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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Two different methods of sperm collection in European catfish (*Silurus glanis* Linnaeus, 1758)

Avrupa yayın balıklarında *(Silurus glanis* Linnaeus, 1758) iki farklı sperm toplama yöntemi

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Abstract: The European catfish (*Silurus glanis* L., 1758) is an important species for the aquaculture sector and the production of quality broodstock in artificial fertilization methods as well. One of the crucial steps determining the success of the reproduction of catfish is to obtain sufficient and good-quality sperm. The aim of this study is to compare two different methods used to obtain sperm from European catfish. The first method is surgery extraction of testicular sperm from taking testicles (CS) and the second method collection of sperm by stripping (SS). The fertilization rate was calculated as a percent for CS and SS groups and the highest fertilization rate was found in the SS group (81.87 ± 17.38%). According to the findings in the present study, it was concluded that it is not necessary to kill male European catfish to get sperm for fertilization.

Keywords: European catfish, fertilization, reproduction, sperm collection method

Öz: Avrupa yayın balığı (*Silurus glanis* L., 1758) su ürünleri sektörü ve yapay üretim yöntemlerinde kaliteli anaç üretimi için önemli bir türdür. Yayın balığı üreme başarısını belirleyen en önemli adımlardan biri yeterli ve kaliteli sperm elde etmektir. Bu çalışmanın amacı, Avrupa yayın balığından sperm elde etmek için kullanılan iki farklı yöntemi karşılaştırmaktır. İlk yöntem, testislerden cerrahi olarak sperm elde edilmesi (CS) ve ikinci yöntem ise sağım yoluyla sperm toplanmasıdır (SS). Döllenme oranı CS ve SS grupları arasında yüzde olarak hesaplanmış olup döllenme oranı en yüksek (%81.87±17.38) SS grubunda elde edilmiştir. Mevcut çalışmadaki bulgulara göre, döllenme için sperm elde etme yönteminde erkek Avrupa yayın balığını öldürmenin gerekli olmadığı sonucuna varılmıştır.

Anahtar kelimeler: Avrupa yayın balığı, döllenme, üreme, sperm toplama metodu

INTRODUCTION

The European catfish (*Silurus glanis* L., 1758) lives as a natural species in the basins of the Aral Sea, Caspian Sea, Black Sea, and Baltic Sea (Copp et al., 2009) and is a valuable fish caught in European waters (Froese and Pauly, 2019). However, European catfish is a commercial target species in the areas where it naturally occurs, thus sustaining and protecting the natural stocks is crucial (Zibiene and Zibas, 2019). European catfish has been listed as a protected species under the Berne Convention and entered into the International Union for Conservation of Nature Red List for species as of 2008 (Linhart et al., 2020).

European catfish is an important species in the fishing and aquaculture industry with its high growth abilities (Linhart et al., 2002; Ulikowski et al., 2003; Alp et al., 2011). In recent years, European catfish, that economic importance continues to increase, has become very attractive to humans for its delicious meat (Paschos et al., 2004) which rapidly makes, it a preferred fish for culture in most European countries. The maximum aquaculture production of European catfish was reported by Uzbekistan with 700 tons in 2019 and by Poland, with 127 tons in Europe (FAO, 2022).

Research on the production of *S. glanis* dates back to 1970 (Çelikkale, 1988; Horvath et al., 1992; Alpbaz and Hoşsucu, 1996; Linhart et al., 2002; Brzuska, 2001). Some authors have studied European catfish biology (Alp et al., 2004), bio-ecology (Akyurt, 1988), age-length and age-weight relationships (Saylar, 1993; Yılmaz et al., 2007), nutrition (Bora and Gül, 2004), growth characteristics (Uysal et al., 2009), and controlled production (Saygı and Güleç, 2019). While this species is naturally found in almost all inland waters in Turkey, its culture study still remains uncommon (Çelikkale, 1994; Geldiay and Balık, 1988; Alpaz, 2005; Saygı and Güleç, 2019). However, the cultivation and production of this species in Turkey are known to slow increase in a limited number of small-scale enterprises. European catfish production was 8.0 tons in 2017, and it was 84.0 tons in 2021 in Turkey (TÜİK, 2022).

Advancements in artificial culture techniques in Asia and Europe have led to progress in European catfish production. Despite the implementation of this reproductive method prior to 2000, significant production issues remain, with limited countries utilizing the technique, mainly in France and Central Europe (Kouril et al., 1996; Krasznai et al., 1980). In European

catfish production, the most important problem is the difficulties in obtaining sperm. Therefore, there is a need to develop different methods of sperm collection to ensure the development of catfish culture (Szabó et al., 2015; Idahor et al., 2018).

In this study, considering the difficulties in obtaining sperm from European catfish males, the effect of two different sperm collection methods on fertilization and hatching rate was investigated.

