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CHANGES IN TESTICULAR MORPHOMETRY IN AMERICAN MINK AS A SEASONAL BREEDER

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ABSTRACT

The *Mustelidae* family is one of the most diverse and numerous ones in the *Carnivora* order and yet, our knowledge about their reproductive biology is scarce. This study was designed to describe and compare the basic characteristics of testicular morphometry of 70 male American mink, taking the seasonal effect into consideration. Additionally, it was tested whether the size of the testes is related to sperm concentration. Mink testes were collected during the reproductive season and out of it. The testes were measured with a calliper and weighted on a precision scale. Spermatozoa were obtained by epididymal slicing and all semen samples were analysed using a CASA-system. A strong correlation between seasons of the year, testicular morphometry and sperm production was found. An additional index was created to normalize the collected data and compare it, showing statistically significant difference between the males in and out of the reproductive season. The average length and width of the testicle from males in breeding season was, respectively, ~1.9 and ~.1.4 cm, compared to ~1.3 and ~0.9 cm in non-breeding males. Testicles collected out of the breeding season had less biomass than the ones collected during the breeding season (~0.7g out of the season and ~3.8g in the season). The study provides new data concerning testicular morphometry in American mink. Their belonging into the seasonal breeders has been further confirmed, indicated by the changes in testes morphometry, as well as cease of sperm production in late autumn/winter.

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1. INTRODUCTION

1.1 Study background

The family *Mustelidae* (commonly called weasels), consisting of over 60 species, is one of the most diverse and species-rich families in the order Carnivora. It includes many endangered species, with the North American black-footed ferret (*Mustela nigripes*) being the most endangered one [1]. Despite being such a large family with many species requiring our attention and help, there is limited information on the reproductive biology of mustelids. Only for seven of over 60 species, data about their reproductive characteristics is available and up to date. These include sea otter, North American river otter, giant otter, wolverine, Siberian polecat, long-tailed weasel and black-footed ferret. American mink (*Neogale vison*) is a semi-aquatic, medium-sized member of the Mustelidae family. It's similar in size to the European polecat and for a long time was believed to be a part of the *Mustela* sub-species, before its classification as the only extant member of the genus *Neovison*. Recently a case has been presented to reclassify it within the genus *Neogale*, with other New World weasel species [2]. Like many other mustelids, mink are sexually dimorphic (males, on average, weight almost twice as much as females) and their reproductive activity is seasonal and photosensitive, with mating period starting in late winter/early spring [3;4]. In the 1920s mink were introduced to European fur farms and quickly gained much popularity due to the exceptional quality of its fur [5].

1.2 Importance of Reproductive Studies

Reproductive biology provides many opportunities to address questions of biology with application to both veterinary and human medicine. Results of studies on animals reproductive traits impact our understanding of the origins of processes occurring during maturation and activity of reproductive organs. Testicular morphometry is an important measurement for evaluating the functionality of the testes. It can represent the quality of spermatogenesis and indicate semen quality of the male [6]. Significant correlations have been reported between the mass of the testes and sperm production in boars and European wild polecats [7;8]. Many seasonally breeding mammals exhibit annual changes in testicular activity, varying from completely regressed to highly active spermatogenesis. Testicular regression during the non-reproductive season is usually associated with significant reduction of testicular mass and cease in sperm production [8; 9; 10; 11].

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1.3 Study Objective

Over the last century American mink, polecats and black-footed ferrets have been used for research in many fields, including physiology of reproduction [4;12; 13; 14; 15; 16; 17; 18; 19]. However, a qualitative characterization of mink testes that would include testicular volume and indexes is, to authors' knowledge, not available.

This study aims to describe and compare detailed characteristics of testicular morphometry of farmed American mink and test whether the size of the testes affects sperm concentration.

2. MATERIALS AND METHODS

2.1 Animals

The study was performed in March 2019 and November 2019, using 70 mink originating from one farm, located in Greater Poland Voivodeship. All males were between 7 months and 3 years old and had reached somatic maturity. The testes with epididymides were obtained after technological slaughter of the animals. The testes were collected during the reproductive season (March) and out of the reproductive season (November). The group from March consisted of 40 animals and the group from November consisted of 30 males. The age distribution in the groups was similar, both of them consisting of both young males and proven breeders, to provide objective data. The farm, which the mink originated from, adhered to the Polish law considering farmed animals' welfare. All mink were fed with freshly made fodder, suitable for their needs and based on animal by-products (mainly coming from poultry production), had constant access to fresh, drinking water and had several environmental enrichments (such as toys, shelves, hiding booths) in their cages to stimulate natural behaviour and prevent boredom. According to Polish law, receiving the research material post-mortem, as a by-product, does not classify the study as an animal experiment, as defined in Article 1 and 2 of the Act on the Protection of Animals Used for Scientific or Educational Purposes. The mink used in this study were not kept nor killed for research purposes; therefore, our study was not considered as an animal experiment and as such it did not require the Research Ethics Committee approval.

