Theriogenology 126 (2019) 177–186

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Testicular morphology and spermatogenesis in harbour porpoises (*Phocoena phocoena*)

T. Kesselring [a], S. Viquerat [a], L.L. IJsseldijk [b], M. Langeheine [c], P. Wohlsein [d], A. Gröne [b], M. Bergmann [e], U. Siebert [a], R. Brehm [c, *]

[a] Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hanover Foundation, Werftstr. 6, 25761, Büsum, Germany
[b] Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584, CL, Utrecht, the Netherlands
[c] Institute for Anatomy, University of Veterinary Medicine Hanover Foundation, Bischofsholer Damm 15, 30173, Hanover, Germany
[d] Department of Pathology, University of Veterinary Medicine Hanover, Foundation, Bünteweg 17, 30559, Hanover, Germany
[e] Institute of Veterinary Anatomy, Histology and Embryology, University of Giessen, Germany

ARTICLE INFO

Article history:
Received 1 May 2018
Received in revised form
22 November 2018
Accepted 27 November 2018
Available online 28 November 2018

Keywords:
Cetacean
Reproduction
Testes
Seasonality
Fertility

ABSTRACT

Knowledge about reproductive parameters in male harbour porpoises such as testicular histology and germ cell maturation as well as seasonal changes in spermatogenesis is scarce. Thus, the aim of the present study was to report changes in the histological appearance of the testicular morphology of neonatal and juvenile harbour porpoises during maturation, to identify stages of spermatogenesis in adult males and to detect seasonal modifications. The identification of these stages can be used to assess the developmental profile of gene expression during spermatogenesis and to identify defects in spermatogenesis arising in pathological conditions. Testes of adult male harbour porpoises from the North and Baltic Sea that became stranded or by-caught in the years 1998–2016 were histologically examined using Haematoxylin and Eosin – staining. The Periodic Acid Schiff (PAS) staining was used for spermatogenic staging and the evaluation of the development of the acrosomic cap. For the identification of changes in testes morphology and morphometry during the course of the year, histological characteristics like germ cell associations and diameter of the convoluted seminiferous tubules were noted for each month. The analysis showed that in adult males more than one stage of spermatogenesis could be found per cross section of the convoluted seminiferous tubules similar to findings in men and some ape species. This rare phenomenon is called multi-stage-arrangement. In sexually active males from the peak breeding season (June and July) eight stages of spermatogenesis were identified and all stages occurred simultaneously, while during the low breeding season (August to May) only residual spermatogenesis or constituent germ cell populations were found. Missing germ cell generations were recorded in specimens from July to September. Our investigations provide a detailed staging of spermatogenesis and give new insight into the reproductive biology of male harbour porpoises. With these new basic parameters, indicators for endocrine disruptors can be developed in the future, aiming to detect how environmental factors could affect male fertility in wildlife.

1. Introduction

The harbour porpoise (*Phocoena phocoena*) is the smallest cetacean species native to German and Dutch waters and exhibits a seasonal pattern in its annual reproductive cycle [1–3]. The conservation status of this species especially in the Baltic area has been of concern for many years, since anthropogenic impacts such as climate change, noise emissions during fishery activities and pile driving, bycatch and pollutants within the aquatic food chain have increased substantially in the North and Baltic Sea [4–8].

As top predators harbour porpoises are sensitive to contaminants [9–12]. Some contaminants might be capable of passing the blood-testes-barrier [12], the placental-barrier and milk – blood - barrier and may harm the development of the living stocks in a yet unpredictable way [7,13–19]. There have been reports on reproductive failure including foetal death, abortion and dystocia in harbour porpoises from Scottish waters that may be connected to the concentration of high toxicants in the blubber of these animals [7], but

* Corresponding author.
E-mail address: Ralph.brehm@tiho-hannover.de (R. Brehm).

https://doi.org/10.1016/j.theriogenology.2018.11.031
0093-691X/

there is no knowledge on how these contaminants influence the reproductive organs of the population. In order to detect changes in reproductive organs due to human impacts, basic knowledge on a cellular level is considered to be a first step towards a better understanding of physiological processes in the reproductive system [20]. Therefore, information on testicular development and histology of male harbour porpoises on a cellular level is urgently required.

