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Age-dependent Enhancement in Fecundity and Ova Diameter of *Schizopyge niger* (Heckel, 1838) in Dal Lake, Kashmir

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Abstract

Understanding the reproduction and recruitment pattern is essential for successful conservation of a species. Accurate estimates of fecundity plays a crucial role in comprehending fish population dynamics. The snowtrout, *Schizopyge niger*, is a Cyprinid fish widely distributed throughout the lakes of Kashmir valley and is one of the most important commercial fish species. In this study, we examined the age-dependent reproductive performance of female *S. niger*, aiming to gain insights into its breeding characteristics for the development of an optimal brood-stock management plan. The study involved 78 female *S. niger* specimens collected from Dal Lake during the spawning season, representing age groups of 2^+, 3^+, 4^+, 5^+, and 6^+ years. Fecundity and ova diameter showed an increasing trend from age 2^+ (3,971 eggs; 1.45 mm) to age 6^+ (10,501 eggs; 2.00 mm). Total fecundity exhibited positive correlations with age, total weight, ovary weight, and ova diameter. Additionally, there was a positive correlation between ova diameter and fish age, with the highest fecundity and largest ova diameter observed in the 6^+ years age group. Among each age group, fecundity and ova diameter varied significantly (p <0.05). Based on these findings, for optimal broodstock management of *S. niger*, it is recommended to use females of the 6^+ years age group after assessing the quality of eggs.

Keywords: *Schizopyge niger*, age, fecundity, ova diameter, Dal lake, Kashmir

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Introduction

The snow trouts, members of the Cyprinidae family and order Cypriniformes, are thought to have migrated into the lakes and streams of Kashmir from watersheds in Central Asia, bordered by the inner and southern slopes of Hindukush, Karakoram, and the inner ends of the northwestern Himalayas and Suleiman Ranges (Sehgal, 1999). In India, these snow trouts inhabit the cold waters of Jammu and Kashmir, Assam, Eastern Himalayas, Bhutan, and Sikkim, situated at an altitude ranging from 1180 to 3000 meters above mean sea level (Chandra, Barat, Singh, Singh, & Matura, 2012). Among them, *Schizopyge niger* (von Hügel, & Heckel, 1838), locally known as "Ael Gad" or "Alghad," resides in the cold streams and rivers of Kashmir, Afghanistan, and Pakistan. Initially classified under the genus Schizothorax by von Hügel and Heckel (1838), it was later reclassified as Schizopyge by Russegger (1843) during the restructuring of schizothoracid classification. This study acknowledges the basionym *Schizopyge* in light of research on fishes in the Kashmir Valley conducted by Kullander, Fang, Delling and Ahlander (1999).

Understanding the reproductive cycle and the factors influencing it are important in fish and fisheries biology (Tomkiewicz, Tybjerg, & Jespersen, 2003). Accurate estimates of fecundity play a crucial role in comprehending fish population dynamics, forecasting abundance trends and assessing spawning stock biomass (Eldridge & Jarvis, 1995). Reproductive potential refers to the capacity of a fish species to reproduce, establishing a connection between the spawning stock and subsequent recruitment (Gundersen, Nedreaas, Kjesbu, & Albert, 2000). Fecundity is the measure of the reproductive capacity of a female fish (Bagenal, 1978) and varies according to many factors, including the age of the female and is influenced by various environmental factors like food availability, water temperature, photoperiod, season, etc. Fecundity estimates are instrumental in fish conservation and management (Shah, Chakraborty, Kumar, & Sadawarte, 2018), since it provides basis for predicting population trends and formulating strategies for stock enhancement.

Knowledge of age dependent reproductive potential is an important factor for standardizing its artificial breeding or seed production strategies of a candidate species. Since age of fish plays a crucial role in determining quality of gametes (Quintero, Durland, Davis, & Dunham, 2011; Aliniya, Khara, Novieri, & Dadras, 2013), considering the age of brooders is essential for obtaining high quality offspring and the selection of brood-stock based on these criteria can significantly influence the success of breeding programs. As older fish grow larger, their reproductive output tends to rise, frequently surpassing what might be expected based solely on their size (Woodhead, 1998; Reznick, Ghalambor, & Nunney, 2002). The significance of old females is widely acknowledged in fish stock assessments (Green, 2008), thus highlighting the importance of maintaining a balanced age structure within fish populations for sustainable fisheries management. The reproductive potential of a population is influenced by the careful selection of broodstock for production purposes (Najar & Qadri, 1999; Najar, Qadri, Wani, & Zargar, 2000).