2.2 Testicular morphometry

Testes were collected post-mortem, within an hour after the animal's death and stored in a cooler at approximately 4°C during their transportation to the laboratory. The testes were measured with a calliper while they were still connected with the epididymis. The testes were weighed both with and without the epididymis. Each testis was measured and weighed separately. The length of each testis was measured from the head of the epididymis to the tail of the epididymis. The width

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of the testes was measured at the middle of the testes and included the tail of epididymis. The measurement points are shown in Figures 1 and 2, the length and width of the testes are given in centimetres, the weight is given in grams. The volume of the testes was calculated using the formula for ellipsoids volume: $\frac{\pi}{6} \times length \times width$ [21], and is given in cubic centimetres. Testes collected in March were collected in the reproductive season, after the males have stopped being used for mating on the farm. The males were well rested and fully recovered from the last mating before their testes were collected. Testes collected in November were collected prior to the reproductive season, when the males are starting to prepare to the reproductive season and to intensify the sperm production. To prevent inter observer bias, all the measurements were done by the same person.

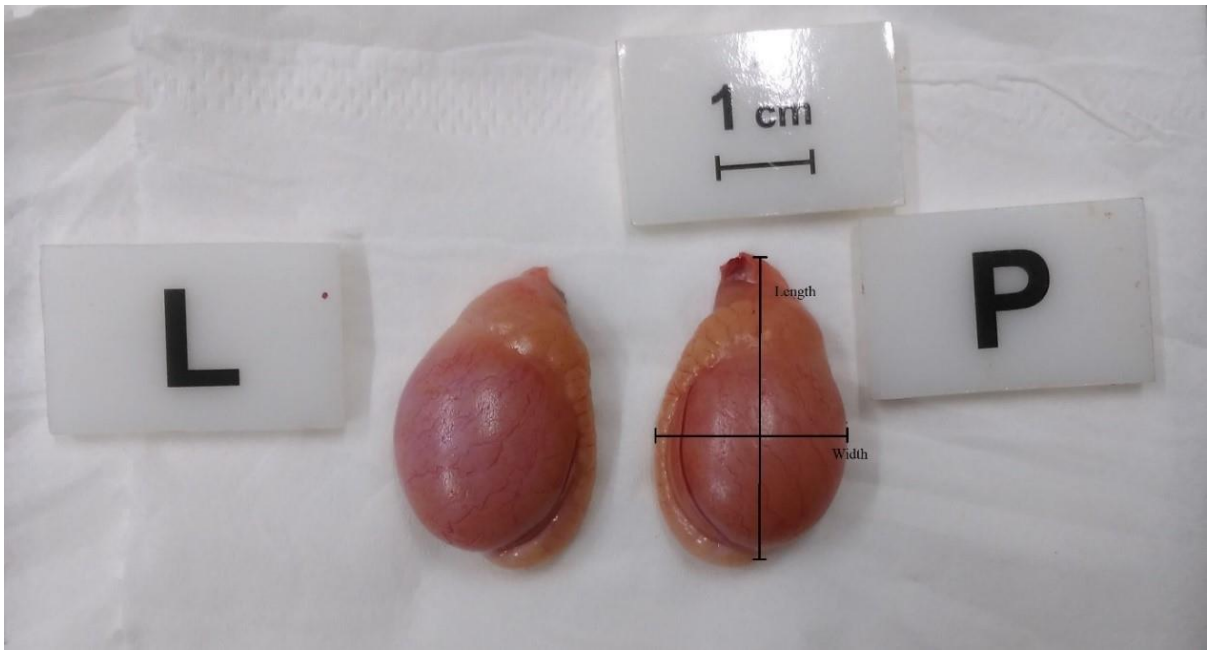

Figure 1: Measurement points for the length and width of the testes. Testes shown in this photo come from the group in the reproductive season (March). "L" stands for the left testis and "P" for the right testis

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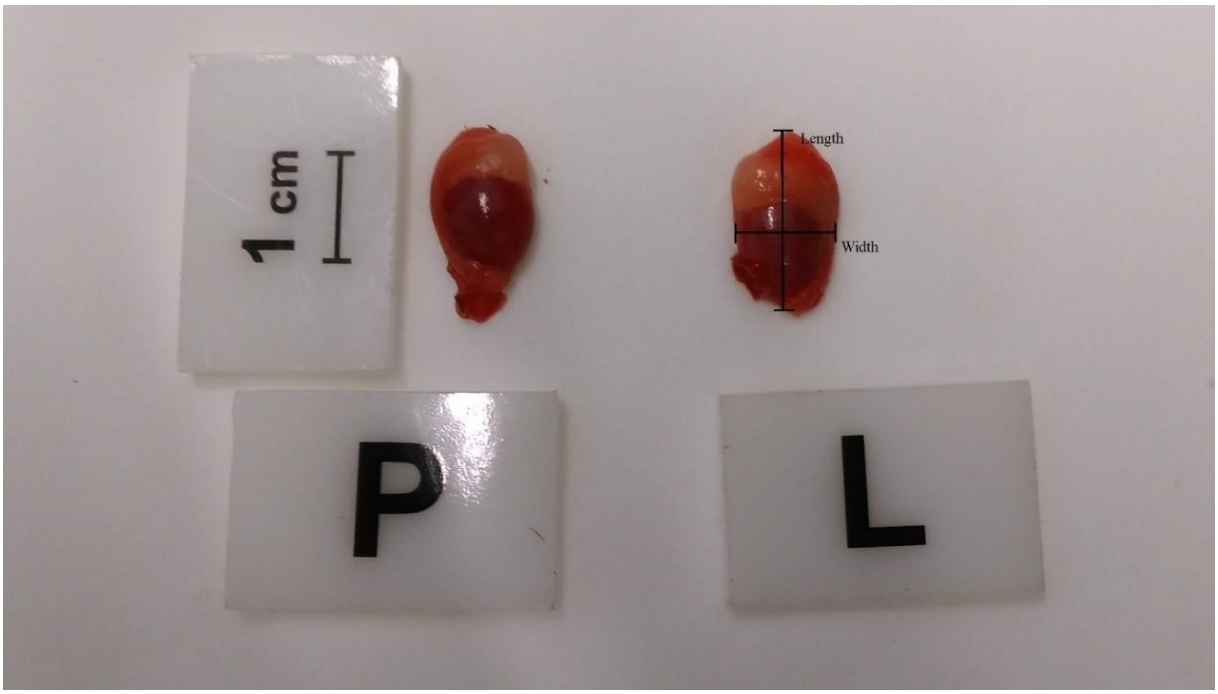

Figure 2: Measurement points for the length and width of the testes. Testes shown in this photo come from the group out of reproductive season (November). "L" stands for the left testis and "P" for the right one.

