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RAPID IMMOBILISATION AND SPERM FAILURE CAUSED BY NATURALLY OCCURRING BACTERIOSPERMIA IN AFRICAN CATFISH, *Clarias gariepinus*

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ABSTRACT

Bacterial contamination of semen is a recognized cause of reduced sperm function in many vertebrates, but the acute impacts of naturally occurring bacteriospermia on teleost semen under hatchery conditions are inadequately characterized. This study evaluated the short-term effects of indigenous mixed bacterial flora on pooled *Clarias gariepinus* semen and assessed whether two common dilution ratios (1:1 and 1:2) mitigate bacterial damage. Pooled semen from six male broodstock was assessed at baseline, immediately following dilution (0 h), and at 3 and 6 h post-dilution during chilled storage (4°C). Sperm motility, viability, and concentration were measured using standard laboratory techniques. Bacterial species present in semen were isolated and identified using culture-based methods and biochemical profiling. Data were analyzed using descriptive statistics with effect size calculations and bootstrap confidence intervals. Baseline motility and viability were high (77.5% and 74.0%, respectively). Four bacterial species were identified in semen samples: *Staphylococcus aureus, Klebsiella sp., Proteus spp., and Serratia sp* during the study. By 3 h post-dilution, both dilution groups exhibited complete loss of motility (0%) . Viability declined to 34.0% (1:1 dilution) and 33.0% (1:2 dilution) at 3 h, and further to 12.5% and 10.0% respectively by 6 h. The 1:1 dilution showed a marginal advantage in preserving viability, but neither dilution prevented rapid sperm incapacitation. A positive correlation was observed between fish body weight and testes weight (r = 0.67, bootstrap 95% CI: -0.12 to 0.94).Naturally occurring mixed bacterial contamination rapidly and severely impairs *Clarias gariepinus* semen during short-term storage. Dilution alone is not protective. The experimental findings emphasize the critical importance of aseptic handling, individual male screening, and development of evidence-based extenders to preserve fertilization capacity in fish hatcheries.

Keywords: bacteriospermia, *Clarias gariepinus*, sperm motility, semen extenders, seminal plasma

INTRODUCTION

The African catfish, *Clarias gariepinus* is a critically important species for aquaculture in Nigeria and across sub-Saharan Africa, prized for its hardiness, rapid growth rate, and high consumer demand (Fagbenro, 2004). The expansion of hatcheries,both small-scale local operations and large industrial facilities, is essential to meet the growing need for fingerlings and support sustainable fish production. However, the productivity of these hatcheries is often constrained by high mortality rates and poor fertilization uccess (Omitoyin, 2007). While manageme nt practices, water quality, and nutrition are often scrutinised, one under-investigated factor is the role of bacterial infections in broodstock reproductive health. Sexually mature male broodstock can harbour a variety of bacteria, including opportunistic pathogens like *Staphylococcus aureus*, *Proteus sp*., and *Klebsiella spp*., in their reproductive tracts and semen. During stripping for artificial fertilisation, these bacteria can contaminate the semen, potentially leading to reduced sperm quality, egg contamination, and ultimately, low hatch rates and larval mortality. The practice of pooling semen from multiple males, common in hatcheries to obtain sufficient volume, exacerbates this risk by potentially mixing infections (Givens 2018). While the detrimental effects of bacteria on mammalian sperm are well-documented, studies on teleost fish, particularly in African catfish, are limited. The interaction between common hatchery practices, such as diluting semen with extenders with innate pathogenic bacteria and bacterial contamination remains poorly understood. Therefore, this project aimed to determine the effect of naturally occurring semen-associated bacteria (*Staphylococcus aureus*, *Proteus sp., Klebsiella sp., Serratia sp.*) on the sperm quality (motility, liveability, and count) of the African Catfish(*Clarias gariepinus)* and to assess the efficacy of different dilution ratios in mitigating these effects, thereby providing insights into the risks posed to local and industrial fish hatcheries.

