

ORIGINAL ARTICLE

Sperm quality and quantity in striped catfish, *Pangasianodon hypophthalmus* (Sauvage 1878) at various broodstock age stages

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Article history:
Accepted 15 April 2025

Abstract

The success of spawning in striped catfish is largely determined by sperm quality, which can be influenced by several factors, including the age of the male broodstock. This study evaluates the influence of broodstock age on critical sperm quality metrics (motility, viability, and abnormalities) and reproductive success (fertilization and hatching rates) in striped catfish. For the treatments, male striped catfish were grouped into four age categories: Group A (1 year), Group B (2-4 years), Group C (5-7 years), and Group D (>8 years). Sperm volume was measured using a 10mL graduated tube, while sperm concentration, motility, viability, and abnormality were assessed microscopically using the Sperm Class Analyzer SCA® CASA system automatic sperm analyzer. To evaluate sperm performance during fertilization, eggs from the same female were used to assess fertilization rate, hatching rate, and larval abnormalities. The findings showed that male striped catfish aged 2-4 and 5-7 years demonstrated the most favorable sperm quality, with an average sperm volume of 51.0mL, concentration of 29.9million/mL, motility of 96.4%, viability of 97.4%, and abnormality rate of 3.6%. In contrast, the 1-year-old group showed the lowest performance, with an average sperm volume of 11.5mL, concentration of 27.7million/mL, motility of 66.5%, viability of 67.4%, and abnormality rate of 1.4%. Based on these results, it is recommended that broodstock aged 2-4 years be prioritized for spawning activities due to their higher sperm production, better sperm quality and they produce offspring that have good survival.

Keywords: Pangasius catfish, Sperm Class Analyzer SCA, Motility, Reproduction

INTRODUCTION

Siamese catfish or striped catfish (*Pangasianodon hypophthalmus* Sauvage 1878) is a species of pangasius known to have high economic value has good water quality tolerance and is easy to cultivate so that it becomes one of the leading commodities in the world fish trade, including in Indonesia. The total world pangasius production in 2020 was 2.5 million tons and continues to increase with an estimated production in 2024 of 3.2 million tons (FAO 2022; Jory 2023). Indonesia as the 3rd largest pangasius producer in the world, also shows a fairly high increase in pangasius production. During the period 2020-2023, pangasius production increased by an average of 6.5% per year with production in 2023 of 348,478.76 tons, with an estimated Indonesian pangasius production in 2024 of 371,095.03 tons (KKP 2024).

The success of striped catfish cultivation is highly dependent on the productivity of its brood spawning,

where profitability and sustainability of the cultivation business require a guarantee of the availability of good-quality seeds. An important factor in striped catfish spawning is the quality and quantity of sperm produced by the male broods. Previous studies on species such as zebrafish and other fish have shown that sperm quality of the male brood has a significant effect on fertilization success and the genetic quality of the offspring produced (Riesco et al. 2019; Pamungkas et al. 2023).

The age of the male brood is thought to affect the level of sexual maturity and sperm quality, including the volume, concentration, motility, fertilizing ability and survival of the larvae produced. However, information regarding the effect of age of male brood's striped catfish on the quality and quantity of sperm produced is not yet known. Several studies on the effect of the age of male brood fish on the level of sexual maturity and sperm production have been conducted, including in the walleye fish *Sander*

vitreus (Casselman et al. 2006), guppy fish *Poecilia reticulata* (Evans et al. 2002), zebrafish *Danio rerio* (Kanuga et al. 2011; Johnson et al. 2018), rainbow trout *Oncorhynchus mykiss* (Shamspour & Khara 2016), Atlantic salmon *Salmo salar* (Erraud et al. 2021), and Arctic charr *Salvelinus alpinus* (Kurta et al. 2023). The results of these studies show that differences in the age of male broods affect sperm production and fertilization success. Studies have shown that younger males often produce higher quality sperm, resulting in better fertilization rates when mating with females. Interestingly, while older males showed reduced fertility rates, the offspring produced from these males exhibited superior survival compared to those sired by younger males. However, research on the impact of differences in the age of male broods on sperm quality and quantity in striped catfish is limited. This study aimed to evaluate the influence of broodstock age on critical sperm quality metrics (motility, viability, and abnormalities), reproductive success (fertilization and hatching rates) and survival of larva in striped catfish. Given the significant role of striped catfish in the fisheries industry and its potential to enhance productivity, research on the effect of broodstock age on sperm quality and quantity is highly relevant. The information obtained from this study could serve as a foundation for improving striped catfish breeding management and developing more effective cultivation strategies, ultimately contribute to the economic growth of the fisheries sector and environmental sustainability.

