blastopore and beginning of embryo formation began nine hours post fertilization. Formation of the optic lens and initiation of a heartbeat occurred between 16.00 to 24.00 hours after fertilization. It confirms the present study as the appearance of the optic vesicle was observed between 24 and 30 hours.

Between 24 and 30 hours, the embryo little elongated and head development started while the blastopore was not closed. Development of embryo in a 0-year parent's is slightly slower than in +1-year-old ones. Though somites were visible in both groups these were clearer in the +1-year-old group. Optic primordium also appeared more prominent in the +1-year-old class. After 30 hours, the embryo became more elongated due to a slight extension of tail end. After 36 hours, the brain differentiated in both groups.

After 42 hours, the optic vesicles were quite elongated and the number of somites and embryos showed further growth. A visible eye lens became more defined, comparatively, in a more advanced stage of +1-year broodstock progeny. The thickness of egg membrane was also reduced and the embryo started an active movement. Finally, the embryo cleaved the egg membrane and began to move freely. Hatching occurred on average after 52 hours of fertilization in the +1-year-old group and after 54 hours in the 0-year group.

In the study, the germinal ring formation occurred after 3.50- 6.30 hours of fertilization, in the present study it was observed lately as compared. The brain became clearly differentiated and the optic vesicle became a little elongated after 7.30-13.40 hours. The embryo cleaved the egg membrane and began to move freely after 38 hours.

The larvae were liberated from the egg membrane due to strong movements of their tail. The small, transparent, cylindrical larva with an oval yolk sac is called a hatchling. At this stage, the mouth was still closed, while the head was clear (60-84 hours). The heart becomes visible. The emergence of the vestigial pectoral fin occurred after 84 hours while the mouth was still closed and the larvae fed on the yolk of yolk sac. The yolk sac was reduced after 96 hours of growth due to the appearance of melanophore cells at the ventral edge. The mouth became more differentiated. The pigment cells in the head region of the larva increased along the mouth.

The appearance of buds for pelvic and dorsal fins occurred after 108 hours. The opening of the mouth was clearly visible. Pigmentation increases over the body. The heart becomes clearly visible in both groups. After 120 hours of fertilization, the number of pigment cells increased to cover most of the larva body. Pair of barbells was visible around the mouth. In both groups, the development process appeared to be similar. Pectoral fins can be easily differentiated as they become fully developed after 212 hours. The larvae start proper swimming and active feeding. The caudal fin appeared incompletely structured after 258 hours. The larva is fully active and developed.

After rearing for around 25 days, these larvae become completely developed and enter the fry stage. The average length and weight of fry of 0-year and +1-year groups were 21.67±1.53 mm, 0.23±0.05 g and 22.00±1.00 mm, 0.25±0.05 g, respectively.

Table 1: Progeny performance of two different broodstock groups

Parameter	0 year	+1 year
Fertilization rate (%)	70.00±1.73 s	74.33±1.15 s
Hatching after fertilization (hrs.)	54.33±3.05	52.66±2.08
Hatching rate (%)	60.33±3.21 s	62.66±2.52 s
Survival rate (%)	97.00±1.00	91.66±2.08
Yolk sac absorption duration (hrs.)	97.33±3.05	110.00±6.00
Length of hatchling at 7th day (mm)	6.73±0.21	6.86±0.05
The average length of fry (mm)	21.67±1.53 s	22.00±1.00 s
The average weight of fry (g)	0.23±0.05 s	0.25±0.05 s

*s = significant

Figure 1. Comparative embryonic development of progeny from two different age groups of *Cyprinus carpio*