A major limitation in commercial fish farming is broodstock productivity, and understanding the factors governing broodstock productivity is crucial for advancing fish culture (Coward & Bromage, 2000). This knowledge can lead to improved techniques in hatcheries, ultimately boosting fish production. The ultimate goal of broodstock management is to maximize seed productivity in hatcheries, with key factors including egg diameter and the fecundity of female breeders.

While existing literature provides abundant information on fecundity and ova diameter in the wild for *S. niger* (Sabha et al., 2017; Hussain et al., 2018; Ali, Shah, Bhat, & Rashid, 2020), there is a notable absence of data regarding the outcomes related to age in this species. Addressing this gap is crucial for developing age-specific brood-stock management strategies that can enhance reproductive success in hatcheries. Such strategies are essential for ensuring long term viability of fish populations, particularly in the context of the changing environmental conditions in Kashmir valley. The main objective of this study was to investigate the impact of age on the reproductive performance of *S. niger* with the aim to suggest potential broodstock management strategies that hatcheries could adopt to improve reproductive indices.

Materials and Methods

Dal Lake, often referred to as the "Jewel of Kashmir," is located in the northeastern region of Srinagar, positioned at a mean latitude of 34°062 N and longitude of 74°522 N, at an elevation of 1584 meters (Fig. 1). Encompassing an approximate area of 11.4 km² and featuring a maximum depth of 5.4 meters, the lake serves as a focal point within the extensive interconnected aquatic ecosystems of the Kashmir Valley (Dar & Romshoo, 2008).

A total of 78 female specimens of *S. niger* were obtained from Dal Lake, Kashmir, during the spawning period from February - April 2021 measuring between 119-293 mm in length and weighing 61-395 g. The age of these samples was determined using opercular bones, a method well-established and widely accepted in temperate waters when compared to other techniques (Le Cren, 1947; Mann, 1973). Opercular bones were carefully extracted from fresh fish samples using a scalpel and briefly boiled in water to remove attached tissues. Subsequently, the bones underwent a 15-minute treatment with 50% H_2O_2, followed by

Table 1. Descriptive statistics showing fecundity and ova diameter in *S. niger*

N	Body weight (g)		Ovary weight (g)		GSI (%)		Absolute fecundity (No. of eggs)		Relative fecundity (per gram body weight)		Ova diameter (mm)	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
78	61	395	6.4	72.5	8.29	30.27	2799	18375	27.12	63.45	0.48	2.32

washing with water and a three-day sun-drying period. The annual growth rings were then counted to ascertain the age of the fish, following the methodology described by Khanna and Singh (2015).

Immediately after dissection, ovaries were carefully removed, measured for length and weight, and subsequently preserved in 10% neutral buffer formalin (NBF) for further analysis. Fecundity was determined using preserved specimens of ovaries through the gravimetric method as outlined by Bagenal (1978). Subsamples were extracted from the anterior, middle, and posterior segments of each ovary with an accuracy of 0.01 g on a counting slide, along with a few drops of water, and the count of mature ova was recorded following the procedure detailed by James, Gupta and Shanbhogue (1978). The average number of mature ova was then calculated from the measured values. Subsequently, this calculated average was employed in the computation of fecundity.

Absolute fecundity was computed using the following equation (Bagenal, 1978)

$$\text{Absolute fecundity} = \frac{\text{No. of ova in subsample x Total ovary weight (g)}}{\text{Weight of sub sample (g)}}$$

The relative fecundity (No. of ova per gram of body weight and ovary weight) was estimated by dividing absolute fecundity with total weight of the fish (g)

$$\text{Relative fecundity} = \frac{\text{Absolute fecundity}}{\text{Weight of fish (g)}} \text{ per gram body weight}$$

The correlation coefficient between fecundity and various parameters was determined using least square method (LSM).