MATERIAL AND METHOD

Research Sites: This research was conducted at the Research Institute for Fish Breeding (RIFB), West Java, Indonesia. The RIFB is one of the research centers of The Ministry of Marine Affairs and Fisheries, of the Republic of Indonesia. This study did not require ethical approval from any institution, and there are no applicable laws in Indonesia. Striped catfish were acquired from the institute's broodstock population.

Experimental Design: Striped catfish male broods

were grouped into four different age categories: A. ≤ 1 year ago (1.02 ± 0.06 kg), B. 2-4 years ago (2.83 ± 0.31 kg), C. 5-7 years ago (3.83 ± 0.32 kg), and D. > 8 years ago (5.17 ± 0.91 kg). Each age category group consisted of 3 broodstock randomly selected from a population of approximately 100 broodstock. During the experiment, broodstock striped catfish were kept in a 200m² rearing pond, with water temperatures maintained at 28-32°C, under a natural photoperiod of approximately 12 hours of light and 12 hours of darkness. The fish were fed a commercial broodstock diet containing 36% protein and more than 5% fat at a rate of 1% of their body weight (BW) twice daily. Each individual was tagged using a microchip implanted intramuscularly in the right dorsal fin muscle to monitor and identify the experimental striped catfish.

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Sperm collection: Before sperm collection is carried out, the broodstock is given hormonal stimulation by injecting ovaprim, which contains sGnRH-a (salmon gonadotropin-releasing hormone analog) at 20µg/ml and domperidone at 10mg/ml, at a dose of 0.2ml per kilogram of broodstock (Kim et al. 2020). Ovaprim is favored over other hormonal stimulants due to its predictability, efficiency in increasing sperm and hatchling yields, reduced handling stress, improved sperm quality, effective hormonal mechanism, and broader applicability across different fish species (Yanong et al. 2022).

Sperm collection was performed using the stripping technique by slowly pressing on the stomach from the base near the pectoral towards the genital area. Stripping was carried out 10 hours

after hormonal stimulation. During the stripping process, no anaesthesia was used; instead, the broodstock was covered with a wet towel to minimize stress during handling and stripping. Stripping was performed carefully to avoid contamination from blood, urine, or feces, and the sperm samples were collected individually in scaled glass tubes. The amount of sperm produced by each male brood was observed on the glass tube scale and then recorded. Sperm is diluted and preserved using a physiological NaCl solution with a ratio of fresh sperm: Physiological NaCl solution of 1:9 (Akçay et al. 2004). The sperm was then stored in a cold box at 4°C until further analysis. Immediately after collection, sperm quality was analyzed.

CASA assessment: The CASA system used was a Sperm Class Analyzer (SCA) 5.2 by Micro-optics (Spain) connected to a CX41 microscope (Olympus) and equipped with a digital camera (U-TV1X-2). The CASA settings were as follows: pH 1 contrast phase, magnification 10×10, and green filter applied to the reflector mirror. The light intensity was carefully adjusted to meet the standard requirements, ensuring optimal visualization, and the results were displayed on a monitor screen. Sperm was diluted by physiological NaCl solution with 1:100 (sperm: NaCl, v/v) before measured and placed on a Leja slide that had been conditioned to 37°C. Then, one drop (1µL) samples activated by aquades with a ratio of 1:1 (sperm:aquades,v/v). Concentration and motility parameters were evaluated after 10 seconds of activation. The motility variables measured included the percentage of motile sperm, progressive motility, non-progressive motility, and static motility. Sperm were classified as motile if they exhibited a velocity of $\geq 20\mu\text{m/s}$. Each measurement was performed in triplicate to ensure accuracy and reliability, and the average results from these replicates were used for data analysis.

