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Research Article

Biological Parameters and Spermatogenesis in Razorfish (*Pelecus cultratus*) Population Inhabiting the Largest Shallow Lake of Central Europe (Lake Balaton): Studies for In Vitro Conservation Purposes

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The study aimed to investigate body parameters and the process of spermatogenesis from April to September. In addition, it sought to test the applicability of sperm cryopreservation for conservation purposes in a razorfish (*Pelecus cultratus*) population of Lake Balaton, the largest shallow lake in Central Europe. During the aforementioned period, measurements were taken for the standard length (SL, cm) and body weight (BW, g), and the sex of specimens was determined. Cells at different stages of spermatogenesis (spermatogonia-SG, spermatocytes-SC, spermatids-ST, and spermatozoa-SZ) were quantified monthly for each male sample. Sperm samples collected at the end of May were cryopreserved using a method designed for common carp. No significant differences were found between males (SL: 25 ± 2 cm and BW: 146 ± 38 g) and females (SL: 26 ± 3 cm and BW: 168 ± 53 g) in terms of measured body parameters. No significant correlations were found between the sex, SL, and BW. High standard deviations were observed for all mean values in all sampling periods, possibly due to the low number of individual samples per month. A significantly higher proportion of SC compared to SZ was found in April. In May, no differences were observed between the four groups. Significantly more SG and SZ than SC and ST were observed in June. In August and September, a slight dominance in the number of SG was recorded, with no differences measured among the cells in different developmental stages. The males studied exhibited a low gonadosomatic index ($0.92\% \pm 0.27\%$). A significant reduction was recorded in motility (MOT), progressive motility (pMOT), and in most of the kinetic parameters (distance curved line-DCL, curvilinear velocity-VCL, straight line velocity-VSL, and beat cross frequency-BCF). The spermiation of males could have started in May and conceivably lasted until the end of June. It is recommended to increase sperm quality and quantity before cryopreservation.

1. Introduction

The razorfish (*Pelecus cultratus*) is a rheophilic, anadromous species endemic to the waters of the Ponto-Caspian and Baltic regions [1–4]. Its distinctive body shape, such as the elongated pectoral fin, resembles that of flying fish species found in tropical waters rather than other cyprinids native to Europe [5]. Razorfish is the sole member of the European subfamily Leuciscinae that inhabits large rivers, lakes, estuaries, and brackish sea basins [4, 6, 7]. Natural populations of the species have significantly declined in several European countries, leading to its protected status in Baltic states (Annex II of the International Association for the Protection of Species of the European Commonwealth of the Baltic

States, Fauna Flora Habitat Directive). Additionally, it is classified as critically endangered in Poland according to The Red List of lampreys and fish of Poland (as of 2009); code: CR) [4, 8–11]. Furthermore, razorfish is listed in the IV Appendix of the EU Bird and Habitat Directive [1, 12]. The regulation of rivers, including activities such as gravel excavation and dam constructions, has resulted in the destruction of natural spawning grounds for the species [10, 13, 14]. To address these challenges, biotechnological methods, including controlled reproduction, larvae rearing, and gamete preservation can assist in the successful recovery and restoration of the natural populations [5, 9, 10, 15, 16].

Lake Balaton, located in Transdanubia, Hungary, is the largest shallow lake in Central Europe. It has a surface area of 594 km^2, a length of 78 km, an average width of 7.7 km, an average depth of 3.2 m, a water volume of 1.9 km^3, and a coastline of 235 km [17, 18]. The lake is divided into four main basins: Keszthely, Szigliget, Szemes, and Siófok [18]. A unique population of razorfish inhabits the lake and its entire life cycle there [1, 19, 20]. Until the 1970s the species was of great economic value, with an annual catch of 100 t [1, 21]. Currently, the species is predominantly characterised by its high angling value (as observed in Gorky Reservoir) and its significant ecological role within the lake's ecosystem [1, 22–24]. The population (and the species) is not protected by the Hungarian government. As a consequence of the aforementioned factors, it is crucial to provide effective support and management for the razorfish population in Lake Balaton [1].