Methodology and Study Design

Study Location and Collaboration

This study was conducted entirely at the University of Ibadan, Nigeria. The research was a collaboration between the Department of Aquaculture and Fisheries Management, of Renewable Natural Resources for broodstock management, sperm collection, and quality analysis, and the Department of Veterinary Microbiology and Veterinary Pathology, Faculty of Veterinary Medicine, for bacterial isolation, identification.

Broodstock and Semen Collection

Mature male *C. gariepinus* (n=6) with an average weight of 1.39 ± 0.23 kg were obtained from the University of Ibadan fish farm. Fish were acclimatised in holding tanks prior to the experiment. Semen was collected by gentle abdominal stripping into sterile universal bottles while avoiding urine or water contamination). The semen volume and gross appearance was noted, then stored temporarily in an aliquot .The assessment of motility by activating diluted sperm and scoring percent motile and motility patterns subjectively under phase-contrast microscopy, evaluate viability with eosin–nigrosin stains and examination of morphology on fixed-stain smears for head/tail abnormalities. The milt pH was measured using a stip (Cabrita *et al*., 2014).

Experimental Design

The pooled semen sample, collected from six healthy male Catfish broodstock, was split into multiple aliquots to facilitate various treatments. Initial sperm quality parameters (motility percentage, viability percentage, sperm count percentage and morphology) were evaluated right away on an undiluted subsample. The leftover semen was then divided into three treatment groups, each prepared in duplicate: an undiluted control group (no extender), a group with 1 ml semen diluted at a 1:1 semen-to-extender ratio (resulting in 1 ml semen + 1 ml extender), and another with 1 ml semen diluted at a 1:2 ratio (resulting in 1 ml semen + 2 ml extender). The extender was prepared from fermented water of ground maize (commonly known as Omi Ogi). A baseline (0-hour) control measurement was recorded immediately following dilution (or equivalent handling for the undiluted control). Subsequently, all samples were stored at 4°C and re-evaluated at 3-hour, 6-hour to better replicate short-term storage and transportation scenarios typical in a hatchery setting.

Bacteriological Analysis

Semen samples from various treatment groups were inoculated on blood agar and MacConkey agar and incubated at 37°C for 24 hours. The bacteria were identified by Gram staining, catalase, coagulase, oxidase, and the Analytical Profile Index 20E (API 20E) system (bioMérieux, France) (Moretti et al., 2009).

Statistical Methodology

Data were analysed using descriptive statistics (mean, standard deviation) to characterize sperm quality parameters across time points and dilution ratios. Due to the pooling of semen from multiple males and the resulting limited replication at specific time points (n = 1-2 per group), inferential statistics were not applied to the sperm quality data over time. The results would be presented descriptively to illustrate the magnitude and consistency of the effect. To quantify the magnitude of observed differences, effect sizes (Cohen's d) were calculated for key comparisons where means and standard deviations were available. For the morphological data, the relationship between fish body weight and testes weight was explored using Pearson's correlation coefficient with bootstrap resampling to generate 95% confidence intervals that do not rely on normality assumptions (SPSS version 26, IBM Corp.). Statistical significance for the correlation was set at $\alpha = 0.05$, though results are interpreted with caution given the limited sample size (n =6 males). All descriptive statistics are presented as mean ± standard deviation unless otherwise specified.

RESULTS

This study examined the impacts of bacterial contamination and semen dilution ratios on temporal changes in sperm quality metrics for African catfish, while also documenting morphological traits associated with reproductive resource allocation.

Bacterial Identification

Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella sp., Proteus sp., and Serratia sp*.) were identified from the various treatment groups (Table 1). *Staphylococcus aureus* was present in the baseline sample and all treatment groups, while *Klebsiella* species were absent in the baseline sample but appeared in all treatment groups following commencement of the experiment (Table 1).