Fertilization and hatching rate: The fertilization rate (FR) and hatching rate (HR) values were measured to assess the ability of sperm to fertilize eggs and the quality of the offspring produced. Fertilization was carried out by mixing 1ml of sperm from each male

parent with 10g of eggs from the same female parent for all treatments. The fertilization rate was calculated 6-9 hours after fertilization, and the hatching rate was measured 24 hours after fertilization. Fertilization and hatching rates were calculated using the following equations:

Fertilization rate/FR(%)= (Number of fertilized eggs/Total number of eggs) × 100 (Tilahun et al. 2016)

Hatching rate/HR(%)= (Number of hatched eggs/Total number of fertilized eggs) × 100 (Hanjavanit et al. 2008)

Survival rate: Survival rate (SR) of larvae was measured to assess the effect of sperm on the quality of the offspring produced. The survival rate was calculated by releasing 30 larvae from each treatment replicated into a container containing 1 liter of water, which was maintained until the third day after hatching (DAH) without additional feeding. The survival rate was calculated using the following equation:

Survival rate/SR(%) = (Number of surviving larvae 3 DAH /Total number of larvae released) × 100 (Tilahun et al. 2016)

Data analysis: The data on volume, concentration, motility, velocity, fertilization rate, hatching rate and survival rate of larvae were statistically analyzed using Microsoft Excel 2016 and the SPSS program (ver. 25). The data were tested for normality using the Shapiro-Wilk test ($P>0.05$), and homogeneity of variances was verified using Levene's test ($P>0.05$). One-way ANOVA with Duncan's multiple range test was applied to determine significant differences among age groups.

RESULTS AND DISCUSSION

Volume and concentration of sperm: The results of the study indicated that male stiped catfish in age groups B (2-4 years) and C (5-7 years) produced the highest and most significant sperm volumes compared to other age groups (Figure 1). These findings suggest that striped catfish within these age ranges have

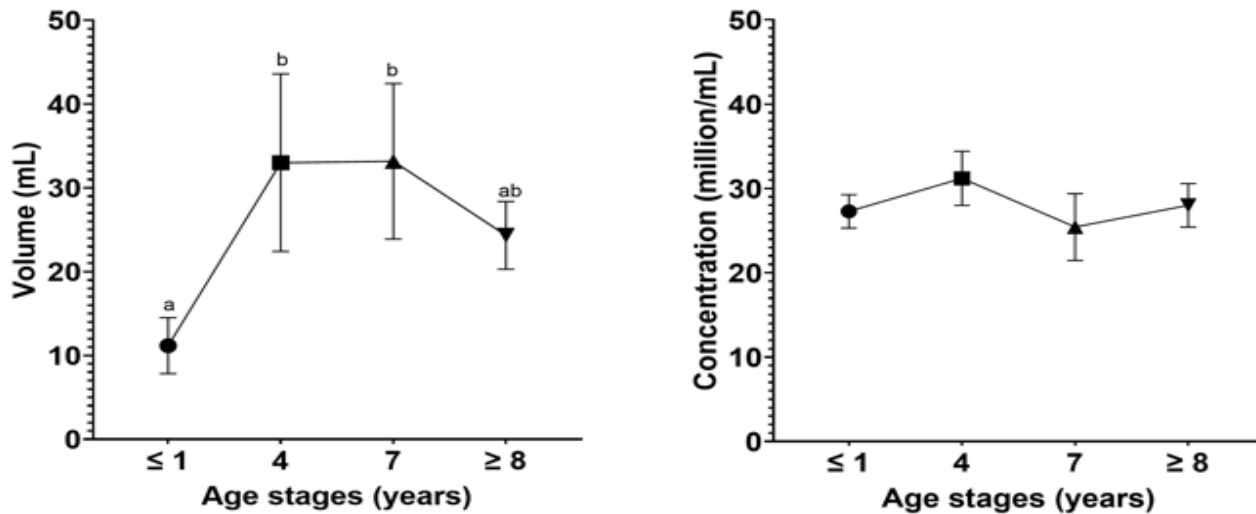


Fig.1. Striped catfish sperm volume and concentration based on age. Error bars represent the standard deviation (SD) of the mean. The superscript letters above the error bars indicate significant differences between the means based on Duncan's multiple range test ($P < 0.05$). Error bars without superscript letters above them indicate means that are not significantly different ($P > 0.05$) between the ages of male parents.