Despite some successful attempts at controlled reproduction and larvae rearing of the species, no developed method has yet been established [1, 5, 9, 10, 16]. Furthermore, information regarding its spawning behaviour, larval development, and the exact time of production of mature gametes (spawning approximately from April to July at 19–22°C in Europe) in Lake Balaton is still unexplored [1, 25–28]. However, the collection of the highest quality gametes from both natural stock and captive broodstock is a crucial aspect for the propagation, maintenance of the genetic background, and the population itself in the lake [15].

Previous studies have shown that developmental potential is encoded in the genotype. However, their expression is determined by various environmental factors [3]. Species are under pressure to adapt to a specific biotope [3, 29]. The morphological variability originates from the phenotypic plasticity and adaptability [3, 30]. Morphological and morphometric studies of razorfish in various biotopes can contribute to understanding of the adaptation and development of the local population [3, 4]. The testis of razorfish has been previously investigated from the perspective of environmental toxicology, particularly mercury content, as well as metabolic indicators, including total moisture, dry matter, lipids, proteins, ash, and nitrogen-free extractive substances [24, 31]. Histological analysis can be used to determine both the stage of gonadal development and the stages of spermatogenesis [32]. The method can help to identify the period when wild caught razorfish males produce the highest number of spermatozoa (SZ). Moreover, sperm quality assessment can be performed using the CASA (computer assisted sperm analysis) system [33]. This technique offers an objective assessment method that focuses on the ratio of effectively moving cells and various kinetic parameters [34]. Cryopreservation can be used to preserve the best sperm samples in the long term, as described by several authors [35, 36]. In this method, a portion of the SZ can be stored at −196°C maintaining their quality and fertilising capacity [37–40]. Quality assessment and sperm cryopreservation have already been used to establish sperm banks and support the restoration of endemic fish populations [15, 35, 41]. In recent decades, successful methods have been developed for several rheophilic fishes such as ide (*Leuciscus idus*), chub (*Squalius cephalus*), and dace (*Leuciscus leuciscus*) [38, 42–44]. No information on spermatogenesis, quality assessment, and cryopreservation of sperm in razorfish is available in the literature to the best of our knowledge. The objectives of the study were: (1) to investigate the body parameters of male and female razorfish during the sampling period from April to September; (2) to examine the spermatogenesis during the aforementioned period; and (3) to apply sperm cryopreservation in samples collected from wild male razorfish.

2. Materials and Methods

The experiments were conducted in compliance with the Government Decree 40/2013 (II. 14.) on Animal Experiments and Act XXVIII of the 1998 Hungarian Parliament on the protection and humane treatment of animals. In addition, the study was performed with the knowledge and approval of the Institutional Animal Welfare Committee of the Hungarian University of Agricultural and Life Sciences, Szent István Campus (Certificate No.: MATE-SZIC/1742-1/2022 and MATE-SZIC/1744-1/2022). Chemicals used in the study were purchased from Reanal (Budapest, Hungary) and Sigma–Aldrich (Budapest, Hungary).

2.1. Fish Sampling. Male and female fish ($N = 127$) were caught from five sampling sites, representing both the northern (Balatonudvari, Sajkod, and Tihany) and the southern (Ordacsehi and Siófok) shores (Figure 1). Monthly sampling was conducted from April to September using four whole water column gillnets (WWCG) with a mesh size of 2.5 cm, measuring 36 m× 3 m. The nets were set for 1.5 hr each. To maintain and transport the captured fish, a 400 L polypropylene tank with a continuous oxygen supply was used.

2.2. The Measurements of Biological Parameters. Body parameters were recorded either immediately at the sampling site or in the laboratory of the Department of Aquaculture (Institute for Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences). Fish were anaesthetized with 2-phenoxyethanol (99%) at a dose of 0.4 mL L^{-1}. Standard length (SL) was measured using a standard tape measure (cm) and the body weight (BW) with a laboratory balance (±0.01 g, Kern EMS 12K1, Kern and Sohn GmbH, Balingen, Germany). The sex ratio was determined visually (based on the gonads) during the dissection of the individuals.