In addition, diameters of intra-ovarian eggs from preserved ovaries were measured after overnight immersion in Gilson's fluid to facilitate the separation of oocytes from ovarian tissue, a process which breaks down the connective tissue as described by Clark (1934). The distribution pattern of eggs was recorded by measuring ova diameter in the anterior, middle, and posterior parts of the ovary using digital vernier calipers (Trusize)™.

The data was analysed using SPSS (ver.19) and MS Excel. The relationships between fecundity, ova diameter, and age were analysed using regression models. Logarithmic transformations were applied to the data to linearize the relationships between variables, which often exhibit exponential or power-law characteristics in biological systems. Linear log regression was selected in this study for its ability to provide a better fit for the data, to provide a more robust and interpretable relationships, ensuring that results were not influenced by outliers.

Results and Discussion

During the present study, marks presumed to be annuli were counted (Fig. 2). It was revealed that the fish samples belonged to age group 2^+ to 6^+ with maximum samples belonging to age group 5^+ (Fig. 3). The total length of the fish varied from 119.39 to 292.17 mm.

The relationship between age (A) and total length (TL) (Fig. 4) has been established as:

Table 2. Average fecundity and ova diameter counts of various age groups in *S. niger*

Age (years)	N*	Body weight (g)	Absolute fecundity (No. of eggs)	Relative fecundity (per gram body weight)	Ova diameter (mm)
2+	9	102.23 ± 10.03	3,971.88 ± 394.89^{a}	38.85 ± 2.30^{a}	1.45 ± 0.013^{a}
3+	14	110.05 ± 5.47	5,115.92 ± 403.11^{b}	46.48 ± 2.06^{b}	1.75 ± 0.007^{b}
4+	12	108.58 ± 8.07	5,435.16 ± 441.15^{c}	50.05 ± 2.45^{c}	1.67 ± 0.009^{c}
5+	28	152.49 ± 6.51	6,512.07 ± 416.75^{d}	42.70 ± 1.66^{d}	1.86 ± 0.006^{d}
6+	15	228.32 ± 21.08	10,501.20 ± 1182.43^{e}	45.99 ± 2.37^{e}	2.00 ± 0.005^{e}

*N in the table depicts sample size of age group

**Means among various age groups with different superscripts (a-e) aligned vertically vary significantly ($p<0.05$); values denote Mean ± S.E.

Log TL = 1.937 + 0.6246 Log A, (R^2 = 0.9859)

The descriptive statistics of overall fecundity and ova diameter of *S. niger* is displayed in Table 1. The absolute fecundity of fish ranged from 2,799 to 18,375 eggs (mean= 6,569) while the relative fecundity ranged from 27.12 to 63.45 eggs/g body weight (mean= 44.38). The ova diameter ranged from 0.48 to 2.32 mm.

Both fecundity and ova diameter exhibited an increase in correlation with the age of the fish, as indicated in Table 2. Fish samples below the age of 2^+ years were excluded from the study as they weren't fecund. Absolute fecundity showed a rise from 3,971 eggs in 2^+ year-old fish to 10,501 eggs in 6^+ year-old fish, while relative fecundity ranged from 38.85 to 50.05 eggs/g body weight, as illustrated in Figures 5 and 6. Additionally, the ova diameter demonstrated an increase with age progression, with the minimum value (1.45 mm) observed in 2^+ year-old fish and the maximum value (2.00 mm) observed in 6^+ year-old fish.

The relationship between fecundity and age (A) (Fig. 7) has been established as:

Log F = 3.3225 + 0.7906 Log A, (R^2 = 0.8378)

The relationship between fecundity and ova diameter (OD) (Fig. 8) has been established as:

Log F = 3.1166 + 2.7512 Log OD, (R^2 = 0.8471)

The relationship between ova diameter (OD) and age (A) (Fig. 9) has been established as:

Log OD = 0.0913 + 0.2594 Log A, (R^2 = 0.8620)