reached optimal reproductive maturity, enabling them to produce larger quantities of sperm. Conversely, the smallest sperm volume was produced by male striped catfish in age group A (<1 year). The sperm volume of male fish is influenced by several factors including ongoing development processes, energy allocation priorities, hormone levels, and broodstock condition factors. Younger male fish usually produce less sperm volume because the reproductive organ development process is not yet perfect, energy allocation priorities are prioritized for growth rather than reproduction, fluctuating hormone levels, and possibly lower condition factors (Kowalski & Cejko 2019). Likewise, in older male broodstock, as they age, their response to reproductive hormones and ATP energy sources begins to decline, leading to reduced sperm production (Woo et al. 2021). This was seen in male broodstock of striped catfish in the age group > 8 years which began to experience a decrease in sperm production, although it was not significantly different from the age groups 2–4 years and 5–7 years.

Interestingly, sperm concentrations across all age groups were relatively similar and did not show significant differences, although age group B (2–4 years old) exhibited the highest sperm concentrations overall. This suggests that although sperm quantity is

comparable across different ages, the sperm quality, measured by concentration, peaks during the 2–4 years age range. These results highlight that the 2–4-year age group is particularly important for breeding programs, as fish in this age bracket produce not only high sperm volumes, but also high sperm concentrations, which are crucial for successful fertilization and subsequent reproductive success.

The results showed that the age of the male striped catfish broodstock did not affect sperm quality, but affected the quantity of sperm (Fig. 1). The difference in quantity can be observed from the volume and concentration of sperm, with younger fish exhibiting lower volumes that increase with age. Likewise, sperm concentrations that were originally low will increase with age. This tendency also occurs in striped bass (*Morone saxatilis*) and zebrafish (*Danio rerio*), where older fish show higher sperm concentrations than younger males, regardless of fish size or mass, especially in middle age (Vuthiphandchai & Zohar 1999; Kanuga et al. 2011). An increase in sperm quantity with age can be attributed to the maturation of reproductive organs and the accumulation of sperm over time, allowing older males to produce a larger volume of sperm during spawning events (Kowalski & Cejko 2019).

Table 1. Sperm quality based on Computer-assisted sperm analysis (CASA) test.

Parameters	Unit	Treatments			
		A	B	C	D
Progression					
Static	%	6.33±3.50 ^a	11.25±5.96 ^a	10.01±6.21 ^a	9.00±2.51 ^a
Non-progressive motile	%	64.59±3.38 ^a	61.24±4.21 ^a	61.16±6.31 ^a	64.35±11.83 ^a
Progressive motile	%	29.08±2.97 ^a	27.51±4.43 ^a	28.83±3.28 ^a	26.65±10.26 ^a
Velocity					
Curvilinear Velocity (VCL)	µm/s	85.87±11.42 ^a	88.08±11.42 ^a	86.31±9.64 ^a	77.91±14.87 ^a
Straight-line Velocity (VSL)	µm/s	47.44±8.69 ^a	50.78±8.17 ^a	49.01±2.41 ^a	42.14±15.49 ^a
Average Path Velocity (VAP)	µm/s	64.66±12.12 ^a	68.55±11.32 ^a	66.15±6.65 ^a	56.14±16.29 ^a
Linearity (LIN)	%	55.04±5.15 ^a	57.73±2.74 ^a	57.17±4.36 ^a	52.43±10.39 ^a
Straightness (STR)	%	73.44±3.12 ^a	74.20±3.13 ^a	74.44±4.30 ^a	73.40±6.62 ^a
Wobble (WOB)	%	74.87±5.27 ^a	77.79±0.98 ^a	76.74±1.82 ^a	70.82±8.04 ^a
Hyperactive					
Hyperactive	%	13.39±4.42 ^a	15.06±3.92 ^a	13.74±2.84 ^a	13.29±4.44 ^a

*The same superscript letter above a value indicates no significant difference between means using the Duncan's multiple range test ($P>0.05$).

Sperm motility of striped catfish at different ages of male broodstock: The results showed that the percentages of sperm motility, progressive motility, non-progressive motility, and static sperm across all age groups were not significantly different. These findings indicate that different age groups do not affect the quality of sperm produced. Sperm quality based on the computer-assisted sperm analysis (CASA) test is presented in Table 1.