2.3. The Investigation of Spermatogenesis. Spermatogenesis was monitored from April to September (April $N = 4$, May $N = 7$, June $N = 5$, August $N = 3$, and September $N = 3$).

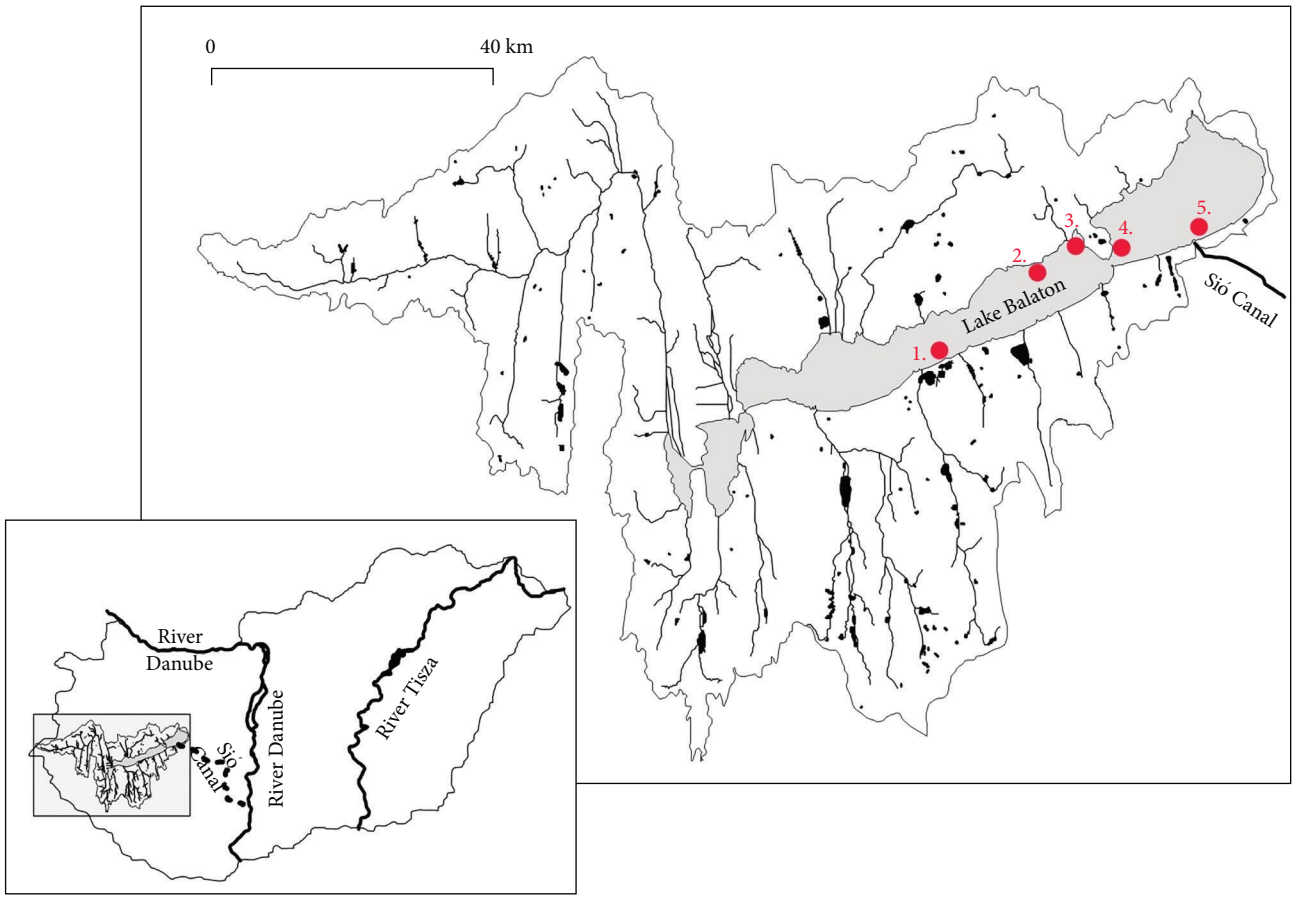

FIGURE 1: Sampling sites in Lake Balaton. (1) Ordacsehi; (2) Balatonudvari; (3) Sajkod; (4) Tihany; and (5) Siófok.

No males were caught in July. The middle parts of the right and left testis were collected for the study. The dissected organ parts were placed in specific histological cassettes (InnoLab Laboratóriumi Innovációs Bt., Dunakeszi, Hungary) and preserved in Bouin's solution. Using an automated tissue processor (Shandon; Citadel 2000 LE11 5RG, Thermo Fisher Scientific, Waltham, Massachusetts, USA), the samples were dehydrated with ethanol (75%–90%) and washed with xylene solutions. The organ parts were subsequently embedded in paraffin using Leica HistoCore Arcadia H equipment (Leica Biosystems, Wetzlar, Germany). Sections of 2–5 μm were then cut from the embedded blocks using a microtome (Leica RM 2245, Leica Biosystems, Wetzlar, Germany). A water bath (Kunz Instruments HP-3, Kunz Instruments Ab, Nynashamn, Sweden) was used to fix the sections to slides at a temperature of approximately 42–45°C. Finally, slides/samples were stained with haematoxylin–eosin (Shandon Varistain 24-4, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Ten fields were captured for each stained sample using a digital camera (QImaging Micro Publisher 3.3, QImaging, Surrey, Canada) connected to a light microscope (Nikon Eclipse 600, Auroscience Kft., Budapest, Hungary) equipped with a 20x objective (magnification: 40x, field of view: 500 μm^2). Cells