2.3 Semen collection and assessment

Within 24 h after the removal of the testes, the spermatozoa were collected by epididymal slicing. Each caudae epididymis was separated from the testis and cleaned from any visible vessels and connective tissues, then placed in 1 ml of prewarmed semen extender in a glass Petri dish where they were minced using a scalpel blade. The cut tissues along with the solution were left to incubate at room temperature for 10 minutes. The tissue was then removed, and a suspension of spermatozoa was filtered into an Eppendorf tube. In order to limit potential any bias and human error while assessing the quality of obtained semen, all samples were analysed using a Computer-Assisted Sperm Analysis system (HTM-IVOS,12.3D, Hamilton-Thorne Biosciences, Beverly, MA, USA). CASA-system allowed us to obtain unbiased and objective data on semen quality and provided us with extensive information about sperm motility and viability. The IVOS software settings were set according to Niżański et al. [22] and are summarized in table 1. Four (4) µl aliquots of extended semen were placed in a prewarmed Leja counting chamber (Leja Products B.V., Nieuw-Vennep, Holland) and placed in a warmer at 39°C. For each sample, five randomly selected microscopic fields were scanned and evaluated to ensure proper evaluation of sperm concentration and motility.

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Table 1: Hamilton Thorne analysis setup used for assessing the motility parameters in the study.

Parameters	Values
Frame rate [Hz]	60
Frames acquired	30
Minimum contrast	75
Minimum cell size [pixels]	4
Nonmotile head size [pixels]	4
Nonmotile head intensity	80
Medium VAP cutoff [µm/s]	100
Straightness cutoff [%]	75
Low VAP cutoff [µm/s]	9
Low VSL cutoff [µm/s]	20

2.4 Statistical analysis

The statistical analysis was performed using R Project 4.0.3 [23]. The basic descriptive statistics of considered testis parameters and sperm concentration were determined with *pastecs* package [24]. The compliance of the distribution of analysed traits with the normal distribution was verified using Shapiro – Wilk test. The statistical significance of influence of reproductive season on testis parameters was verified with Wilcoxon test for independent samples with the significance level $\alpha = 0.05$. The statistical significance of differences for right and left testis parameters were verified with Wilcoxon test for dependent samples with the significance level $\alpha = 0.05$.

The relationship between the testis parameters and sperm concentration was investigated using Pearson's correlation with *psych* package [25]. The correlation whose $100\%(1-\alpha/k)$ confidence intervals did not overlap, where considered as statistically significantly different between groups [26].

The principal component analysis (PCA) of considered traits was performed with *ade4* [27] and *factoextra* [28] packages.

3. RESULTS

3.1 Descriptive statistics and correlations

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Both testes were collected from 66 males. Four (4) males from the November group lacked one of their testes, probably due to technological issues encountered during pelting. Live spermatozoa were found in all testes collected during the reproductive season (40 males). However, live spermatozoa were found in only one set of testes (one visible moving spermatozoid) from testes collected out of the breeding season, thus only morphometric measurements of those testes were taken into consideration and analysed statistically. An index was created to present morphometric measurements in a more objective way. The index was created by dividing the length by the width of each testes included in the study. The data acquired was analysed for seasonal differences and correlation between testes size and sperm concentration, when possible. Morphological measurements for the full dataset with the basic descriptive statistics are shown in table 2. The analysis of the full dataset showed that the mean testis length for American mink was 1.69 cm (1.68 and 1.7 cm for right and left testis accordingly). The mean width of the testes was 1.24 cm (1.23 and 1.26 for right and left testis). The variations for those two traits were the lowest from all the measurements taken (19.66% for testes length and around 24.7% for width), the only lower ones being variations for the created indexes (11.33% for right and 13.83 for left testis index). The mean volume of the right testis was 1.13 cm^3, with the minimum and maximum values being 0.39 and 2.05 cm^3, respectively. The values for the left testis volume ranged from 0.4 to 2.26 cm^3, with the mean value being 1.16 cm^3. The weight of the testes proved to be the parameters with the highest variations (all four VC above 60%). The mean values for the testes weight with the epididymis were 2.57 and 2.51 g for right and left testes respectively, with the medians being 3.29 and 3.14 g. The means for the testes weight without the epididymis were around both 1.9 g. The left testes showed a slightly wider range of values (minimum and maximum being 0.27 and 4.16 g) then the right ones (0.28 minimum and 4.07 g maximum). It's interesting to note the difference between the means and medians for the weight of the testes, both with and without the epididymis. The medians for testes weight with epididymis were 3.29 and 3.14 g (right and left testis) compared to 2.57 and 2.51 g means of the same parameters. The medians for the RTnoEPW and LTnoEPW were 2.41 and 2.44 g, with their means being around 1.9 g both.