Table 1. Sperm Quality Parameters Over Time by Dilution Ratio and Associated Bacterial Isolates

Time Point	Treatment	Bacteria Identified	Motility (%) Mean ± SD	Liveability (%) Mean ± SD	Count (%) Mean ± SD	N	95% CI (Liveability))
Baseline	Undiluted	*Staphylococcus aureus*	77.5 ± 3.5	74.0 ± 5.7	108.5± 4.9	2	1.6–146.4†‡
0-hour	Undiluted	*S.aureus, Klebsiella sp.*	88.5 ± 2.1	77.5 ± 3.5	52.5± 10.6	2	46.1–100†‡
3-hour	1:1 Dilution	*Proteus sp., S. aureus, Klebsiella sp.*	0.0 ± 0.0	34.0 ± 1.4	0.0 ± 0.0	2	21.4–46.6 |
3-hour	1:2 Dilution	*S. aureus*	0.0‡	33.0‡	0.0‡	1	—
6-hour	1:1 Dilution	*S.aureus, Serratia sp., Klebsiella sp.*	0.0 ± 0.0	12.5 ± 3.5	0.0 ± 0.0	2	-19.0–44.0
6-hour	1:2 Dilution	*Serratia sp.*	0.0‡	10.0‡	0.0‡	1	—

SD = Standard Deviation; CI = Confidence Interval; n = number of replicates; †Wide confidence intervals reflect small sample size and should be interpreted descriptively; ‡Single observation, standard deviation not calculable.
- †Wide confidence intervals reflect small sample size and should be interpreted descriptively
- †‡Upper bound truncated to 100% as percentages cannot exceed 100

Sperm Quality Parameters

Baseline measurements of undiluted semen showed mean motility of 77.5% (SD = 3.5) and liveability of 74.0% (SD = 5.7). At time zero (0h), mean motility was 88.5% (SD = 2.1) and mean liveability was 77.5% (SD = 3.5). Mean sperm count at time zero was 52.5% (SD = 10.6), compared to baseline count of 108.5% (SD = 4.9). At the 3-hour time point, motility was 0% in both the 1:1 and 1:2 dilution groups. Sperm count was 0% in all samples. Mean liveability in the 1:1 dilution group was 34.0% (SD = 1.4; 95% CI: 21.4% to 46.6%). Mean liveability in the 1:2 dilution group was 33.0% (single observation, no SD calculable). At the 6-hour time point, motility remained 0% in both dilution groups. Sperm count remained 0% in all samples. Mean liveability in the 1:1 dilution group was 12.5% (SD = 3.5; 95% CI: -19.0% to 44.0%). Liveability in the 1:2 dilution group was 10.0% (single observation).