at different stages of spermatogenesis (spermatogonia-SG, spermatocytes-SC, spermatids-ST, and spermatozoa-SZ) were identified on each slide and counted using Image J 1.48v open source software (Image J for Windows, National Institutes of Health, USA; Figures 2(A)–2(C)). The relative proportions of cells at different developmental stages and their standard deviation were determined for each sampling month [32].

2.4. Sperm Motility Assessment. Based on the analysis of spermatogenesis, sperm was collected the following year at the end of May. The testes of six males (SL: 25 ± 1 cm and BW: 123 ± 12 g) were dissected and weighed on a laboratory balance (± 0.01 g, Kern EMS 12K1, Kern and Sohn GmbH, Balingen, Germany). As it was not possible to obtain sufficient sperm from the testes of the males, the whole organ was cut into small pieces and pressed through a mesh with a pore size of 200 μm. The sperm suspension was collected in 1.5 mL test tubes. Sperm motility was assessed in both fresh and cryopreserved/thawed samples using a computer assisted sperm analysis system (CASA, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA). Razorfish SZ (1–100 μm^2) were identified by a digital camera (JAI CV-A10 CL, Minitube of America, Venture Court Verona, USA, frame rate of 60 s^{-1}). In our experiments, the following sperm parameters were recorded: total motility (MOT, %), progressive motility (according to the criteria of the producer: straight line distance 5 μm, pixel to μm ratio: 151–100, pMOT, %), distance curved line (DCL, μm), curvilinear velocity (VCL, $\mu m\ s^{-1}$), straight-line velocity (VSL, $\mu m\ s^{-1}$), linearity (LIN, %), amplitude lateral