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Table 2: Basic descriptive statistics of mink testis morphometric parameters for the full dataset

n = number of individuals, SD = standard deviation, VC = variation coefficient (presentedas a percentage)
Medians did not differ statistically significantly depending on the part of the testis (right, left) (Wilcoxon test, p-value > 0.05)

testis parameter	statistics						
	n	minimum	maximum	median	mean	SD	VC [%]
right testis length [cm]	67	1.01	2.30	1.80	1.68	0.33	19.66
left testis length [cm]	69	1.09	2.40	1.80	1.70	0.33	19.66
right testis width [cm]	67	0.70	1.70	1.40	1.23	0.30	24.50
left testis width [cm]	69	0.70	1.89	1.30	1.26	0.31	24.88
right testis volume [cm^3]	67	0.39	2.05	1.32	1.13	0.46	40.52
left testis volume [cm^3]	69	0.40	2.26	1.29	1.16	0.46	39.90
right testis index	67	1.01	1.85	1.36	1.39	0.16	11.33
left testis index	69	0.71	1.94	1.38	1.38	0.19	13.83
right testis and epididymis weight [g]	67	0.36	5.12	3.29	2.57	1.60	62.43
left testis and epididymis weight [g]	69	0.43	5.15	3.14	2.51	1.64	65.54
right testis (without epididymis) weight [g]	67	0.28	4.07	2.41	1.90	1.31	68.83
left testis (without epididymis) weight [g]	69	0.27	4.16	2.44	1.91	1.34	70.24

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A summary of the measurements for the two groups (in and out of reproductive season) with their descriptive statistics are presented in table 3. The mean values of every measured parameter differ statistically significantly between the two groups, with the biggest differences observed within testicular mass (both with and without the epididymis). The mean RTEPW and LTEPW in the reproductive season were both 3.94 g, with the median value around 3.8 g. The mean values for out of the season group were around 0.71 g, and the medians 0.65 g. The weights of the testes without the epididymis for males out of the season were both around 0.42 g, with the medians equalling 0.36 g both. For the group in the season the mean weight of the testes was around 3 g, and the median value was around 2.96 g.

Table 3: Basic descriptive statistics of testis morphometric parameters of minks in reproductive season and out of reproductive season

n = number of individuals, SD = standard deviation, VC = variation coefficient (presentedas a percentage)
Medians differing statistically significant between groups were marked by different letters (Wilcoxon test, p-value < 0.05)

testis parameter	statistics						
	n	minimum	maximum	median	mean	SD	VC [%]
OUT OF REPRODUCTIVE SEASON							
right testis length [cm]	27	1.01	1.78	1.35[a]	1.33	0.18	13.22
left testis length [cm]	29	1.09	1.70	1.39[a]	1.37	0.17	12.49
right testis width [cm]	27	0.70	1.31	0.90[a]	0.90	0.13	14.19
left testis width [cm]	29	0.70	1.89	0.91[a]	0.98	0.27	27.60
right testis volume [cm^3]	27	0.39	1.22	0.62[a]	0.63	0.17	26.31
left testis volume [cm^3]	29	0.40	1.56	0.70[a]	0.71	0.25	35.24
right testis index	27	1.01	1.85	1.48[a]	1.49	0.18	12.09
left testis index	29	0.71	1.94	1.46[a]	1.45	0.26	17.85
right testis and epididymis weight [g]	27	0.36	1.58	0.64[a]	0.70	0.25	36.34
left testis and epididymis weight	29	0.43	1.73	0.65[a]	0.72	0.27	37.51

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[g]							
right testis (without epididymis) weight [g]	27	0.28	1.15	0.36[a]	0.41	0.18	43.43
left testis (without epididymis) weight [g]	29	0.27	1.28	0.36[a]	0.43	0.21	47.73
REPRODUCTIVE SEASON							
right testis length [cm]	40	1.60	2.30	1.90[b]	1.91	0.15	7.94
left testis length [cm]	40	1.50	2.40	1.90[b]	1.94	0.18	9.55
right testis width [cm]	40	1.20	1.70	1.45[b]	1.46	0.12	7.91
left testis width [cm]	40	1.10	1.80	1.50[b]	1.46	0.14	9.38
right testis volume [cm^3]	40	1.07	2.05	1.43[b]	1.46	0.22	14.95
left testis volume [cm^3]	40	0.86	2.26	1.48[b]	1.49	0.26	17.54
right testis index	40	1.13	1.54	1.31[b]	1.31	0.08	6.24
left testis index	40	1.13	1.62	1.33[b]	1.33	0.10	7.17
right testis and epididymis weight [g]	40	2.62	5.12	3.94[b]	3.82	0.51	13.29
left testis and epididymis weight [g]	40	1.14	5.15	3.94[b]	3.80	0.76	19.89
right testis (without epididymis) weight [g]	40	1.94	4.07	2.99[b]	2.94	0.47	15.97
left testis (without epididymis) weight [g]	40	1.29	4.16	3.04[b]	2.98	0.55	18.42
sperm concentration [$x10^6$/ml]	40	8.70	215.50	86.55	93.00	42.14	45.32

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Pearson's correlation test for the full dataset (70 males) showed that all measurements taken were positively and statistically significantly correlated with each other (table 4). The only two exceptions were the created indexes which showed negative correlation with every other variable, excluding themselves. The index created for the left testis showed very few significant correlations, namely with LTW (-0.67), LTV (-0.44) and RTI (0.52). RTI showed significant correlations with almost every measurement, excluding only RTL, but the coefficients were lower than -0.7. The vast majority of coefficients are positive and higher than 0.8 which indicates that all the testicular measurements are highly correlated with each other.