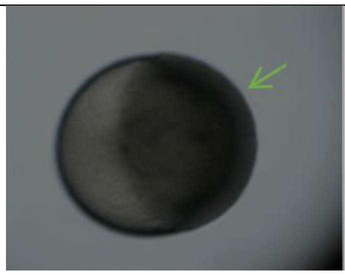
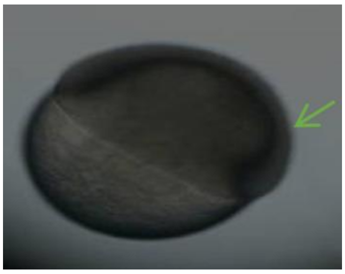
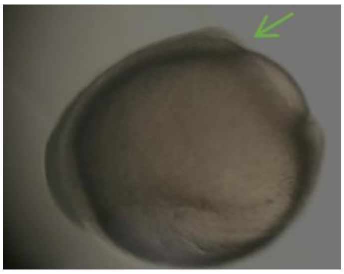
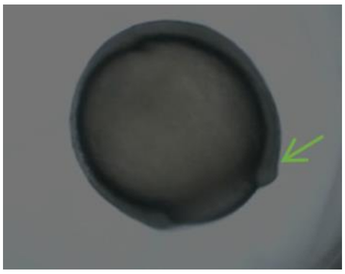
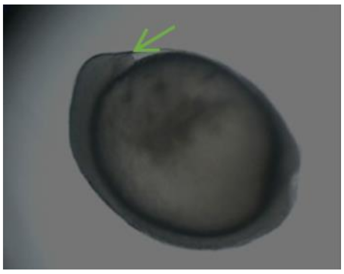
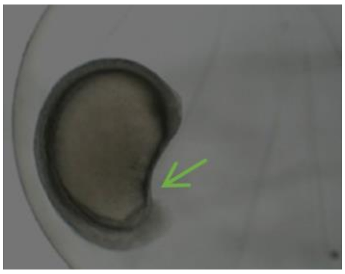
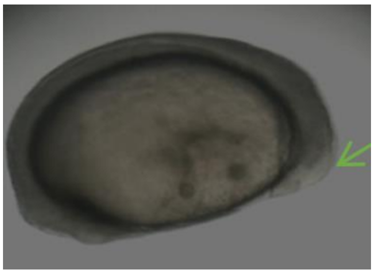
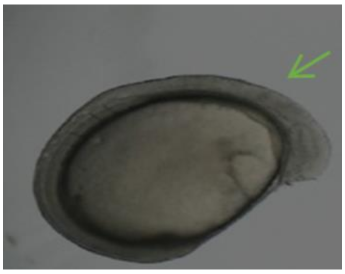
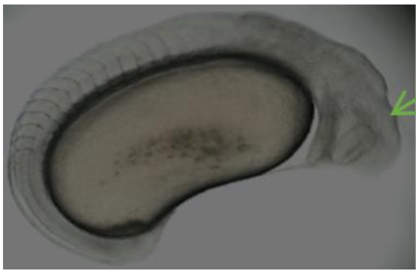
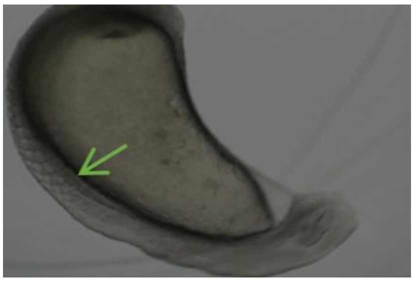
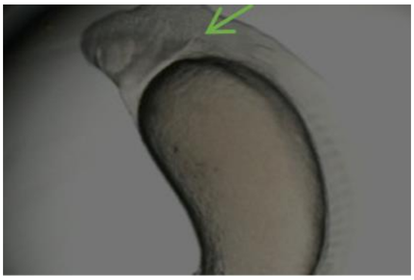
Time (in hrs.)	0-year	+1-year
8	 Development of blasto-disc	 Development of blasto-disc
12	 Germinal ring formation	 Yolk plug stage
16	 Initiation of embryo over yolk sac	 Embryo formation begin
24	 Developing embryo	 Developing embryo with well developed head
30	 Developing embryo with well developed head	 Developing embryo with well differentiated head and somites
36	 Developing embryo elongated showing head region, somites and brain	 Developing embryo well defined somites, brain and yolk sac