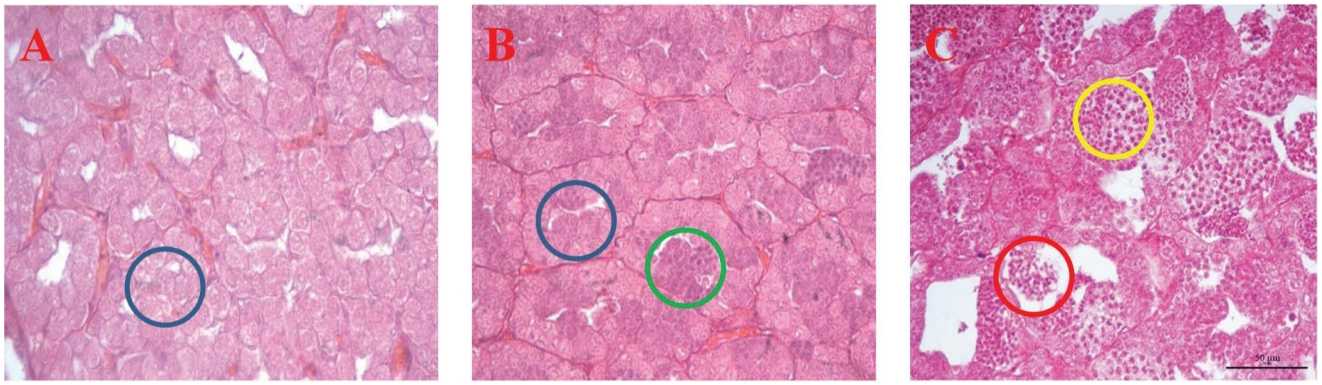

FIGURE 2: (A–C): The histological examination of razorfish testis. (A) Spermatogonia (blue marker), (B) spermatogonia (blue marker), spermatocytes (green marker), and (C) spermatids (yellow marker), spermatozoa (red marker).

head displacement (ALH, μm), and beat cross frequency (BCF, Hz) [34]. SZ were activated (in duplicate per sample, average number of sperm cells per activation: ~300−500 SZ) using a simple saline solution (50 mM NaCl, 30 mM Tris, pH: 8.0 ± 0.02 [45]) mixed with bovine serum albumin (BSA, 0.01 g^{-1} mL).

2.5. Sperm Cryopreservation. The gonadosomatic index (GSI, %, average weight of testis per average body weight multiplied by 100) was calculated prior to sperm cryopreservation [46]. Sperm samples from six males were diluted (1 : 9, sperm : extender + cryoprotectant) in a simple sugar-based extender (350 mM glucose, 30 mM Tris, pH: 8.0 ± 0.02 [47]). Methanol (10%) served as cryoprotectant. Diluted samples were loaded into 0.5 mL straws (Minitüb GmbH, Tiefenbach, Germany). Sperm samples were frozen in liquid nitrogen vapour (3 cm above the surface) for 3 min [47]. Cryopreservation was conducted in a controlled-rate freezer (IceCube 14 s, IceCube Series v. 2.24; Sy-Lab, Neupurkersdorf, Austria) using the specified cooling programme: from 7.5 to −160°C, at a cooling rate of 56°C min^{-1} [48]. Straws were thawed in a water bath at 40°C for 13 s [47].

2.6. Statistical Analysis. Statistical analyses were implemented with SPSS 22.0 (SPSS Inc., Chicago, USA), GraphPad Prism for Windows (GraphPad Software version: 5.0 and 8.0, La Jolla, California, USA) and R Statistical Environment (R Core Team 2022) software. Normal distribution was tested using the Shapiro–Wilks test at a significance level of $P < 0.05$. Data not displaying normal distribution were transformed with logarithmic and arcsine square root functions. Linear regressions (on log-transformed data) were employed to model length–weight relationships in both sexes. Analysis of covariance (ANCOVA) was applied to the interaction between sex, BW, and SL. The comparison of different body parameters was carried out using independent Student's *t*-test. Comparison of the proportion of cells in the four different stages of spermatogenesis in a given month (based on the number of males) was performed by one-way analysis of variance (ANOVA) or Kruskal–Wallis test followed by Tukey, Dunnett T3, or Dunn's multiple comparison test (significance level $P < 0.05$). Motility (MOT and pMOT) and kinetic parameters (DCL, VCL, VSL, LIN, ALH, and BCF) in fresh and cryopreserved groups were compared using Student's *t*-test (independent samples) at a significance level of $P < 0.05$.