Table 4: Pearson's correlation matrix of mink testis morphometric parameters for the full dataset

Coefficientsmarked with *were statistically significant (p-value < 0.05)

	RTL	LTL	RTW	LTW	RTV	LTV	RTI	LTI	RTEPW	LTEPW	RTNOEPW	LTNOEPW
RTL	1	0.89*	0.93*	0.75*	0.98*	0.86*	-0.35	-0.24	0.94*	0.89*	0.93*	0.91*
LTL		1	0.88*	0.82*	0.90*	0.95*	-0.43*	-0.15	0.89*	0.87*	0.89*	0.91*
RTW			1	0.84*	0.98*	0.90*	-0.66*	-0.40	0.95*	0.92*	0.95*	0.94*
LTW				1	0.81*	0.95*	-0.62*	-0.67*	0.80*	0.79*	0.80*	0.82*
RTV					1	0.90*	-0.52*	-0.33	0.96*	0.92*	0.96*	0.95*
LTV						1	-0.55*	-0.44*	0.89*	0.87*	0.89*	0.91*
RTI							1	0.52*	-0.54*	-0.52*	-0.54*	-0.54*
LTI								1	-0.32	-0.32	-0.33	-0.33
RTEPW									1	0.95*	0.99*	0.97*
LTEPW										1	0.95*	0.97*
RTNOEPW											1	0.97*
LTNOEPW												1

RTL = right testis length [cm], LTL = left testis length [cm], RTW = right testis width [cm], LTW = left testis width [cm], RTV = right testis volume [cm^3], LTV = left testis volume [cm^3], RTI = right testis index, LTI = left testis index, RTEPW = right testis and epididymis weight [g], LTEPW = leftt testis and epididymis weight [g], RTnoEPW = right testis without epididymis weight [g], LTnoEPW = left testis without epididymis weight [g]

The correlation matrix of testes morphometry and sperm concentration for mink in and out of the reproductive season is shown in table 5. The group out of the season didn't show many statistically significant correlations. RTV is highly correlated with RTL and RTW; and LTV shows significant correlations with LTL and LTW, although the correlation between testis volume and its length is stronger for right testis (0.64 for the left and 0.88 for the right one). The only significant correlation for the left index is with the testis width. It's worth noting that for the full dataset, the strongest correlation for LTI was also with LTW, and they were both negative (-0.67 and -0.79 for full dataset and out of season males, respectively). The LTI-LTW is also the only statistically significant negative correlation for the out of season group. Every weight measurement variable (both with and without the epididymis) is significantly correlated with the dimensions of right testis (RTL, RTW, RTV). Interestingly enough, those correlations occur for the weight of both right and left testes and have similar coefficient values (>0.6).

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Table 5: Pearson's correlation matrix of testis morphometric parameters and sperm concentration for minks in reproductive season (lower triangle) and out of reproductive season (upper triangle)

Coefficientsmarked with *were statistically significant (p-value < 0.05)
Correspondingcorrelation coefficients marked with different letters were statistically significantly different

	RTL	LTL	RTW	LTW	RTV	LTV	RTI	LTI	RTEPW	LTEPW	RTNOEPW	LTNOEPW	SC
RTL	1	0.49	0.61	0.10	0.88*	0.25	0.41	0.06	0.72*	0.73*	0.63*	0.65*	-
LTL	0.70*	1	0.42	0.38	0.49	0.64*a	0.05	0.21	0.41	0.50	0.40	0.36	-
RTW	0.72*	0.55*	1	0.52	0.91*	0.57	-0.47	-0.37	0.88*	0.78*	0.84*	0.81*	-
LTW	0.60*	0.72*	0.69*	1	0.33	0.95*	-0.45	-0.79*	0.44	0.40	0.44	0.37	-
RTV	0.93*	0.68*	0.92*	0.70*	1	0.44	-0.06	-0.18	0.91*	0.87*a	0.86*	0.86*	-
LTV	0.72*	0.93*b	0.68*	0.92*	0.76*	1	-0.35	-0.58	0.51	0.50	0.51	0.44a	-
RTI	0.33	0.16	-0.42	-0.14	-0.04	0.01	1	0.45	-0.21	-0.08	-0.24	-0.17	-
LTI	0.13	0.39	-0.18	-0.36	-0.02	0.02	0.39	1	-0.33	-0.28	-0.31	-0.32	-
RTEPW	0.80*	0.64*	0.61*	0.54*	0.76*	0.64*	0.21	0.15	1	0.90*a	0.90*	0.89*	-
LTEPW	0.44	0.47	0.42	0.49	0.47b	0.50	0.02	-0.01	0.56*b	1	0.82*	0.88*	-
RTNOEPW	0.77*	0.66*	0.64*	0.63*	0.76*	0.70*	0.14	0.06	0.92*	0.52	1	0.96*a	-
LTNOEPW	0.62*	0.76*	0.62*	0.80*	0.67*	0.83*b	-0.03	-0.04	0.71*	0.74*	0.71*b	1	-
SC	0.00	0.23	-0.02	0.16	-0.02	0.18	0.03	0.09	0.28	0.24	0.21	0.26	1