Figure 1. *Continued*

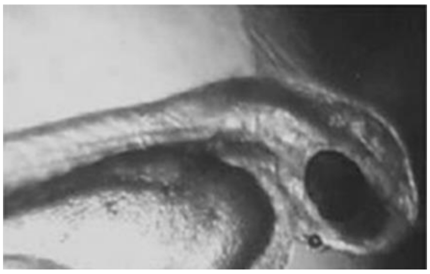
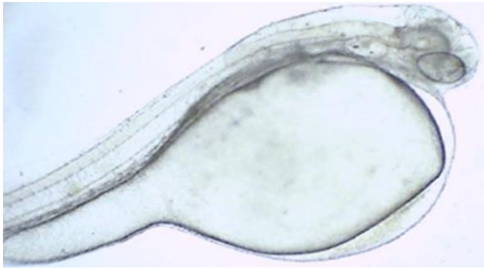
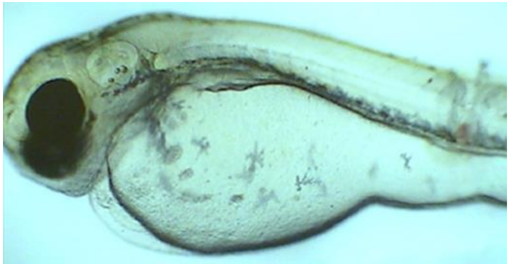
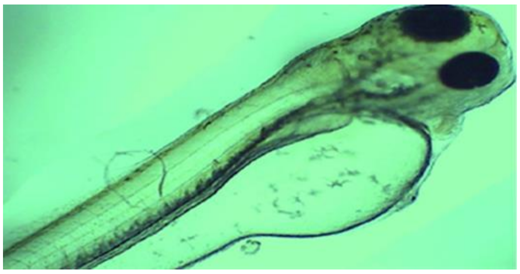
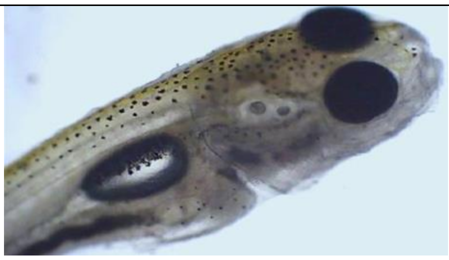
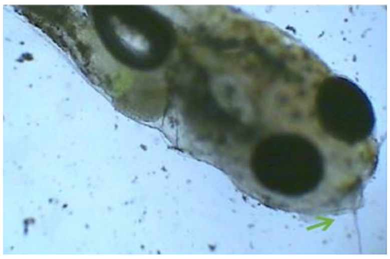
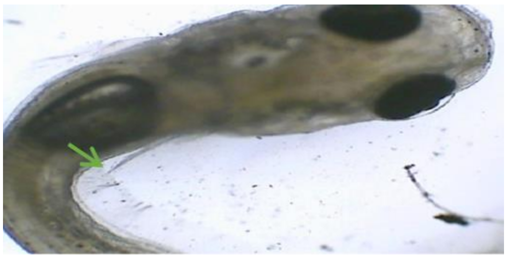
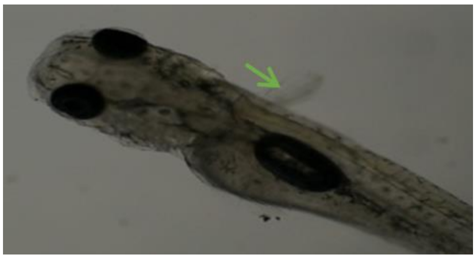
Time (in hrs.)	**0-year**	**+1-year**
42	 Eyes appeared and tail bud stage	 Eyes visible , yolk sac appeared in oval shape, hatching stage
52	 Hatching stage	 Hatchling just after hatching
54	 Embryo showing well developed yolk sac and heart	 Embryo showing well developed yolk sac and heart, pigmentation started
60	 Embryo showing well developed yolk sac and heart, initiation of pigmentation	 Embryo showing vascular bed of vessels and heart, yolk became thin
72	 Embryo showing vascular bed of vessels and heart	 Pigmentation spread over the body, vascular bed of vessels visible distinctly
84	 Pigmentation spread over the body, yolk sac became thin	 Emergence of vestigial pectoral fin occurred, feeding on yolk sac