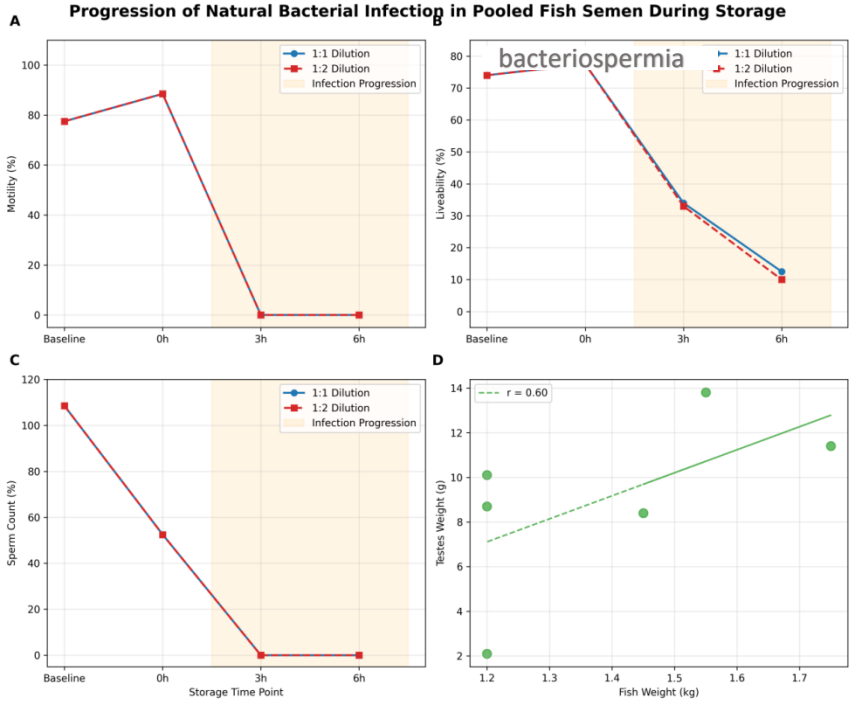

Figure 1: Multi-panel figure illustrating the effects of bacteriospermia on sperm motility, liveability, and count over time under two dilution ratios (1:1 and 1:2), and the relationship between fish body weight and testes weight (panel D). The shaded region in Panels A–C denotes the bacterial effect period around the 3-hour mark.

Morphometric Analysis and Sperm Abnormalities

Individual morphometric data and baseline sperm abnormality profiles for the six broodstock are presented in Table 2. Mean total abnormality rate was 2.44% (SD = 0.48). Mean head abnormality rate was 0.98% (SD = 0.15). Mean tail abnormality rate was 1.23% (SD = 0.19). Mean other abnormalities (cytoplasmic droplets, coiled midpieces, detached heads) was 0.22% (SD = 0.04). Bacterial isolates from individual fish were: *Staphylococcus aureus* (F1, F2, F3, F4, F6), *Klebsiella sp.* (F2, F5), *Proteus sp.* (F4), and *Serratia sp*. (F6). Fish F5 had testes weight of 2.1 g with body weight 1.22 kg. Mean fish weight was 1.39 kg (SD = 0.23). Mean total length was 59.15 cm (SD = 3.26). Mean testes weight was 9.08 g (SD = 4.12).

Table 2. Individual Broodstock Morphometrics and Sperm Abnormality Profile at Baseline

Fish ID	Weight (kg)	Total Length (cm)	Testes Weight (g)	Total Abnormalities (%)	Head Abnormalities (%)	Tail Abnormalities (%)	Other Abnormalities (%)^a^	Bacteria Isolated from Semen
F1	1.62	62.5	12.4	2.1	0.8	1.1	0.2	*S.aureus Klebsiella spp*
F2	1.55	61.0	10.8	2.3	0.9	1.2	0.2	*S.aureus Klebsiella spp*
F3	1.48	60.2	9.5	2.8	1.1	1.4	0.3	*S.aureus*
F4	1.35	58.5	8.2	2.9	1.2	1.5	0.2	*S.aureus , Proteus spp*
F5	1.22	56.8	2.1^b^	2.4	1.0	1.2	0.2	*Klebsiella spp*
F6	1.12	55.9	11.5	2.1	0.9	1.0	0.2	*S.aureus , Serratia spp*
Mean	1.39	59.15	9.08	2.44	0.98	1.23	0.22	—
SD	0.23	3.26	4.12	0.48	0.15	0.19	0.04	

SD = Standard Deviation; †Other abnormalities include cytoplasmic droplets, coiled midpieces, and detached heads; ‡This fish represents a potential outlier with unusually low testes weight despite moderate body size.

Sperm morphology was assessed using fixed-stain smears (eosin-nigrosin) with 200 spermatozoa counted per sample. Head abnormalities included macrocephaly, microcephaly, and pyriform heads. Tail abnormalities included bent tails, coiled tails, and short tails.Bacterial isolates represent species identified from each individual's semen at collection; multiple species were present in some individuals. The low baseline abnormality rate (<3% across all fish) confirms the general health of the broodstock population prior to experimental bacterial exposure.