3. Results

3.1. The Investigation of Biological Parameters. During sampling, an exceedingly high mortality rate (~90%) was observed attributed to water column gillnets and fish handling. The sex ratio in the captured razorfish population was 48%–52% (*N* male: 61 and *N* female: 66). No significant difference was found between males (SL: 25 ± 2 cm and BW: 146 ± 38 g) and females (SL: 26 ± 3 cm and BW: 168 ± 53 g) in the measured parameters. No significant correlation was identified between sex, SL, and BW.

3.2. The Investigation of Spermatogenesis. In addition to the mean values, high standard deviations were observed in all sampling periods, possibly due to the low number of individual samples per month. A significantly higher proportion of SC was found in April compared to SZ. No differences were recorded between SG, ST, and SZ as well as between SG, SC, and ST. In May, no distinctions were observed among the four groups. A significantly higher number of SG and SZ were observed in June compared to SC and ST. However, no difference was found between SC and ST. In August and September, a slight dominance in the number of SG was recorded. Nevertheless, no difference (attributed to the low number of males and high standard deviation) was measured between (Table 1).

3.3. The Results of Sperm Cryopreservation. The males studied exhibited a low GSI of 0.92% ± 0.27%. Significant reductions were recorded in MOT, pMOT, and most of the kinetic parameters (DCL, VCL, VSL, and BCF). However, no statistically significant difference was observed for LIN and ALH parameters (Table 2).

4. Discussion

4.1. The Investigation of Biological Parameters. Data collected monthly from April to September suggested an almost 1 : 1

Table 1: Proportion of the cells at different developmental stages in testis samples collected from April to September (April $N = 4$, May $N = 7$, June $N = 5$, August $N = 3$, and September $N = 3$).

	Spermatogonia (SG, %)	Spermatocytes (SC, %)	Spermatids (ST, %)	Spermatozoa (SZ, %)
April	20 ± 21^{ab}	53 ± 34^{a}	27 ± 25^{ab}	0^{b}
May	29 ± 6^{a}	6 ± 11^{a}	28 ± 16^{a}	37 ± 39^{a}
June	53 ± 28^{a}	1 ± 1^{b}	2 ± 3^{b}	45 ± 28^{a}
August	97 ± 25^{a}	0^{a}	0^{a}	3 ± 6^{a}
September	98 ± 16^{a}	2 ± 3^{a}	0^{a}	0^{a}

Different superscript letters indicate significant differences in the proportion of cells at different developmental stages at a given month (row; $P < 0.05$). The table presents the mean and standard deviation.

Table 2: The motility and kinetic parameters measured in fresh and cryopreserved razorfish sperm ($N = 6$).

	Fresh	Cryopreserved
MOT (%)	35 ± 16^{a}	13 ± 17^{b}
pMOT (%)	19 ± 11^{a}	3 ± 4^{b}
DCL (μm)	25 ± 4^{a}	13 ± 11^{b}
VCL ($\mu m\, s^{-1}$)	55 ± 11^{a}	28 ± 23^{b}
VSL ($\mu m\, s^{-1}$)	44 ± 11^{a}	24 ± 19^{b}
LIN (%)	79 ± 6^{a}	56 ± 44^{a}
ALH (μm)	1.3 ± 0.3^{a}	1.0 ± 0.9^{a}
BCF (Hz)	20 ± 4^{a}	10 ± 8^{b}

Different superscript letters indicate significant differences between fresh and cryopreserved groups at given motility and kinetic parameters $P < 0.05$. The table presents the mean and standard deviation.