RTL = right testis length [cm], LTL = left testis length [cm], RTW = right testis width [cm], LTW = left testis width [cm], RTV = right testis volume [cm^3], LTV = left testis volume [cm^3], RTI = right testis index, LTI = left testis index, RTEPW = right testis and epididymis weight [g], LTEPW = leftt testis and epididymis weight [g], RTnoEPW = right testis without epididymis weight [g], LTnoEPW = left testis without epididymis weight [g], SC = sperm concentration [$x10^6$/ml]

The group in the reproductive season showed much more statistically significant correlations, with most of them having values above 0.6 (LTL-RTW; LTW-RTEPW; RTEPW-LTEPW being the only three exceptions). No statistically significant negative correlations were observed. The basic measurements (length, width and volume) were correlated with every variable, excluding RTI, LTI and LTEPW, for both right and left testis. The indexes for both left and right testis show no statistically significant correlations for the males in the reproductive season. Interestingly, the weight of the left testis with epididymis showed only two significant correlations, with RTEPW (value 0.56) and LTnoEPW (0.74), but the LTnoEPW showed significant correlations with almost every other variable (excluding the indexes). The sperm concentration showed no statistically significant correlations with any of the variables.

The correlation matrix showed five statistically significant differences between the corresponding correlation coefficients. The LTV-LTL and LTV-LTnoEPW are significantly stronger for the males in the reproductive season; the LTV-LTnoEPW correlation for the out of season group was statistically insignificant with coefficient value 0.44. The correlations for RTV-LTEPW, RTEPW-LTEPW and RTnoEPW-LTnoEPW were stronger for the group out of reproductive season, with the RTV-LTEPW correlation being insignificant for the group in the reproductive season (value 0.47)

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3.2 Principal Components Analyses

Table 6 presents the eigenvalues, percentage of cumulative explained variance and loadings of first four principal components. According to the Kaiser rule, only PCs whose eigenvalue was equal or higher than 1 were used for further analyses [29]. Two of them fulfilled this criterion; PC1 and PC2, and combined they explain more than 90% of the total variability in the dataset. The loadings shown in table 6 indicate that PC1 is highly negatively correlated with all the variables, except RTI and LTI (all values close to or above -0.9). The second principal component is correlated with RTI (-0.56) and LTI (-0.82).

Table 6: Eigenvalues, percent of cumulative explained variance and loadings of first four principal components of PCA of mink testis morphometric parameters

	PC1	PC2	PC3	PC4
eigenvalue	9.63	1.29	0.52	0.30
cumulativevariance percentage	80.26	10.78	4.30	2.53
loadings				
RTL	-0.93	-0.26	0.14	0.11
LTL	-0.92	-0.25	0.01	-0.28
RTW	-0.98	0.00	-0.10	0.07
LTW	-0.89	0.32	0.20	-0.23
RTV	-0.97	-0.12	0.00	0.08
LTV	-0.96	0.04	0.12	-0.26
RTI	0.60	-0.56	0.57	0.06
LTI	0.43	-0.82	-0.33	-0.16
RTEPW	-0.97	-0.12	-0.04	0.13
LTEPW	-0.95	-0.11	-0.03	0.13
RTnoEPW	-0.97	-0.12	-0.04	0.11
LTnoEPW	-0.98	-0.10	-0.03	0.06

RTL = right testis length [cm], LTL = left testis length [cm], RTW = right testis width [cm], LTW = left testis width [cm], RTV = right testis volume [cm^3], LTV = left testis volume [cm^3], RTI = right testis index, LTI = left testis index, RTEPW = right testis and epididymis weight [g], LTEPW = leftt testis and epididymis weight [g], RTnoEPW = right testis without epididymis weight [g], LTnoEPW = left testis without epididymis weight [g]

The effect of original variables on the principal components and loading vectors are presented on figure 3. The placement of the vectors on the graph shows high positive correlations between all of the measurements taken (length, width, mass, volume). The variable with the smallest correlation to the rest of the measurements is LTW. The indexes for both testes show significant negative correlation with LTW, LTV and RTW. RTI and LTI don't show any other significant correlations. The PCA scatterplot (figure 4) shows a stark difference between the two groups

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taking part in the study. The variables from both groups were negatively correlated with PC1, with the out of season group (right side of the plot) showing smaller values than the one in the reproductive season. The scatter plot shows strong separation between the two groups in terms of their correlation to PC1. They showed much less variation in terms of their correlation to PC2, with the out of season group being the one more scattered on the Y axis. The group out of the reproductive season (green circles on the plot) is more dispersed and has more outliers. The group in the season (blue triangles on the plot) is more uniform and forms a closer cluster on the plot. The variability in this group is more visible for the X axis but it's still relatively small. It can be clearly seen that the males in the reproductive season had greater values for all variables concerning testicular morphometry (length, width, volume and mass of the testes).