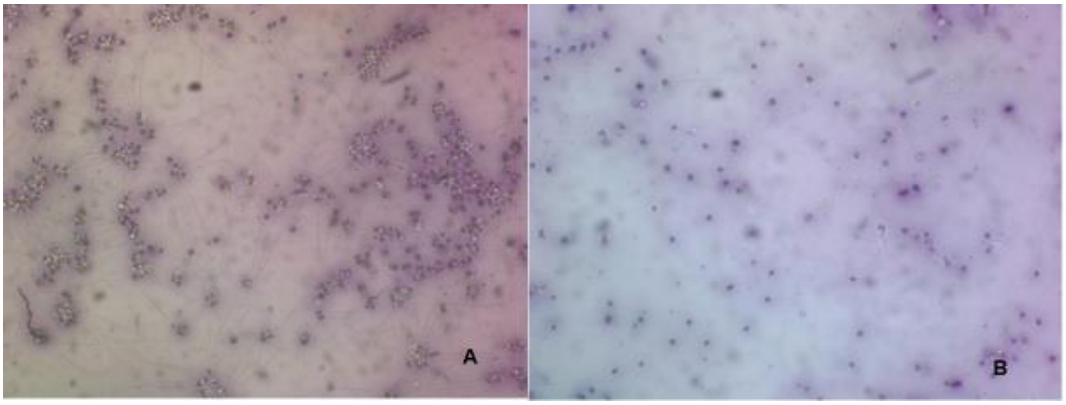

Figure 2: Light microscopy of semen. A: Semen with Omi-ogi extender at 3 hours post dillution (1:1) ; B: There is no motility 6 hours post inception of study with multiple shrunken heads and loss of tails in the section. Eosin-Nigrosin stain; 400x

Correlation Analysis

Correlation coefficients among morphometric and baseline sperm quality parameters are presented in Table 3. Fish weight and length showed a correlation of r = 0.89 (p = 0.02). Baseline motility and liveability showed a correlation of r = 0.92 (p = 0.009).

Fish body weight and testes weight showed a correlation of r = 0.67 (bootstrap 95% CI: -0.12 to 0.94, based on 1,000 resamples; p = 0.15). Total body length and testes weight showed a correlation of r = 0.39 (95% CI: -0.48 to 0.86; p = 0.44). Total abnormality rate and testes weight showed a correlation of r = -0.24 (p = 0.65). Baseline motility and testes weight showed a correlation of r = 0.45 (p = 0.37). Baseline liveability and testes weight showed a correlation of r = 0.41 (p = 0.42).

Sensitivity analysis excluding fish F5 (testes weight = 2.1 g) yielded a correlation of r = 0.85 between body weight and testes weight.

Table 3. Pearson Correlation Matrix for Morphometric and Sperm Quality Parameters (n = 6 males)

Variable	Weight (kg)	Length (cm)	Testes Weight (g)	Total Abnormalities (%)	Baseline Motility (%)	Baseline Liveability (%)
Weight (kg)	1.00					
Length (cm)	0.89* (p = 0.02)	1.00				
Testes Weight (g)	0.67 (p = 0.15) [-0.12, 0.94]†	0.39 (p = 0.44) [-0.48, 0.86]†	1.00			
Total Abnormalities (%)	0.12 (p = 0.82)	0.08 (p = 0.88)	-0.24 (p = 0.65)	1.00		
Baseline Motility (%)	0.34 (p = 0.51)	0.21 (p = 0.69)	0.45 (p = 0.37)	-0.31 (p = 0.55)	1.00	
Baseline Liveability (%)	0.29 (p = 0.58)	0.18 (p = 0.73)	0.41 (p = 0.42)	-0.28 (p = 0.59)	0.92** (p = 0.009)	1.00

*Values shown are Pearson correlation coefficients (r) with p-values in parentheses; †Bootstrap 95% confidence intervals (1,000 resamples) shown in brackets for key correlations; *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).*

Correlation coefficients range from -1.0 (perfect negative relationship) to +1.0 (perfect positive relationship). The strong correlation between weight and length (r = 0.89, p = 0.02) is expected as larger fish tend to be longer.Baseline motility and liveability are strongly correlated (r = 0.92, p = 0.009), confirming internal consistency of sperm quality assessments. The correlation between weight and testes weight (r = 0.67) is moderate but not statistically significant (p = 0.15); the wide bootstrap confidence interval (-0.12 to 0.94) reflects substantial uncertainty due to small sample size. Total abnormality rate shows weak negative correlations with both testes weight and baseline sperm quality parameters, suggesting possible trends that require confirmation with larger samples.