MATERIALS AND METHODS

Broodstock characteristics

This experiment carried out in this study was performed in compliance with the national legislation for fish welfare and approved by the institutional ethics committee. The suitable 7 male and 7 female European catfish broodstocks (6-9 years old) were selected for experiments and stocked in soil ponds within the Mediterranean Fisheries Research, Production, and Training Institute, Antalya. The fish were anesthetized using 2-phenoxyethanol at a concentration of 0.5 ml/l before each manipulation (hormone injection, gamete collection, and surgical intervention) (Neiffer and Stamper, 2009). The collection of egg and sperm was applied according to Linhart et al. (2004). Both female and male broodstock of European catfish were stimulated by intramuscular injection of carp pituitary hormone at a dose of 5 mg/kg^{-1} before gamete collection. The eggs were stripped into plastic bowls 24 hours after hormonal injection.

Experimental design

Sperm collection in male European catfish was carried out in two methods. In the first method, the laparotomy method which is surgical was used to remove the surgical testicular tissue (CS method). For the surgical intervention was used a surgery kit of sterilized surgical instruments. The surgical method of testicular sperm operation included separate stitches of the peritoneum and skin to create a supplemental anastomosis between inner organs and prevent infections. The abdomens of the three male individuals were cut approximately 5 cm. The intervention followed the division of skin from the peritoneum, peritoneum incision, taking of testicular tissue (Figure 1), and suturing of the injury creating intermittent eight-shaped stitches on the peritoneum. The operation time lasted 10-12 minutes. The testis was carefully removed so that other organs were not damaged. After testis removal, the testicle particles were tightened using a 200 µm net.

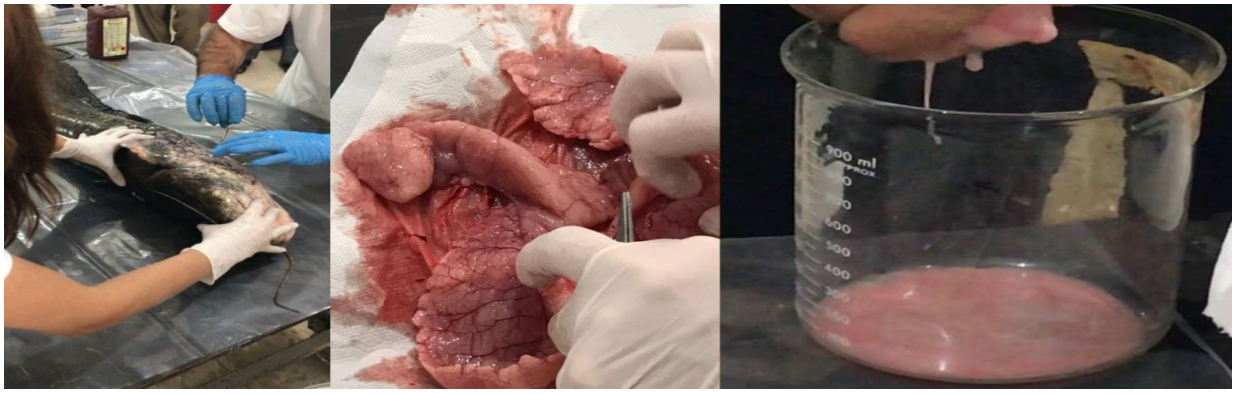

Figure 1. Surgical removal of the testis in European catfish (Photos by Merve Tınkır)

In the second method used in the study, sperm were collected from four male European catfish by hand stripping method (SS method) using a catheter (Figure 2).

Figure 2. Getting the sperm of the European catfish through stripping (vacuum method) (Photos by Merve Tınkır)

Fertilization procedure

European catfish is generally contaminated by urine during sperm collection, and contaminated sperm is activated (Linhart et al., 2004). Therefore, sperm samples taken by stripping different methods were kept in an immobilization solution (30 mM TRIS-HCl and 200 mM NaCl adjusted to pH 7) in order to sperm remain immotile. The sperm was added to an immobilizing solution at a volume ratio of approximately 1:1 and kept on ice.

Immediately after the sperm samples were added to the egg, the activation solution was added (Horvath and Tamas, 1976; Linhart et al., 2004). 300 g of eggs taken from each female were fertilized at 22 °C with 1 ml of sperm samples which were activated using 150 ml of hatchery water. After one minute of fertilization, 0.5 g/l of tannic acid for 20 seconds was used to remove the stickiness of the eggs. Fertilized eggs

treated with tannic acid are washed with hatchery water and transferred to plastic boxes filled with chlorine-free tap water. Plastic boxes measuring 18.0 cm × 18.0 cm × 6.0 cm filled with chlorine-free water were used. Each group (total 8 boxes) was replicated four times. In a laboratory, these plastic boxes (with fertilized eggs) were kept at 22 °C.