It is known from other mammalian species, that spermatogenesis is a very sensitive process and vulnerable to environmental disturbances. It has been shown in humans and rats, that cytotoxic effects and hormonal changes can be a result of toxicants that have entered an individual's physiological endocrine system [21,22]. Additionally, knowledge about the general process of spermatogenesis and seasonal effects in harbour porpoises is restricted to a few studies [23–26] and detailed staging of spermatogenesis based on germ cell morphology and their specific associations within the seminiferous epithelium have not been reported. It has been noted, that various cell associations (stages) can be observed in one tubular cross-section [26], but no specific number of stages has been given. Staging is a common approach to assess the cycle of the seminiferous epithelium [27]. Based on this procedure, alterations in the testicular development due to environmental changes or disturbances can be recognised. These changes can occur seasonally and thus be considered as a physiologic phenomenon or have a pathological origin. With a detailed staging of spermatogenesis in harbour porpoises, the detection of potential influences of endocrine disruptors on male reproductive biology can be possible [28]. It is known from other species, that the influence of certain noxae can cause alterations in the development of germ cells and might be capable of paralyzing the germ cell differentiation. This failure in the spermatogenetic process leads to the presence of missing generations of germ cells within one cross-section of a convoluted seminiferous tubule [29] and might lead to reduced fertility of the individual.

To define a baseline for investigations on testicular structures as an indicator for an individual's reproductive status, the histological arrangement of the seminiferous epithelium of juvenile and adult individuals has to be considered. It has been observed in harbour porpoises from other waters that more than one spermatogenetic stage can be found in one cross section of a seminiferous tubule [24–26], but no fundamental staging has been performed yet.

Furthermore, macroscopic and microscopic morphometric parameters of the testes and the diameter of seminiferous tubules undergo a seasonal cycle [24,25,30–34].

Seasonal breeding is efficient as it conserves energy and benefits natural selection [35]. Male harbour porpoises from different regions show full sexual activity from June to July (North Sea and Baltic Sea, Gulf of Maine, Bay of Fundy) [25,33,34]. These periods coincide with the time of ovulation in female harbour porpoises [33,35–39]. Thereby, the parturition occurs under the best climatic and nutritional circumstances and offers good environmental conditions for raising the neonates.

The testes of harbour porpoises appear to be 'inactive' from October – April [24,25,30]. Investigations on this testicular cycle including testes mass and diameter of convoluted seminiferous tubules reveal a maximum in the peak breeding season (Bay of Fundy, North and Baltic Sea: July) and might be slightly earlier or later in our study areas depending on the ovulation time of female individuals reflecting environmental differences. Furthermore, the examination of testicular tissue might have the potential to add information on the status of sexual maturity of an individual to the tooth age determination.

The aim of this study was to generate fundamental data on testicular morphology, time of initiation of spermatogenesis and seasonal changes with histological methods as a baseline for further studies that might focus on the effects of environmental influences on male reproduction in harbour porpoises.