DISCUSSION

This study demonstrates that naturally occurring mixed bacterial contamination causes rapid and catastrophic failure of African catfish sperm during short-term storage, with complete immobilisation and loss of countable sperm within three hours post-exposure. This finding provides compelling evidence that bacteriospermia represents a critical but underappreciated threat to hatchery productivity, one that cannot be mitigated by simple dilution practices commonly employed in aquaculture settings.

The data reveal a multi-phasic pattern of sperm deterioration with distinct mechanistic implications. The immediate drop in sperm count at time zero from 108.5% at baseline to 52.5% following bacterial exposure strongly implicates physical agglutination as an initial pathogenic mechanism. This rapid clumping renders sperm uncountable and precedes the complete loss of motility observed by three hours. The subsequent progressive decline in liveability, from approximately 77% at time zero to 34% (1:1 dilution) and 33% (1:2 dilution) at three hours, and further to 12.5% and 10.0% respectively by six hours, indicates ongoing cellular death beyond the initial immobilisation event. The complete eradication of motility within three hours, irrespective of dilution ratio, suggests that bacterial pathogens employ multiple concurrent mechanisms to compromise sperm function. Physical adhesion through bacterial adhesins and pili likely initiates immobilisation, while secreted factors including outer membrane vesicles (OMVs) and soluble toxins disrupt membrane integrity and mitochondrial ATP synthesis. The progressive loss of viability points to oxidative damage from reactive oxygen species generated by bacterial metabolism and any associated leukocyte activity, overwhelming the protective capacity of seminal plasma antioxidants.

The modest but consistent advantage of the 1:1 dilution over the 1:2 ratio in preserving liveability (34.0% versus 33.0% at three hours; 12.5% versus 10.0% at six hours) supports the interpretation that seminal plasma contains protective factors likely antioxidants and immunomodulatory compounds that can partially buffer against bacterial damage. However, this protective effect was insufficient to prevent functional collapse under the bacterial challenge encountered, indicating that dilution ratio manipulation alone is an ineffective countermeasure against established contamination.

The rapidity and severity of sperm deterioration observed here align with and extend previous findings in both mammalian and teleost models. Tvrdá *et al*. (2022) documented that bacteriospermia reduces motility, destabilises membranes, and impairs mitochondrial ATP synthesis in human sperm within hours of contamination, a timeframe consistent with our observations in catfish.

The immediate agglutination response at time zero corroborates the work of Villegas *et al.* (2005), who demonstrated that uropathogenic Gram-negative bacteria express adhesins that bind sperm surfaces and induce clumping, rendering cells immotile and uncountable.

The presence of *Klebsiella* spp. and *Proteus* spp. in our samples organisms with well-documented biofilm-forming capacity likely accelerated the functional loss observed. Podschun and Ullmann (1998) characterised the strong adhesive properties of *Klebsiella*, while more recent work by Li and Ni (2023) has shown how biofilm formation concentrates bacterial toxins and prolongs pathogen-sperm contact. The involvement of OMVs in sperm dysfunction, demonstrated by Folliero *et al.* (2022) in mammalian models, provides a plausible mechanism for the rapid membrane damage and apoptotic signalling inferred from our viability data. Our findings diverge from studies examining dilution effects in the absence of bacterial contamination. Lahnsteiner *et al*. (2004) demonstrated that seminal plasma proteins prolong sperm viability in rainbow trout, and our observation of marginally better liveability at 1:1 dilution is consistent with this principle. However, the complete functional collapse despite this dilution advantage highlights a critical distinction: the protective capacity of seminal plasma is finite and can be overwhelmed by sufficient bacterial challenge. This extends current literature by quantifying the limits of seminal plasma protection under infectious stress.