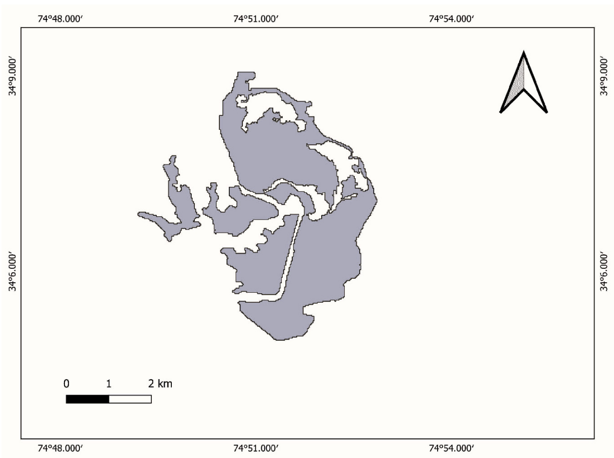

Fig. 1. Map showing the sampling sites (Dal lake)

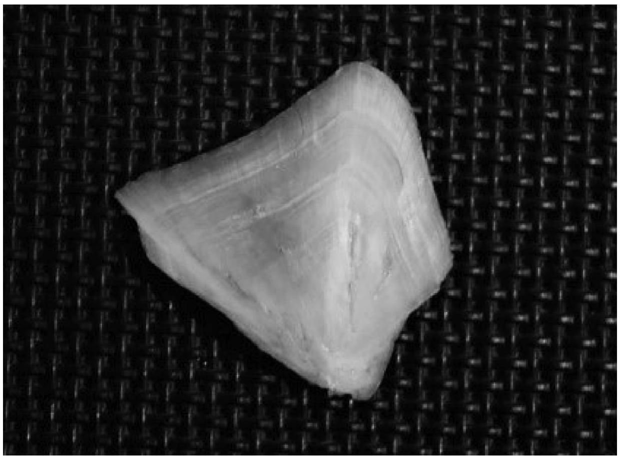

Fig. 2. Opercula showing annuli (3+ year old)

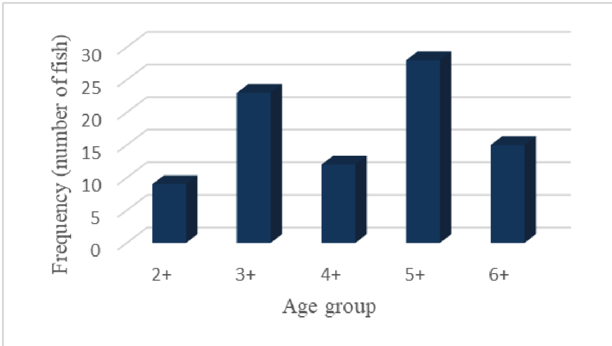

Fig. 3. Age-distribution of *S. niger*

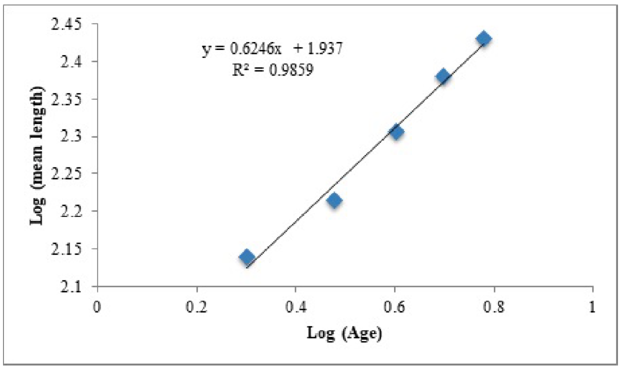

Fig. 4. Logarithmic relationship between age and mean length of *S. niger*

In almost all teleost, the relationship of fecundity with fish weight and ovary weight are found to be linear, as these factors influence fecundity (Bhat et al., 2018). Fecundity is affected by age, size, fish species, feeding of fish, season and environmental conditions (Nikol'skij, 1980; Thorpe, Miles, & Keay, 1984). In the present study, absolute fecundity was found to increase with the advancement of fish age