2. Materials and methods

2.1. Sample collection

A total of 127 testes of male harbour porpoises stranded and bycaught along German and Dutch coasts between 1998 and 2016 were sampled during necropsies. The study was supervised in Hanover by Prof. Dr. Ralph Brehm (Department for Anatomy, University of Veterinary Medicine Hanover, Germany) and in Büsum by Prof. Dr. Ursula Siebert (Institute for Terrestrial and Aquatic Wildlife Research (ITAW), Büsum, Germany). Specimens were collected within the German stranding network, which conducts work (collect and hold carcasses and samples from European protected species) on German strandings following appropriate licenses from the relevant authorities (Ministry of Energy, Agriculture, the Environment, Nature and Digitalization, Ministry of Agriculture, Environment and Rural areas). Most of the animals were found stranded along the coast of Schleswig Holstein of the North Sea and western Baltic Sea, only a small number of animals has been identified as bycatch. Most specimens were sampled during necropsies in Büsum, Germany and Utrecht, The Netherlands. The necropsies in The Netherlands are commissioned by the Dutch Ministry of Agriculture, Nature and Food Quality. An additional approval for animal experiments by German or Dutch authorities was not necessary. Based on the initial classification from the necropsy protocol (Supplemental table 1 [40]), we grouped specimens into two maturity classes: juveniles and neonates as non-adult ($n = 93$) and adult specimens ($n = 34$). Depending on organisational and logistical conditions, the porpoises were stored at −20 °C until necropsy or were dissected immediately after death. Necropsies were performed according to a standardised necropsy protocol for harbour porpoises [40]. Tissue preservation status of samples was assessed using a scale from 1 (very fresh) to 4 (decomposed). Only samples originating from carcasses with a decomposition status of 3 or better were included into the analysis. Testes morphometry ($n = 127$) was measured and their weight (without epididymis) was noted. All testes were then fixed in 4% buffered formalin or Bouin's solution and prepared for histological examination by two sections of 1 cm in diameter - one section of the testes surface and one from the centre. Only one section covering the centre and the periphery was taken from individuals with smaller testes (neonates and juveniles).

2.2. Histological preparation

Tissue samples were dehydrated and embedded in paraffin. Using a Leitz 1512 microtome (Techno Med GmbH Bielefeld), three 3–5 μm slices of each sample were produced. The slices were mounted on glass layers and stained with Haematoxylin and Eosin (HE) ($n = 127$).

2.3. Age determination

Six teeth from 86 animals of these specimens (34 adults, 52 non-adults) were removed from the lower jaw and preserved in 4% buffered formalin. Tooth preparation was performed using a standard protocol [31].

2.4. Histological examination

For the qualitative assessment of the testes, a staging of the

seminiferous epithelium of adult harbour porpoises from the peak breeding season was performed with a microscope (Olympus BH2, Olympus optical Co., Ltd). Based on acrosomal development after Periodic Acid Schiff (PAS) staining [41] (from 3 specimens), the staging was analysed according to [42]. For this, specimens that showed no tissue alterations due to freezing or decomposition were chosen (n = 3).

In order to detect seasonal changes during the course of the year, at least one individual of each maturity class was examined per month. At least 10 tubular cross sections per histological slice and specimen were selected for the investigation. For the assessment of the reproductive status of adult males only specimens with a sufficient tissue preservation status and no tissue alteration due to freezing or decomposition were chosen (n = 25). Individuals that displayed all stages of spermatogenesis were classified as 'active'. If only some stages of spermatogenesis or single germ cell types were present within the tubules, the individual was classified as 'inactive'. Within this 'inactive' group, specimens with abundant germ cells that indicate the beginning of the breeding season were attributed with 'increasing activity', while specimens that show residual germ cells that remained from former spermatogenic waves of the breeding season were attributed with 'decreasing activity'. Based on the histological examinations, the reproductive status of the individual was then determined.

For morphometric analysis of the testes, the diameters of 20 seminiferous tubules per individual were measured in transverse section using a Zeiss Axioskop (Carl Zeiss Microscopy GmbH, Jena, Germany) with an Olympus DP 70 camera and the Olympus DP Soft software (Olympus Deutschland GmbH, Hamburg, Germany). The tubules were randomly selected out of those with a circular profile to minimise measuring errors. In samples of overall poor quality, the best available tubules were selected with a minimum of 10 tubules. The shortest distances between the peritubular membranes were measured and the diameter in each individual sample was averaged.