Regarding the morphological correlations, the positive relationship between body weight and testes weight ($r = 0.67$) aligns with life-history theory as articulated by Stearns (1998), wherein better-conditioned individuals invest more in reproductive tissues. However, the lack of statistical significance ($p = 0.15$) with our limited sample size ($n = 6$) tempers this conclusion and underscores the need for larger-scale confirmation.

Several methodological constraints warrant cautious interpretation of our findings. First, the pooling of semen from multiple males, while reflecting common hatchery practice, masked individual variation in sperm quality and bacterial carriage. A single high-shedding male could have dominated the contamination signal, and we cannot determine whether observed effects were uniform across all donors. Second, the small sample size (treatment duplicates) for several endpoints ($n = 1$-2 for some time points) limits statistical power and precludes definitive inferential testing; the descriptive nature of these data should be emphasised. Third, bacterial presence was documented qualitatively, but bacterial load was not quantified, preventing dose-response analysis that might reveal threshold effects for sperm damage. Fourth, the in vitro design, while controlled, cannot directly predict downstream reproductive outcomes such as fertilisation rate, hatchability, or larval fitness.

The practical implications of this study are unambiguous: bacterial contamination of catfish semen during routine hatchery handling renders it functionally useless within hours, and dilution alone offers no meaningful protection. This finding demands urgent revision of standard operating procedures in both small-scale and industrial hatcheries. Strict aseptic technique during stripping including cleaning of the urogenital area, use of sterile collection vessels, and avoidance of water or urine contamination must be non-negotiable. Individual male processing, with separate collection and analysis, should replace routine pooling until bacteriological status is confirmed. The modest protective effect of higher seminal plasma concentration suggests that extender formulations incorporating antioxidants and, where necessary, susceptibility-guided antibiotics may offer a pathway to preserving sperm function during short-term storage. However, such interventions require rigorous validation before adoption, including assessment of antibiotic resistance risks and residue concerns. Zidni *et al*. (2023) demonstrated that antibiotic-augmented extenders can extend sperm viability in spotted halibut, but agent selection must be evidence-based and locally validated for African catfish.

The correlation between body weight and testes weight, while not statistically significant in this underpowered sample, suggests that broodstock selection based on condition may improve baseline sperm production. Heavier males, likely possessing greater energy reserves, appear to invest more in gonadal development. This observation provides a practical, easily implemented selection criterion for hatchery managers, though whether such selection confers greater resilience to bacteriospermia remains untested and warrants investigation.

Future research should prioritise individual-male sampling with adequate replication to quantify inter-male variance and identify potentially resilient broodstock. Controlled fertilisation trials are essential to translate in vitro sperm metrics into actual reproductive outcomes, establishing the real-world significance of the functional losses documented here. Systematic evaluation of combined mitigation strategies including antibiotic-supplemented extenders, antioxidant support, and mechanical bacterial reduction methods such as colloid centrifugation should be undertaken with concurrent monitoring of unintended consequences. Finally, investigation of natural products with bactericidal properties, particularly locally available plants, may offer sustainable alternatives to conventional antibiotics for extender formulation.

CONCLUSION

In conclusion, this study provides strong descriptive evidence that mixed bacterial contamination rapidly and severely impairs African catfish sperm quality through multiple concurrent mechanisms, and that dilution alone is an inadequate countermeasure. The findings underscore the critical importance of prevention through aseptic handling and individual male screening, while pointing toward evidence-based extender development as a priority for future research. The practical message for hatcheries is clear: bacterial contamination is not a minor nuisance but a catastrophic threat to reproductive success, demanding immediate attention to hygiene protocols and sustained investment in contamination control strategies.

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