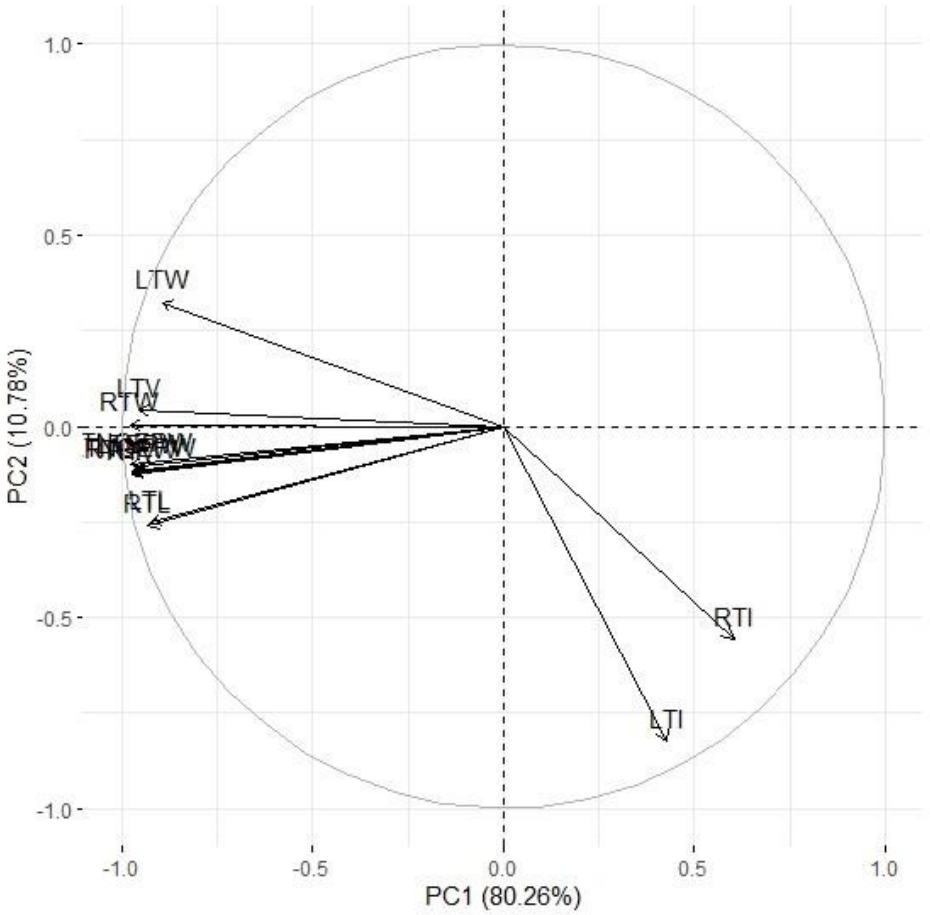

Figure 3: Bi-plot from PCA of mink testis morphometric parameters

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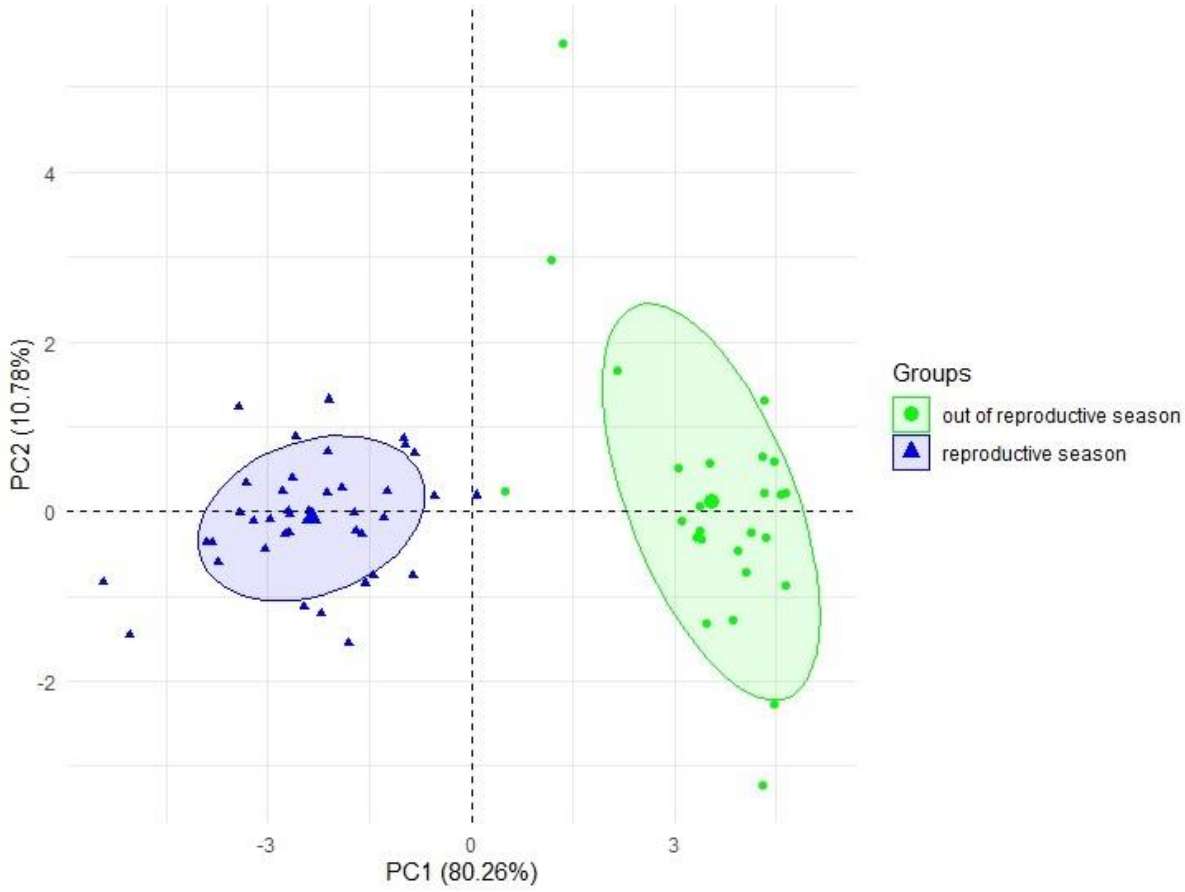