2.5. Modelling the diameter of the convoluted seminiferous tubules over the Julian day of the year

In a first step, the Gonadosomatic Index (GSI) [43] as the percentile ratio of testes weight versus body weight of each specimen was calculated. Using the age determination from growth layer group analysis (GLG) and the post-mortem examinations, the dataset was classified into two maturity classes comprising adults (specimen older than or equal to 4 years of age) and non-adults (specimen younger than 4 years of age). As each of the 127 specimen includes an assessment of age class by the experts present during the dissection (neonate, juvenile and adult), we used this data to assess the validity of our post necropsy age classification. Only carcasses with a decomposition status of 1–3 were used in the analysis to minimise bias from the unknown time difference between the time of death and finding the carcass (for lack of better measure, we therefore assumed the date of the stranding to be near equivalent to date the animal died).

Using a set of 68 samples, we modelled the (log-transformed) diameter of the convoluted seminiferous tubules (assessed according to 2.4.) using a generalized additive model. The included covariates were body length (as measured from rostrum to peduncle) as proxy for developmental age, using a thin plate smoother restricted to 5 knots available to smoothing. We tested for an effect of the Julian day of the stranding event/report date (with the 1st of January defined as the 1st day of the year, i.e. the 31st of December being the 366th Julian day of the year) for each specimen as a cyclic covariate smooth with 4 knots as a proxy for seasonality. We tested each covariate individually and as interaction terms. We also tested for group effects on smoothing parameters using the maturity class as a 'by' condition. A regular smoothing function was used for single variables and a tensor smooth was used for interaction terms. Models selection was performed using generalized cross validation (gcv) scores and deviance explained measures. All analysis was conducted in R [44] using mgcv [45–47].

3. Results

3.1. Macroscopic findings of the testes during the course of the year

Our classification based on GLG was matched by both the average diameter of seminiferous tubules as well as the mean testis mass in relation to the overall body weight (Gonadosomatic Index, GSI). We found a clear distinction between specimens classified as adults and non-adult specimens. Adult specimen (GLG $\geq$ 4 years) did not include any juvenile or neonate specimen, whereas non-adult (neonate and juvenile) specimen (GLG < 4 years) did not include any specimen previously assessed as adults (Table 1) during the necropsies. The average age determined from GLG was 5.73 (±1.11) years in all (n = 86), 9.56 (±1.14) years in adults (n = 34) and 0.5 (±1.16) years in non-adult specimen (n = 52). The average GSI was 0.951 (±0.179) % in adult specimens, 0.048 (±0.004) % in non-adult specimens and 0.282 (±0.064) % over all age classes (Table 1). The mean testis mass in relation to the overall body weight (GSI) reached a peak in July, while the lowest values were found in September to February. Non-adult individuals showed no significant variation in the course of the year (Table 1).

3.2. Testicular maturation and staging of the seminiferous epithelium

Histological examination indicated that in seminiferous cords of neonatal harbour porpoises only Sertoli cells and gonocytes, which mostly have not reached the basement membrane, can be found. The seminiferous cords displayed no lumen. There was no evidence of mitosis and no other cell types of spermatogenesis were present (Fig. 1A). In juvenile harbour porpoises aged older than one year, a lumen in the seminiferous tubules was identified and spermatogonia were abutting the basement membrane (exemplified in Fig. 1B). In animals older than three years, first signs of germ cell activity like cell divisions were found (exemplified in Fig. 1C and D). In individuals older than four years, the seminiferous epithelium showed a seasonal cycle (Fig. 2, seasonal cycle shown in Fig. 3).

Fully active males that showed all stages of spermatogenesis were found only in the months June and July. During this time, one cross-section contained several stages of spermatogenesis at once (Fig. 4). One specimen from mid of June showed an increasing activity, but no elongated spermatids in the lumen of the convoluted seminiferous tubules. In individuals that have been found dead between September and April, only some stages or residual germ cells were identified (Figs. 2C and 3). In individuals that were classified as inactive adults (Figs. 2A and 3), either only the epithelial layer with spermatogonia and Sertoli cells was found, or in the case of one specimen, the epithelial layer and some residual germ cells were found. In individuals that were listed with an increasing activity, abundant germ cell types were observed, but no elongated spermatids or stages of spermatogenesis were present (Figs. 2B and 3). Individuals listed with 'decreasing activity' showed some residual germ cells from former spermatogenic waves like spermatids in the lumen of the convoluted seminiferous tubules (Figs. 2C and 3).