The eggs in every plastic box were counted and recorded on the first day. Dechlorinated water was gently changed in the plastic boxes 48 hours after post-fertilization, non-developing embryos were controlled counted, and removed. Also, the final total number of eggs was recorded. The malformed larvae and hatched larvae were manually counted immediately after hatching. It has completed an incubation period of approximately 4 days at 22 °C. The fertilization rate for each Petri dish was determined by dividing the number of eye-stage embryos at 48 hours post-fertilization by the initial number of eggs. The hatching rate was calculated by dividing the total number of hatched larvae by the initial number of eggs, while the malformation rate was calculated by dividing the number of malformed larvae by the initial number of eggs. The embryo development process was observed under a stereomicroscope (Leica Microsystems). The experimental data were analyzed using (SPSS 20.0) and expressed as mean ± SD, followed by paired sample t-test.

RESULTS

The result of the first group, testicular sperm (CS) >14 ml of fresh sperm was collected from each individual male during sampling. In the second group, stripping sperm (SS) >11.5 ml of sperm was collected from each individual during the sampling. For fertilization, over 1 kg of eggs was taken from each female during the stripping process (Table 1).

Table 1. Male and female catfish numbers, amount of sperm and eggs

Number of broodstocks female	Number of egg (g/total)	Number of broodstocks male	Amount of sperm (ml)
1	1390	1 ^{s}	13.5
2	1275	2^{c}	16.4
3	1380	3^{c}	14.0
4	1450	4 ^{s}	12.8
5	1225	5^{c}	14.5
6	1600	6 ^{s}	12.0
7	1720	7 ^{s}	11.5

^{c} surgical method testicular amount of sperm, ^{s} stripping method amount of sperm

Fertilization and hatching rates of 81.87 ±17.38% and 71.26 ±13.48%, were attained through insemination using the stripping method (SS). The surgical obtained of testicular sperm (CS) has found a fertilization rate of 66.18±11.39% and a hatching rate of 50.23±11.80%. The malformation rate post-hatching in the larvae was found to be similar between the two methods. With the addition of malformations larvae, the total larvae hatching rate was determined to be 76.10±12.39% in the stripping group and 53.83±11.28% in the surgical group respectively. Statistical analysis showed a significant difference in fertilization, hatching, and total hatching between the two different sperm collection methods ($p<0.05$) (Figure 3).

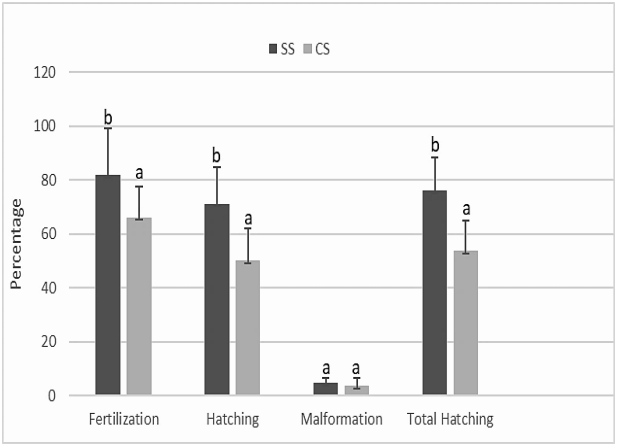

Figure 3. Fertilization (%), hatching (%), malformation (%), total hatching (%) rate of European catfish

The results were presented as the mean ± SD. Different superscripts indicate statistical significance differences (p <0.05).

DISCUSSION

In some locations where animal conservation regulations have not been improved, injuries from offensiveness have been decreased by suturing the fish's mouth later a bore is made in the jowl throughout sperm collection (Horvath et al., 1992). This method is likely to prevent breathing and is against EU legislation. As previously stated in many European and Asian countries, the killing of males and the acquisition of gametes in the production of European catfish is against Bern's convention to preserve and maintain genetic diversity. With the exception of the Czech Republic and France, stripping of male European catfish is still not widely practiced (Linhart et al., 2000).