Figure 4: Scatter plot of PCA of testis morphometric parameters for minks in reproductive season and out of reproductive season

4. DISCUSSION

Photoperiodic cycle regulates lives of many mammals, both carnivorous and herbivorous. It governs over the availability of food, the density of their fur, and their reproductive biology. Changes in the daylight exposition trigger hormonal response which in turn can change some aspects of animals' morphology, such as density and colour of the fur, body mass and activity of reproductive organs. Seasonal cycles of testicular activity that include annual transitions between full productivity and total arrest occur in many mammalian species, including rats, bats, common voles and many mustelids. Even after years of research, this cycle still is an interesting phenomenon of reproductive biology, studied by many researchers [30; 31; 32; 33; 34; 35].

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American mink is of a one of the few farm animals with only one reproductive season throughout the year, triggered by decreasing day length [36]. This study further proved that male mink produce little to no spermatozoa during non-breeding season, and that some alterations in testicular morphometry occur during the annual cycle, which confirms it's belonging to the seasonal breeders. Several studies confirm that the mass of mink testes regress after the breeding season in March and remain small until the start of spermatogenesis in November. Both Blottner and Sundqvist report that mink testis in March weight ~ 3 g [30; 37]. The weight of paired testes in late March reported by Onstad averaged 11 g [38]. Comparisons of the data for the measurements taken out of the reproductive season are hampered because the few references available were based on relatively small sample sizes. However, paired testes weight in November were reported to be on average 1.34 g (sample size: 6 males) [38] similar to the results of this study (0.699 g and 0.723 g for right and left testis, respectively). An extensive study on male reproductive system was carried out on wild mink by Persson et al. (2011) [39]. They reported that the weight of paired testes in autumn and winter were, respectively, 0.7 ± 0.6 and 1.8 ± --1.3 g, which is much less than the weight of testes from the November sample in this study (table 3). This example only confirms that farmed American mink and their wild counterparts have different masses and body dimensions due to years of selective breeding.

A study on the reproductive patterns of wild European polecats was conducted by Kristiansen et al., including testes weights and occurrence of spermatozoa throughout the year [8]. The testes weights show a similar pattern to the results of this study, with peak weight during the reproductive season (April/May for polecats) and a steady decline till late autumn (November). The study included 147 males of different ages, ranging from few months to 5 years old, and all of the adult males had active sperm cells through February-August, with a singular exception. No spermatozoa were found through September-January (out of the reproductive season). The paper also reported a strong correlation between testicular weight and spermatozoa density (r_s= 0.79, *n* = 147, P<0.0001). This study showed no significant correlation between sperm concentration and of the testes measurements taken (table 5). The correlation values with the weight of the testes were never above 0.3 and were the highest coefficients for SC in this study but they were not statistically significant. The stark difference may originate from the fact that, while similar in their anatomy and biology, the mink and polecats are different species, but further study on the correlation between testicular morphometry and sperm concentration are recommended to explore this case.

Only one study concerning the testes dimensions in mink was found and even then, the testes were measured by palpation through the scrotum and assigned in to one of four categories [18]. Of the males used in that study 12 out of 31 males were assigned to 3rd category (>1.7 cm in diameter), and the second largest group (11 males) was assigned to the 2nd category (testes 1.2–

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1.7 cm in diameter). Comparing these results with the results obtained in this study is relatively difficult because measuring testes via palpation can be very subjective and the results obtained included the skin thickness which may have biased the results. Dimensions which were considered the diameter of the testes were unclear in that study because an explanation of the measuring method used was not provided. The measurements were taken just prior to the beginning of, or during, the reproductive season so the time of year the data was collected was similar to this study. Depending on whether we consider the "diameter" to be the length or width of the testes, the results of both studies are quite similar, given that the average length and width of testicles in this study were, respectively, ~1.9 and ~1.4 cm for the group measured during the reproductive season (table 2). Another study by Neal et al. concerning male ferrets reported that the smallest testis length (1±0.1 cm) was found in October. The testes showed a gradual increase in length until a peak was reached in April (2.1±0.1 cm). Those results were obtained by measuring only the right testis of an animal through scrotum, using callipers [16]. Although results obtained by Neal et al. include the thickness of the scrotum skin, their measurements are similar to the ones presented. In this study, the morphometric differences between the testes collected during and out of the reproductive season may be enhanced by the fact that the males in November were younger than the ones slaughtered in March, by at least 4 months, even though all the animals included in this study had finished their somatic growth.

5. CONCLUSIONS

The study provides new data concerning testicular morphometry in American mink. Belonging of mink into the seasonal breeders has been further confirmed, indicated by the changes in testes dimensions and weight, as well as cease of sperm production in late autumn/winter (out of the reproductive season). No significant correlation was shown between sperm concentration and any of the measurements taken which is contrary to previous studies on other mustelids. This study might be a base for further research on male reproductive biology in mustelids and can be a first step in establishing semen quality norms for American mink in the future. The results of the research suggests that attempts to obtain semen samples from male American mink outside of the reproductive season is futile, therefore establishing a suitable window of time for further projects concerning sperm properties and quality in this species. While the results presented provide more insight into male reproductive biology of mustelids it might be beneficial to consider a more extensive study on the anatomy of the testes throughout the year, which may provide a broader view on the causes of the phenomenon of seasonal breeding.

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