In specimens from the breeding season, different germ cell associations within one cross section of a convoluted seminiferous

Table 1
Summary of average age, diameter of convoluted seminiferous tubules and GSI per maturity class. Subset: identifies the maturity class as assessed by experts during the dissection (all: all specimen regardless of maturity class; adults; non-adults); Ø age: mean age in respective subset using GLG (±standard error); Ø convoluted seminiferous tubules: diameter of convoluted seminiferous tubules (±standard error) [μm]; Ø GSI: gonadosomatic index (±standard error) [%] (sensu Barber & Blake 2006) [43].

Subset	Ø age (n = 86)	Ø convoluted seminiferous tubules (n = 68)	Ø GSI (n = 127)
All	5.73 (±1.11) years	73.9 (±5.66) μm	0.282 (±0.064) %
Adults	9.56 (±1.14) years	125.27 (±10.17) μm	0.951 (±0.179) %
non-adults	0.5 (±0.16) years	43.08 (±1.67) μm	0.048 (±0.004) %

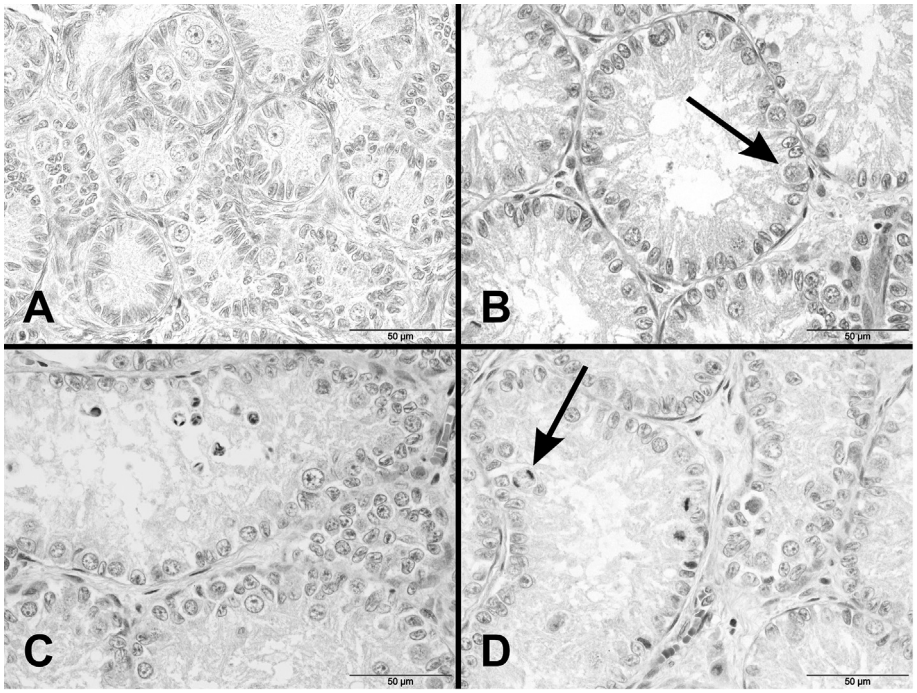

Fig. 1. Cross section through the seminiferous epithelium of a neonate (A) and juvenile (B, C & D) harbour porpoise. The arrow in (B) signals a spermatogonium and the arrow in (D) indicates a mitotic germ cell. The juvenile individual was found in April, length: 118 cm. Staining: H&E, primary magnification: X 40.