sex ratio among razorfish inhabiting Lake Balaton. In contrast, another study revealed a slight female dominance (62%, male: 38%) in the population of the lake [20]. Raczyński et al. [3] presented a shifted proportion of males and females (~1 : 4) based on samples collected in Vistula Bay, Poland. Furthermore, the dominance of males over females (~3 : 1) was recorded by Sliskovic et al. [4] in razorfish originating from the River Mur (Slovenian section of the river close to Ceršak). Former studies and our findings suggest that the sex ratio shows high variability in various razorfish populations across Europe including those inhabiting rivers, estuaries/bays, and lakes. The nearly balanced male–female ratio discovered in Lake Balaton can serve as a foundation for the successful maintenance of the natural population, ensuring a sufficient number of spawners of both sexes. Similarity was observed in the measured body parameters of males (SL: 25 ± 2 cm and BW: 146 ± 38 g) and females (SL: 26 ± 3 cm and BW: 168 ± 53 g). Comparable results for both male (~26 cm) and female (~27 cm) SL at Lake Balaton were previously published by Staszny and Paulovits [20]. A slightly higher mean SL was recorded in males (29 cm) and females (28 cm) of the razorfish population inhabiting Vistula Bay, Poland. However, the total BW of both sexes (150 g) displayed similarities with our results. A comparable total length was measured in males (29 ± 6 cm) and females (29 ± 4 cm) collected from River Mur in Slovenia. In contrast to our findings, a modest BW was found in both sexes (males: 139 ± 98 g, females: 135 ± 52 g) compared to the population in Lake Balaton [4]. Inter- and intrapopulation differences in body shape can be observed in several fish species, such as salmonids [3, 49]. Different populations may have adapted to various environments (freshwater lakes, estuaries, and rivers), resulting in slight phenotypic variation [3, 30]. To summarize, the systematic (annual) monitoring of body parameters in relation to changing environmental conditions is recommended for the razorfish population of Lake Balaton [4, 10, 16, 20]. In our study, no significant correlation was detected between SL, BW, and sex of individuals. Similar observations were made by Raczyński et al. [3] in the Vistula Bay population (Poland). However, a weak significant correlation was observed between the sex of the fish and the predorsal length parameter. Former studies have suggested that the sexual dimorphism in razorfish is weak [3, 50]. Nevertheless, further detailed morphometric analysis of the body shape can reveal unique features and differences between males and females in the population of Lake Balaton.

4.2. The Investigation of Spermatogenesis. Based on the results of the investigation of spermatogenesis in male razorfish, the varying proportion of cells in different developmental stages each month clearly indicates proliferation. According to histological analysis, spermiation in males could already commence in May and conceivably last until the end of June. Similar to our study, spermatogenesis and the pattern of spawning season were investigated in winter flounder (*Pseudopleuronectes americanus*). The maturation stage was determined by the size and age of males. However, the spermatogenesis showed no temporal or geographical differentiation [51]. Our analysis of spermatogenesis showed an extended period, reflecting a mosaic pattern of spawning. Histological analysis of the testis of Atlantic bluefin tuna (*Thunnus thynnus*) was performed both during the spawning season (May–June) and off spawning season (October–November). The testis exhibited a higher proportion of cells in early and late spermatogenesis. In addition, the period off of the spawning season was characterised by an increased number of SG and a reduced proportion of SC and SZ [52]. A similar trend was observed in razorfish males in our study, confirming the occurrence of expanded natural spermatogenesis in various freshwater and marine species as well. Nevertheless, the data collected in our study cannot be considered complete due to the absence of samples from July and the modest number of samples in April, August and September. Further annual sampling and detailed investigation of male testes, combined with assessment of female ovaries can provide comprehensive knowledge regarding the spawning season and behaviour of this unique razorfish population.