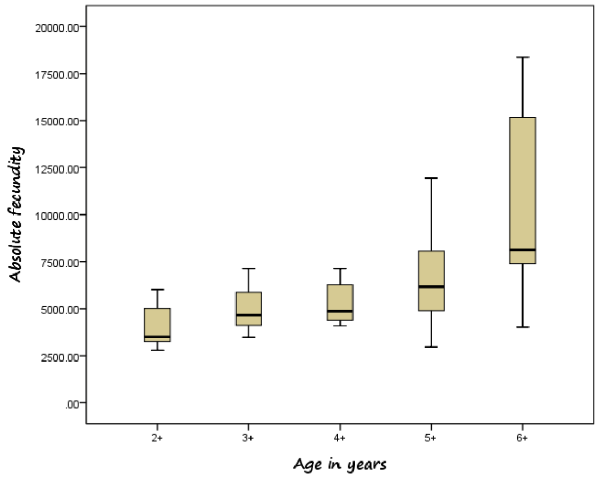

Fig. 5. Variations in the absolute fecundity of *S. niger* with age

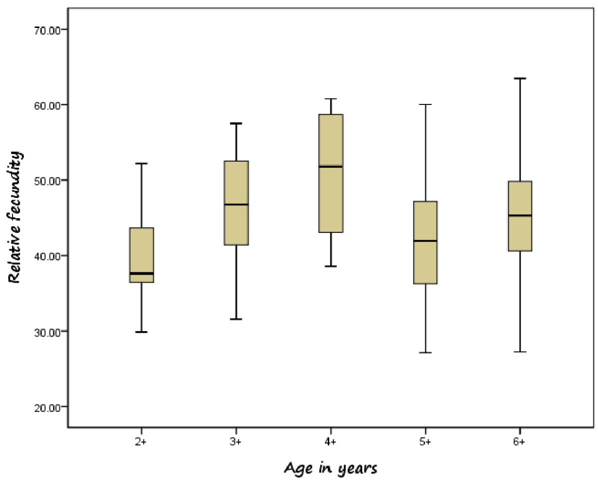

Fig. 6. Variations in the relative fecundity of *S. niger* with age

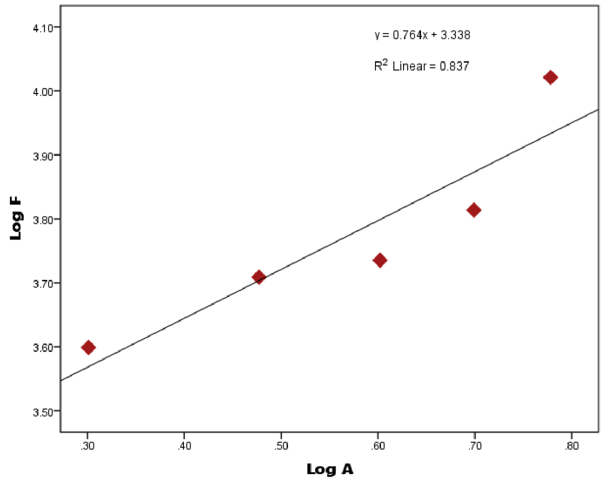

Fig. 7. Relationship between age and fecundity of *S. niger*

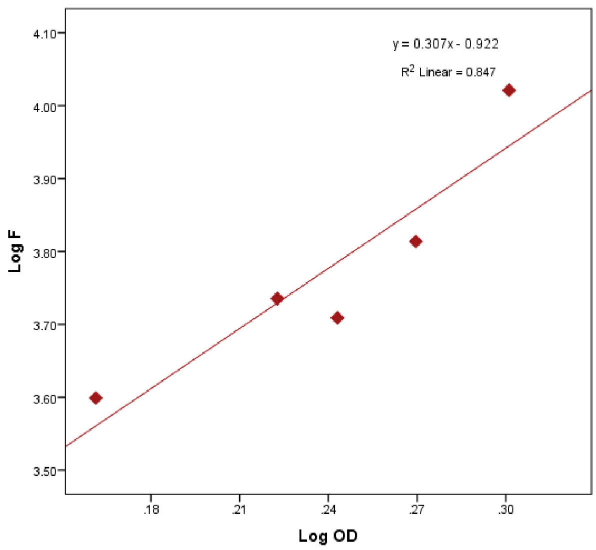

Fig. 8. Relationship between fecundity and ova diameter of *S. niger*

from age 2^+ year to 6^+ year. The results are in agreement with works of Jyoti and Malhotra (1972); Sunder and Subla (1984) for some schizothoracid species, (Schafhauser-Smith & Benfey, 2001) for brook trout (Bandpei et al., 2011) and Savaddkouhi and Khara (2017), for *Rutilus frissi*. In the present study, a positive correlation was noticed between fecundity and age (r^2= 0.8382). Similar results have been reported by Yuce and Sen (2003) and Coban et al. (2013) for some other fish species. Absolute fecundity and ova diameter among various age groups also revealed a positive correlation (r^2= 0.8471). Similar findings were observed by Gandotra, Shankar and Singh (2009) for *S. richardsonii* with correlation coefficient value (r) of 0.915. Younger fish (4^+ year old) exhibited better relative fecundity than older groups. These findings align with observations of Getinet (2008) suggesting that younger females, relative to their weight, possess a greater capacity for egg production.