tubule were detected with a maximum of eight stages during June and July (Fig. 3). The PAS staining of three representative specimens that showed no tissue alterations due to freezing or decomposition confirmed eight different and recurrent germ cell associations during the peak breeding season as follows (Fig. 5, based on classification shown in Fig. 6): Stage I was featured by the presence of two generations of spermatids, such as round and elongated spermatids. Elongated spermatid bundles were more packed and some were located deep within the epithelium. Additionally, young pachytene spermatocytes were located between round spermatids and spermatids with an acrosomal vesicle in which only occasional proacrosomal granules were identified. At the end of this stage, the small proacrosomal vesicles are located towards the basal lamina. Type A spermatogonia were observed in the HE-staining. The second stage (stage II) of spermatogenesis showed early round spermatids containing single acrosome granule in contact with the nucleus. The elongated spermatids were lined along the tubular lumen prior to spermiation and Pachytene spermatocytes, intermediate- and Type A spermatogonia were observed. Stage III of the cycle showed round spermatids with an acrosome that was spread over the nucleus and a round acrosome vesicle. The elongated spermatids were located very close to the tubular lumen and residual bodies were present. A number of type A spermatogonia and intermediate spermatogonia have been identified, along with pachytene spermatocytes with larger nuclei. In the following stage IV of the seminiferous cycle, the round spermatids show an extensive acrosomal vesicle. Elongated spermatids were undergoing spermiation towards the tubular lumen and large residual bodies were observed. Type B spermatogonia were present near to the basal lamina. Type A spermatogonia and preleptotene spermatocytes have been observed. Stage V showed preleptotene, leptotene and pachytene spermatocytes as well as spermatogonia of the type A and spermatids that line the apical parts of the Sertoli cells (sperm release). The following stage VI is featured by a generation of elongating spermatids, since it is the stage immediately after the sperm release. Pachytene spermatocytes that enter the diplotene level of the meiosis and spermatogonia type A were identified. Stage VII of the cycle shows bundles of elongated spermatids that were deeply inserted in the epithelium, head orientated to the basement membrane. Diplotene spermatocytes and primary leptotene spermatocytes were present. Finally, stage VIII of the seminiferous cycle showed meiotic figures of the first and second divisions and secondary spermatocytes. Additionally, zygotene spermatocytes, spermatogonia type A and spermatids still located in the epithelium were found in this stage. Depending on the seasonal state, a certain number of stages or germ cell types are apparent. Our results showed, that re-initiation of spermatogenesis proceeds in May, while in August and September the spermatogenic processes are ceasing. A number of missing germ cell generations from July to September as a sign of decreasing efficiency of spermatogenesis has been encountered (example in Fig. 7). While in July only a few areas with missing germ cell generations within