4.3. The Results of Sperm Cryopreservation. The efficacy of sperm cryopreservation was likely influenced by the relatively low GSI (0.92% ± 0.27%) and the initial quality of fresh sperm. GSI in males is influenced by various factors, especially seasonal changes such as water temperature [46, 53]. In two cyprinid species, roach (*Rutilus rutilus*) and rudd (*Scardinius erythrophthalmus*), inhabiting the oligotrophic Lake Sapanca (similarly to Lake Balaton) in Turkey, the GSI of males can vary in a wide range (∼0.2%–10%) depending on the sampling period [53]. Bokor et al. [46] also reported a notable difference in GSI of wels catfish (*Silurus glanis*). The study, GSI was estimated both in male broodstock reared and hormonally induced in commercial fish farms in Hungary, both during and off the spawning season. Compared to males analysed in the off season (0.765% ± 0.21%), GSI was notably higher in individuals during the spawning (2% ± 0.05%) season [46]. In addition, a remarkable variation in GSI (0.3% ± 0.188%–1.756% ± 0.049%) was found in the Mediterranean horse mackerel (*Trachurus mediterraneus*) in relation to the sampling period [54]. The spawning season of the razorfish is relatively long, in Europe it takes place from April to July at temperature ranging from 19 to 22°C [1, 25–28]. Based on the gametogenesis study results, it is recommended to systematically collect more samples from males from May to the end of June in order to identify individuals with high GSI at Lake Balaton. In addition, the continuous monitoring of further sperm quality parameters, such as sperm concentration, in the aforementioned period (May–June) can support the development of controlled reproduction, preservation methods, and conservation programs by razorfish [33]. Through our analysis, a decreased quality (motility and kinetic parameters) of squeezed fresh sperm was observed, which had significant impact on post-thaw motility and kinetic parameters. The results of GSI evaluation suggested that razorfish males had already spawned or were not prepared for spawning. To enhance both the sperm quality and quantity of sperm, hormonal stimulation of spermiation can be employed [55]. Previous studies have shown that various agents, including carp pituitary extract, human chorionic gonadotropin hormone and Ovopel have been applied for controlled reproduction of razorfish [1, 9]. Ovopel has been identified as a suitable candidate for hormonal stimulation in both male and female razorfish. However, its high stress sensitivity poses a challenge, resulting in elevated mortality during handling and manipulation of individuals [1, 9]. In our study, a similar high mortality rate (∼90%) was also recorded during fish capture using water column gillnets and subsequent handling. To improve the effectiveness of sperm cryopreservation in wild caught razorfish males, the development of an applicable hormonal stimulation method is highly recommended. Optimal processes for spermiation induction, requiring minimal manipulation, such as single dose injection, are obviously desirable [9].

5. Conclusion

Based on our findings, the razorfish population of Lake Balaton exhibits a balanced sex ratio which may support its future maintenance. Our results, combined with previous studies, suggest that systematic annual morphometric studies can reveal in detail the unique phenotypical features of a population adapted to this freshwater oligotrophic habitat. The onset of male spermiation could be as early as May, with the possibility of continuation until the end of June. Prior to sperm cryopreservation, sperm quality and quantity enhancement are strongly recommended.

Data Availability

The dataset presented in this study is available from the corresponding author following direct request.

Conflicts of Interest

The authors have no relevant financial or nonfinancial interests to disclose.

Authors' Contributions

Zoltán Bokor contributed in the supervision, conceptualisation, investigation, methodology, and writing–original draft. Ferenc Fodor contributed in the investigation and data curation. Levente Várkonyi contributed in the investigation and data curation. Borbála Nagy contributed in the investigation and data curation. Zete Levente Láng contributed in the investigation and data curation. Árpád Ferincz contributed in the investigation and data curation. Ádám Staszny contributed in the investigation and data curation. József Molnár contributed in the investigation and data curation. Kinga Katalin Lefler contributed in the investigation and data curation. Balázs Csorbai contributed in the investigation and data curation. Zoltán Vancsura contributed in the investigation and data curation. Zsolt Szári contributed in the project administration and funding acquisition. Urbányi Béla contributed in the project administration and funding acquisition. Gergely Bernáth contributed in the supervision, conceptualisation, investigation, methodology, writing–original draft, and writing–review and editing.

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