The egg size is part of reproductive strategy of the fish. In this study, the mean ova diameter was found to increase with increasing age. The Kruskal-Wallis test revealed that the mean ova diameter between various age groups differed significantly ($p < 0.05$). Increase in ova size with increasing age has also been reported by Szczepkowski, Szczepkowska, Krzywosz, Wunderlich, and Stabinski (2010) for

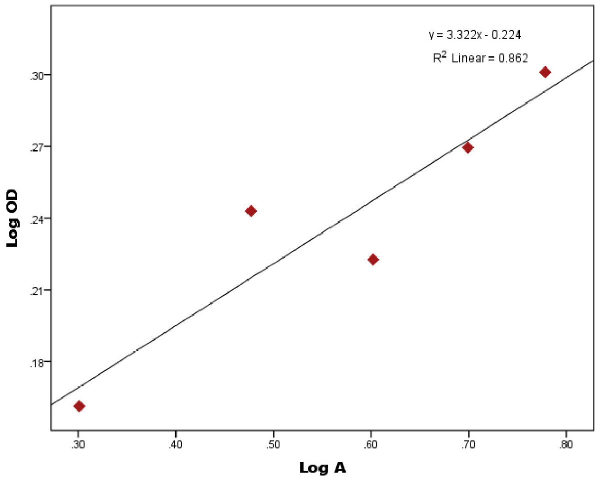

Fig. 9. Relationship between age and ova diameter of *S. niger*

European whitefish. Ova diameter dependency on fish age has been noted in various fish species (Kamler, 2005) and Dupuis and Sutton (2011) suggested that older fish tend to produce larger eggs. The overall fecundity values of *S. niger* observed during this study is in accordance with the results of earlier works on schizothoracids (Qadri et al. (2015) for *S. curvifrons*; Sabha et al. (2017); Ali et al. (2020) for *S. niger*). The mean relative fecundity values of 44.38 eggs/g body weight recorded in the present study is lower than earlier works of Yousuf and pandit (1992) (53.23 eggs/g body weight), Shafi, Yousuf and Parveen (2013) (53 eggs/ g body weight) and Shafat, Bhat, Balkhi, Najar and Mudasir (2016) (48.90 eggs/g body weight). The possible reason for this decline in relative fecundity over the years may be a manifestation of the changing water quality parameters of Dal Lake (Mansour, McNiven, & Richardson, 2006) as water quality exerts a profound effect on fecundity in fish (Burger & Gochfeld, 2005; Krishnani et al., 2003). During the past few decades, concentration of harmful substances has increased in Dal Lake (Mushtaq, Qadri, & Yousuf, 2018; Rather & Dar, 2020; Kumar, Parvaze, Huda, & Allaie, 2022) and prolonged exposure to environmental stressors effects fish metabolism, growth and development including reproductive potential (Barton, Morgan, & Vijayan, 2002; Benejam, Benito, Ordóñez, Armengol, & García-Berthou, 2008). Ova diameter values recorded for *S. niger,* during the present study were in accordance with previous works conducted by CIFRI (1977) and Sabha et al. (2017) for the same species.

This study on *S. niger* suggests a positive association between the age of the fish and both fecundity as well as egg diameter. Notably, the 6^+ year age group exhibited the highest capacity for egg laying and the largest ova diameter. This highlights the importance of this size class for egg collection in culture practices. For practical purposes, fish of age 6+ (260-293 mm length range and 228g weight) should be prioritised for harvesting. However, it is necessary to conduct studies to assess further the egg quality, which is crucial for determining the potential of the eggs to yield viable offspring. Therefore, while the study supports the use of 6^+ year olds for egg production in cultural practices, the overall success hinges on further research to evaluate the quality of the eggs and their ability to produce healthy offsprings.

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