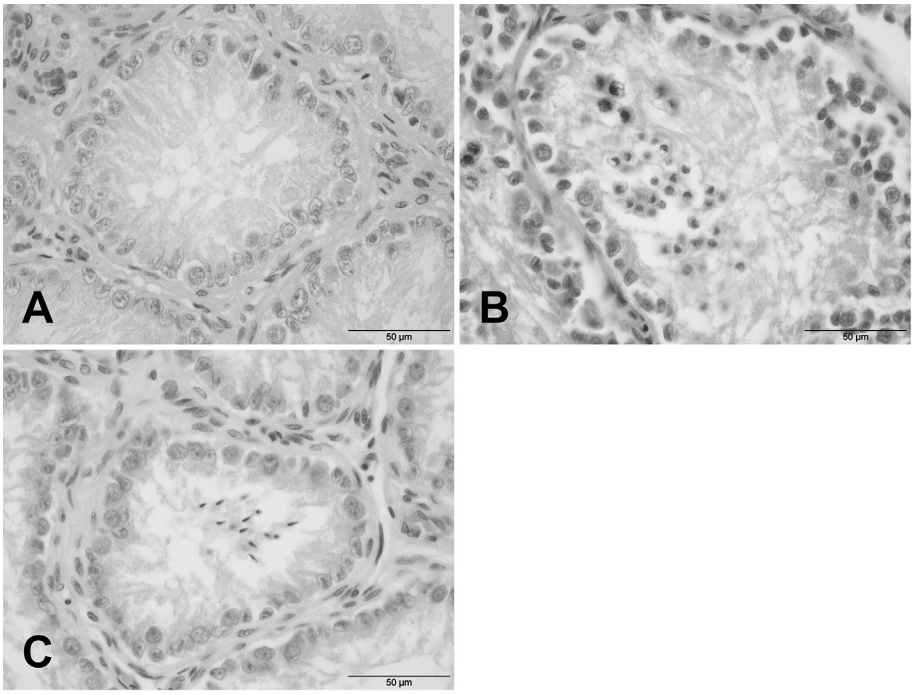

Fig. 2. Cross section through the seminiferous epithelium of adult harbour porpoises found in January (A), May (B) and September (C). (A) Sexual inactive adult individual found in January: tubules contain only a single layer of spermatogonia and Sertoli cells along the basement membrane. Magnification: X 40. (B) Additional germ cells can be found in a harbour porpoise from May. Primary magnification: X 40. (C) Activity of spermatogenesis is decreasing in August and September, only some residual spermatozoa can be identified in the lumen of the tubules. Staining: H&E, primary magnification: X 40.