The quality of sperm in striped catfish in this study did not show any influence on the age of the male brood. The results showed that the four treatments of male brood age had no significant effect on various larval quality parameters ($P>0.05$) (Table 1). Analysis of Computer-assisted sperm analysis (CASA) test data showed that the parameters related to kinetic movement, in the form of total motility levels at all observation age levels, reached $90.85\pm5.83\%$, with a progressive motility of $28.02\pm5.23\%$. This result is in accordance with what was reported by Pamungkas et al. (2023) in striped catfish with different feed treatments which had a total motility of 77.32-96.00% with a progressive motility of 12.35-32.10%.

Sperm quality can also be evaluated through the characterization of spermatozoa motility, which is presented by several parameters, including curvilinear

velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), and wobble (WOB). VCL is the average speed of sperm along the curved line, VSL is the average speed of sperm along a straight line between the first and last positions, and VAP is the average speed of spermatozoa across the flat path from the beginning to the end of the period of their flow in µm/s. VCL, VSL, and VAP values are important variables to assess the fertility ability of spermatozoa, which is positively correlated with sperm motility ability. Furthermore, the LIN parameter is the relationship between the straight-line velocity and the curved-line velocity during the measurement period calculated from the division of VSL with VCL, while the STR is the relationship between the straight-line velocity and the average velocity during the measurement period and is an indicator of the spermatozoa's swimming pattern. This STR value was calculated from the results of the distribution of VAP and VCL. The last parameter is WOB, which is the strongest spermatozoa shake indicator for one second obtained from the result of the division of VAP and VCL. LIN, STR, and WOB are indicators of progressive movement of spermatozoa expressed in percentage (%). In this study, the results of the calculation of the average

values of all age groups of VCL parameters were $84.54 \pm 11.84 \mu\text{m/s}$, VSL $47.34 \pm 8.69 \mu\text{m/s}$, VAP $63.88 \pm 11.60 \mu\text{m/s}$, LIN $55.60 \pm 5.66\%$ and STR $73.87 \pm 4.29\%$.

Furthermore, using the parameters above, sperm can be differentiated between hyperactive and non-hyperactive. Hyperactive sperm is characterized by very aggressive motility and movement, which in natural spawning is indispensable so that sperm can reach and penetrate the egg until fertilization occurs (Kholodnyy et al. 2020). Hyperactive sperm usually show very fast and agile swimming ability with irregular patterns and are characterized by VCL values greater than $97 \mu\text{m/s}$, and LIN less than 50% (Schmidt & Kamp 2004). In this study, the results of the analysis of hyperactive parameters in all age groups showed no significant difference with an average value of $13.87 \pm 3.41\%$.

Although it did not show a significant difference, the highest average parameters of VCS, VSL, VAP, LIN, STR, and WOB were shown in 2-4 years old (treatment B) and decreased in the >8 years old (treatment D). Apart from internal influences in the form of maternal age, sperm quality can be affected by environmental conditions, such as heavy metal pollution of selenium (Seyedi et al. 2021) and cryopreservation treatment (De Almeida-Monteiro 2020). This is because sperm quality varies significantly between species, depending on environmental and nutritional factors. Seen in the Rainbow trout, *Oncorhynchus mykiss*, the older one shows a decrease in sperm function and viability (Risopatrón et al. 2018). However, sperm in older guppies (*Poecilia reticulata*) (Gasparini et al. 2014) and carp (*Cyprinus carpio*) (Betsy et al. 2016) did not show any decline in function or survival. Similarly, the results of a study by Chalde et al. (2014) on a pejerrey fish, *Odontesthes bonariensis*, proved that sperm quality is not significantly affected by age but is more influenced by other environmental or physiological factors. Shazada et al (2023) also obtained results that sperm motility and viability in goldfish (*Cyprinus carpio*) are influenced by health, age, and environmental conditions.

The results showed that the age difference in the male broodstock of striped catfish did not have a significant effect on the fertilization rate and hatching rate ($P > 0.05$) but had a significant effect on the survival rate of the larvae ($P < 0.05$) (Fig. 2). Previous research on Roughscale sole fish *Clidoderma asperrium* showed that under natural conditions younger male parents have higher levels of reproductive hormones (Luteinizing Hormone (LH) and testosterone), resulting in better sperm quality than older males, whose hormone levels decrease with age (Woo et al. 2021). In addition, as the male parent ages, the production of ATP energy sources can decrease due to reduced mitochondrial efficiency and increased Reactive Oxygen Species (ROS) which cause increased oxidative stress and have an impact on the quality of sperm produced (Risopatrón et al. 2017; Otoh & Udoh 2018; Kurta et al. 2023). However, in a cultivation environment as conducted in this study, hormonal manipulation and optimal feeding strategies in older broodstock can maintain the stability of their reproductive hormone levels, so that the quality of the sperm produced remains good and provides fertilization and hatching rates that are relatively not significantly different from younger male broodstock (Mylonas et al. 2009; Otoh & Udoh 2018).