One reason why male European catfish is killed during production is due to insufficient sperm production during spermiation. Two methods are used for obtaining sperm from male European catfish. The surgical method involves the use of non-lethal surgical techniques for complete (Sanap et al., 2018) or partial (Diyaware et al., 2010) resection of testicular tissue in male catfish through laparotomy. However, laparotomy can cause post-operative diseases, necessitating continuous monitoring (Romanova et al., 2017). While gonadectomized fish can be sold, they cannot be used for breeding, and they may die after a period of time. The non-surgical method involves obtaining sperm through stripping. This method involves obtaining sperm through vacuum aspiration without killing the male individual. This practice is more advantageous and allows the individual to continue reproductive activity for years. However, contamination of sperm with urine during stripping is inevitable in some fish species due to the close proximity of the sperm canal and ureter or the presence of a single urogenital pore through which

both sperm and urine are released. Sperm collection through stripping cannot prevent contamination with urine, which causes the loss of sperm motility within two minutes (Linhart et al., 1987). Spermatozoa suddenly activate during the sperm-collecting procedure when urine is mixed with the sperm. This early activation of spermatozoa is caused by urine contamination and has a negative impact on artificial reproduction (Linhart et al., 1987; Linhart et al., 2004). Similar corruption of sperm quality, likely related to infection with urine, has been reported in pikeperch (Křišťan et al., 2014) and European catfish (Legendre et al., 1996). Sperm motility can be preserved through the use of an immobilizing solution (Linhart et al., 2004). Sperm can be directly aspirated into an immobilizing solution through vacuum pumping into the papillae of male broodstock, minimizing bacterial contamination and other microflora (Linhart et al., 2020; Tinkir et al., 2021).

It has been observed that when spermatozoa are activated due to urine infection, a small amount of energy content is depleted a few seconds before exposure to the immobilizing solution (IS) (Alavi et al., 2019). The motility parameters of sperm undergo rapid changes due to the high energy consumption after activation, just before being affected by IS. The relationship between ATP production and motility for European catfish has been investigated by Billard et al. (1997). Within the first 5 to 10 seconds of activation, catfish sperm loses 50% of its ATP (Boryshpolets et al., 2009). However, by allowing the sperm to develop in the IS or seminal plasma, this lost energy can be restored within a matter of minutes, whether through artificial or natural means. Also, this situation has also been reported for carp (*Cyprinus carpio* L., 1758) and sturgeon (*Acipenser ruthenus* L., 1758) (Linhart et al., 2008; Xin et al., 2020).

The quality of gametes is a major factor in the success of fertilization, embryo quality, and larval growth performance (Linhart et al., 2020). Various abnormalities and embryonic malformations have been linked to sperm from males that have been exposed to pesticides or other toxic compounds (Labbé et al., 2017). However, no correlation was found between sperm aging and embryonic malformations (Linhart et al., 2020). There is no significant correlation between the rate of larval malformation and the method of sperm collection. However, sperm collection methods affect the rate of fertilization and hatching (Linhart et al., 2020). A study comparing the use of sperm from male African catfish (*Clarias gariepinus*) broodstock found that fertilization percentages were 71.5% and 81% for sperm obtained by stripping and from testicular tissue (fifty percent of testicular tissue), respectively (Adebayo et al., 2012). In northern pike, the mean hatching rates were found 79 ± 6% and 78 ± 6% for eggs fertilized with stripped sperm and testicular sperm, respectively (Kristan et al., 2020). The mean concentration of stripped sperm was found to be lower than that of testicular sperm in northern pike (Hulak et al., 2008). In European catfish, the fertilization rate was found to be higher when using sperm obtained through stripping (85.1%) compared to sperm collected from the testicular tissue of killed fish (65.6%) (Linhart et al., 1987). A study comparing fertilization rates of sperm obtained through stripping and from the testicular sperm of rainbow trout male broodstock found an average of 81% and 39.2%, respectively (Geffen and Evans, 2000). The results in the present study are in accordance with the other studies above, while it was discovered that the fertilization rate achieved through sperm obtained via stripping was 81.87%, whereas the fertilization rate obtained from sperm in testicular tissue was 66.18%. The success of fertilization in European catfish, the quality of the embryos, and the subsequent performance of the offspring are largely dependent on the quality of the gametes and the method of obtaining sperm.

CONCLUSION

According to the results of the current study, it can be concluded that it's not essential to kill male European catfish to obtain sperm by vacuum method for fertilizing female eggs. Consequently, successful fertilization was achieved at a rate of 81.87% through the use of stripping (vacuum method) in this investigation. This practice is more advantageous and allows the individual to survive and continue their reproductive activity the next reproduction season.

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AUTHORSHIP CONTRIBUTIONS

Merve Tınkır and Devrim Memiş: Conceptualization, methodology. Merve Tınkır: Data curation, writing- original draft preparation. Merve Tınkır: Visualization, investigation. Devrim Memiş: Supervision, project administration, resources, funding acquisition. Merve Tınkır, Adil Yılayaz, and Devrim Memiş: Writing-reviewing and editing.

CONFLICTS OF INTEREST

The author declares that there is no conflict of interest in this manuscript.

ETHICS APPROVAL

In order for the experimental design to be carried out on the basis of an ethic, the approval numbered 09/2020 was obtained by the local ethics committee for animal experiments at the Mediterranean Fisheries Research, Production, and Training Institute.

DATA AVAILABILITY

All relevant data are inside the article.

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