Figure 1. *Continued*

Time (in hrs.)	0-year	+1-year
96	 Mouth became more differentiated and open, showing active digestive system	 Mouth became more differentiated and open, showing active digestive system
108	 Appearance of buds for pelvic and dorsal fins	 Appearance of buds for pelvic and dorsal fins, active feeding started
124	 Pair of barbels visible around mouth	 Pair of barbels visible around mouth
212	 Pectoral fins can be differentiated easily	 Pectoral fins distinctly visible
258	 Development of body structure and all fins complete	 Development of body structure and all fins complete

Discussion

In the present study, a higher fertilization as well as hatching rates has been recorded in +1 year group. Similar to the present findings, Aliniya et al. (2013) also recorded a higher fertilization rate in the older age group of common carp. The fertilization rate of *Salmo trutta abanticus* was reported in the range of 72 - 80% with a mean value of 76.0±4.0% (Bozkurt, 2006). It is believed that larger and older female broodstock spawn bigger eggs (Brooks et al., 1997; Jhingran, 2006) resulting in higher fertilization and hatching rates (Trippel et al., 1998). Hence, egg size is an important parameter for the success of fertilization. The range of survival rate in the present study is confirmed by the survival rate of four different age group's brooder progeny of Kutum fish, which ranged between 85 to 95% (Savakoudhi & Khara, 2017).

In common carp, generally, hatching occurs between 38 and 72 h of post-fertilization (Trippel et al.,

1998) while in the present study, it occurred at 54.33±3.05 and 52.66±2.08 hours of post-fertilization in the 0-year and +1 year groups, respectively. The variation in the incubation period and hatching rates is possibly due to differences in the physicochemical condition of water at various places (Kala et al., 2020). Like present study, Mojer (2015) also recorded similar results with regards to embryonic development of common carp. In accordance with present findings, it was confirmed that time required for absorption of the egg yolk after hatching differed, being 4-5 days in *Cyprinus carpio* (Han et al., 2001)

In catfish, *Clarias gariepinus,* the effect of broodstock age was determined on fry growth and found that fry from 24 and 30 months old broodstock had a significantly ($p<0.05$) higher weight (18 g and 24 g) at four weeks of age compared to fry obtained from 15 and 18 months old female broodstock which weighed 8.33 g and 10.00 g, respectively (Jokthan, 2013). Park et al. (2017) reported that in *Cyprinus carpio,* the mouth is not opened just after hatching. Melanophores appear all over the body in the shape of tree branches after 13 days of hatching, whereas pigmentation was started all over the body after about 7 days in the current study. The length of juvenile fish after 30 days of hatching, was 21.8–22.4 mm (Park et al., 2017) while in the present study, the mean length of larvae of 0-year group broodstock is observed around 21.67±1.53 mm and of 1-year broodstock is 22.00±1.00 mm.

Haniffa *et al.* (1991) reported that, in common carp the fertilized eggs were transparent and adhesive with a diameter ranging between 0.9 mm and 1.10 mm. Hatching occurred after 73.00 hours. The hatchlings were transparent and measured an average of 2.7-2.9 mm, with a large oval-shaped head and well-defined yolk sac. The yolk got fully absorbed within 3 days in the larva and the larvae initiated exogenous feeding. Berkeley et al. (2004) stated that in *Sebastes melanops* the progeny from the older age group showed higher survival under a broader range of environmental conditions than the progeny from the younger group, which is also found in the present study.

Conclusion

Present study results revealed that the +1-year-old group showed better gamete quality and progeny performance in comparison to the 0-year group of common carp. Accordingly, it found that +1-year female is more suitable for breeding and production purposes and in females; the effect of age on the reproductive profile is evident. It also proves that age plays an important role in overall reproductive performance, which affects upcoming generations as well. Therefore, consideration of age is important during the selection of quality broodstock.

Ethical Statement

All procedures were performed in compliance with relevant laws and institutional guidelines and that the appropriate institutional committee has approved them vide synopsis number CFSc/ FRM/3550.

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Author Contribution

First Author: Conceptualization, methodology, data curation, visualization, writing original draft and formal analysis

Second Author: Investigation, supervision, review and editing

Third Author: Investigation, supervision, review and editing

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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