Interestingly, the survival rate of larvae produced from spawning using sperm from male broodstock older than 4 years old tends to be lower and significantly different from those produced from broodstock younger than 4 years old. These results are similar to previous studies conducted on African Catfish *Clarian gariepinus* which showed that embryos from older parents experience decreased resistance to environmental stressors, such as temperature fluctuations and lack of oxygen, resulting in low survival in the early larval stage (Lyubomirova et al., 2021). The mechanism by which sperm from older parents affects survival rates in fish larvae is multifaceted, involving declines in sperm quality, increased oxidative stress, and hormonal changes associated with aging (Johnson et al. 2018; Riesco et al. 2019; Halvaei et al. 2020). These factors contribute to lower fertilization success and increased mortality

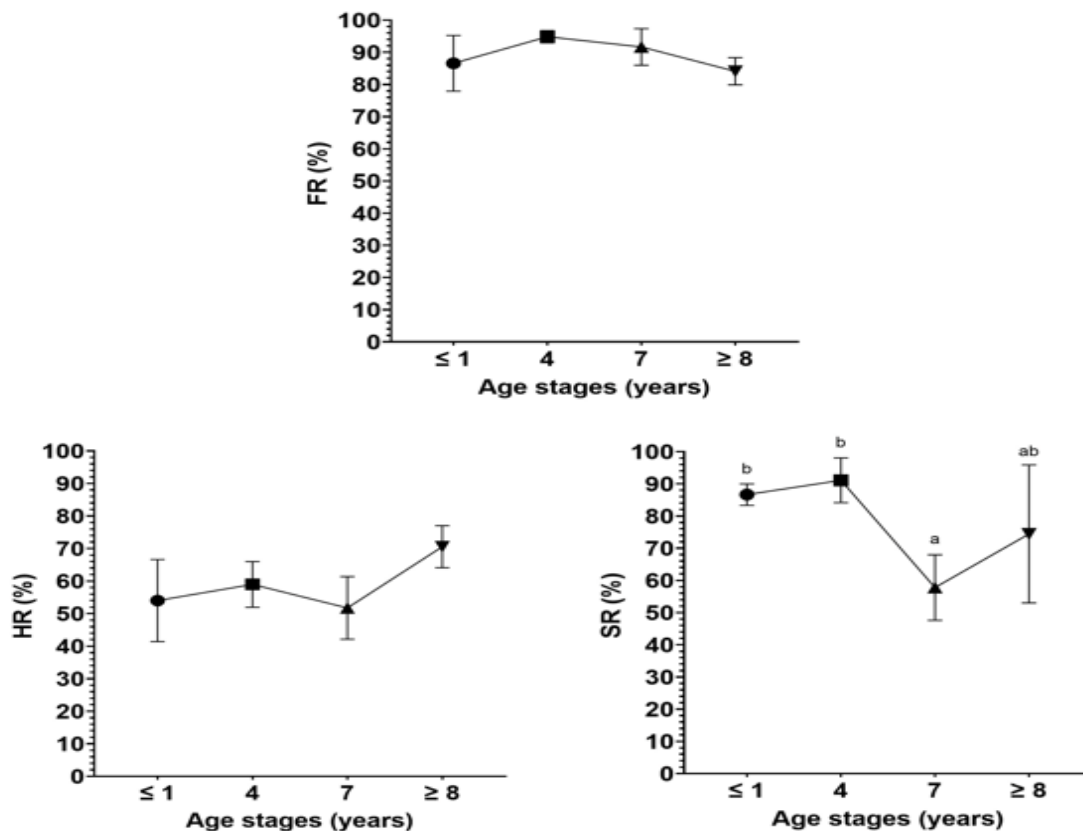


Fig.2. Fertilization rate, hatching rate and survival rate (3 day after hatching) of striped catfish larvae based on sperm from different ages of male parents. Error bars represent the standard deviation (SD) of the mean. The superscript letters above the error bars indicate significant differences between the means based on Duncan's multiple range test ($P < 0.05$). Error bars without superscript letters above them indicate means that are not significantly different ($P > 0.05$) between the ages of male parents.

rates among larvae, highlighting the importance of selecting appropriately aged broodstock for optimal reproductive outcomes in aquaculture settings. Understanding these dynamics is essential for improving fish farming practices and ensuring higher survival rates for offspring.

CONCLUSIONS

The different ages of male broodstock in striped catfish does not affect sperm concentration and quality, but it significantly affects sperm quantity and survival rate of larvae. Research indicates that the optimal age for male striped catfish to achieve the best reproductive outcomes is between 2-4 years. In this age range, males tend to produce a higher quantity of sperm and survival rate of larvae (3 DAH), which leads to increased fertilization and hatching rates, and ultimately, the offspring produced can be more numerous and survive. Older striped catfish males are

still able to provide optimal reproductive performance but require effective application of reproductive-stimulating hormones and optimal feeding strategies.

ACKNOWLEDGEMENTS

This study was supported by the Research Institute for Fish Breeding, West Java, Indonesia. We also recognized valuable technical assistance from the striped catfish commodity team at the Research Institute for Fish Breeding.

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مقاله کامل

کیفیت و کمیت اسپرم در گربه‌ماهی راه‌راه، *Pangasianodon hypophthalmus* (Sauvage 1878) در مراحل مختلف سنی مولدین

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چکیده: موفقیت تخم‌ریزی در گربه‌ماهی راه‌راه تا حد زیادی توسط کیفیت اسپرم تعیین می‌شود که می‌تواند تحت تأثیر عوامل متعددی از جمله سن مولدین قرار گیرد. این مطالعه تأثیر سن مولدین را بر معیارهای مهم کیفیت اسپرم (حرکت، زنده ماندن، و ناهنجاری‌ها) و موفقیت باروری (نرخ لقاح و تخم‌ریزی) در گربه‌ماهی راه‌راه ارزیابی می‌کند. برای تیمارها، گربه‌ماهی‌های راه‌راه نر به چهار دسته سنی گروه‌بندی شدند: گروه A (۱ سال)، گروه B (۲-۴ سال)، گروه C (۵-۷ سال) و گروه D (بیش از ۸ سال). حجم اسپرم با استفاده از یک لوله مدرج ۱۰ میلی‌لیتری اندازه‌گیری شد، در حالی که غلظت اسپرم، تحرک، زنده ماندن و ناهنجاری به‌صورت میکروسکوپی با استفاده از آنالیزگر خودکار اسپرم CASA® SCA سیستم آنالیزگر کلاس اسپرم ارزیابی شد. برای ارزیابی عملکرد اسپرم در طول لقاح، از تخم‌های یک ماده برای ارزیابی میزان لقاح، سرعت تفریح و ناهنجاری‌های لارو استفاده شد. یافته‌ها نشان داد که گربه‌ماهی نر راه‌راه ۲-۴ و ۵-۷ ساله با میانگین حجم اسپرم ۵۱ میلی‌لیتر، غلظت ۲۹/۹ میلیون در میلی‌لیتر، تحرک ۹۶/۴ درصد، زنده ماندن ۹۷/۴ درصد و نرخ غیر طبیعی ۳ درصد، مطلوب‌ترین کیفیت اسپرم را نشان داد. در مقابل، گروه یک ساله با میانگین حجم اسپرم ۱۱/۵ میلی‌لیتر، غلظت ۲۷/۷ میلیون در میلی‌لیتر، تحرک ۶۶/۵ درصد، زنده ماندن ۶۷/۴ درصد و نرخ غیر طبیعی ۱/۴ درصد کمترین عملکرد را نشان داد. براساس این نتایج، توصیه می‌شود مولدین ۲-۴ ساله به‌دلیل تولید بیشتر اسپرم، کیفیت بهتر اسپرم و تولید فرزندان که بقای خوبی دارند، برای فعالیت تخم‌ریزی در اولویت قرار گیرند.

کلمات کلیدی: گربه‌ماهی راه‌راه، آنالیزر کلاس اسپرم SCA، تحرک، تولیدمثل