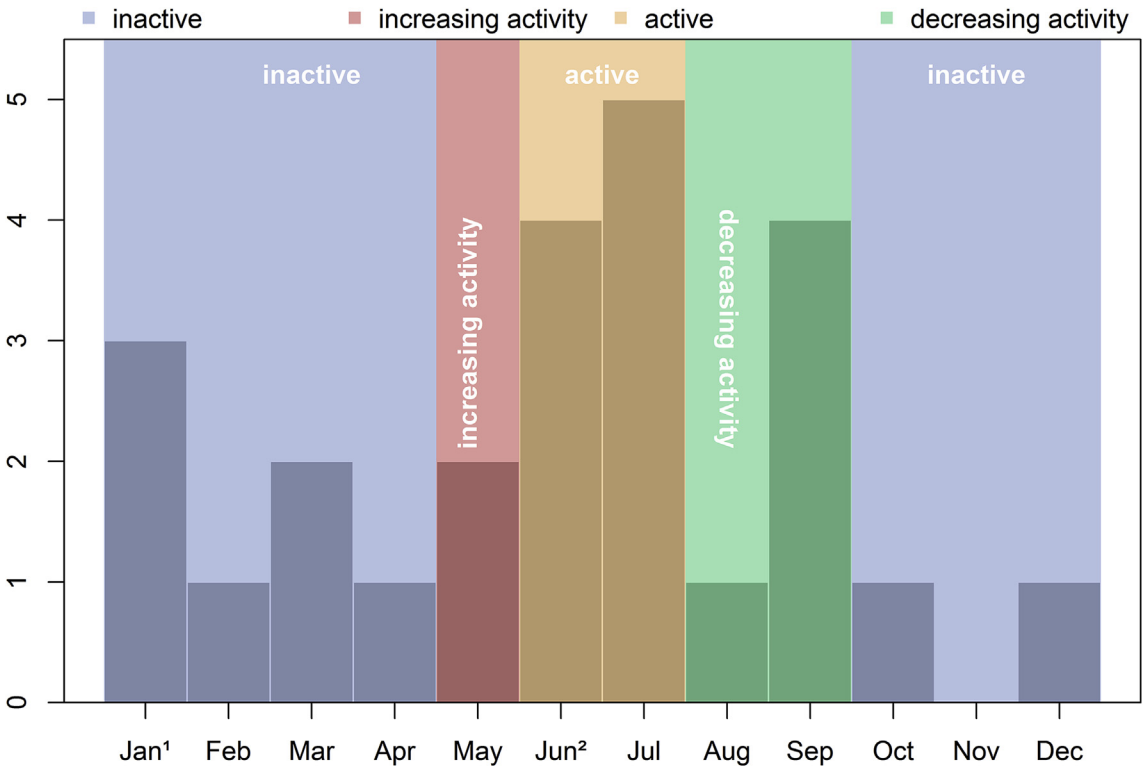

Fig. 3. Summary of samples from adult harbour porpoise testes from the North and Baltic Sea and their reproductive status based on testicular activity, that has been investigated through histological evaluation; the x-axis indicates the month the carcasses have been found (Jan[1] indicates one specimen within January that was attributed with 'decreasing activity', Jun[2] indicates there was one animal found in June that showed 'increasing activity'); the y-axis indicates the number of specimens studied; the background colours identify the conclusion of the reproductive status drawn based on the morphological histological appearance of testes: blue/inactive: no sign of activity or only a few isolated germ cells present in the convoluted seminiferous tubules; pink/increasing activity: increase in abundance of germ cells and some spermatogenic stages can be identified; yellow/active: active reproduction with all eight stages present; green/decreasing activity: only isolated residual germ detected.

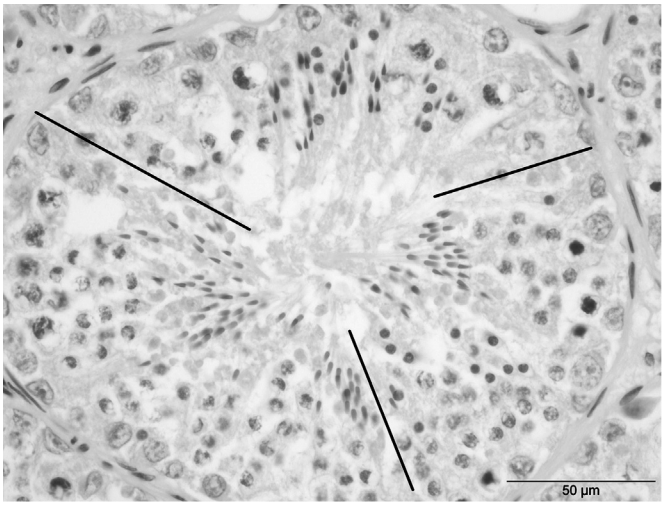

Fig. 4. Cross section through the seminiferous epithelium of an adult harbour porpoise found in July, showing a multi-stage-arrangement in one cross section. Bars indicate borders between the stages. Staining: H&E, primary magnification: X 40.

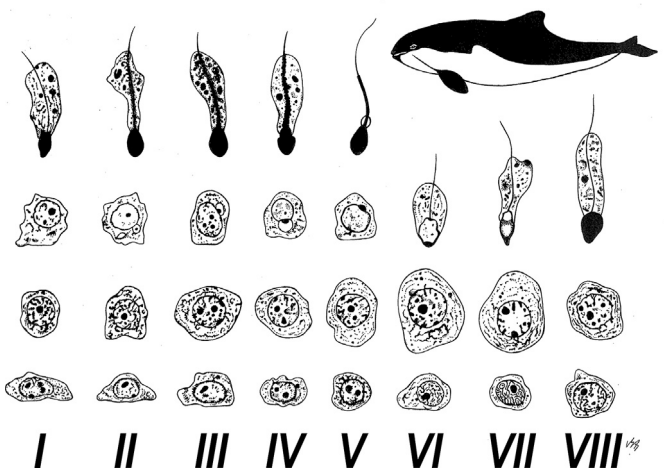

Fig. 6. Stages of spermatogenesis in the harbour porpoise (modified according to [42].

one convoluted seminiferous tubule were found, specimens from August and September showed more absent germ cell populations, leading to tubules containing only some residual spermatids in the tubular lumen and Sertoli cells. This indicates that spermatogenic waves are interrupted and only residual cells from former waves can be observed (Fig. 2C).

3.3. Morphometrical analysis and maturity classes

Average diameter of the convoluted seminiferous tubules in adult animals was 125.27 (±10.17) µm, in non-adult animals 43.08 (±1.67) µm and the average across all age classes was 73.90 (±5.66) µm. In non-adult animals, the average mean tubular diameter corresponds to a body length of at least 114 cm. The average diameter of the convoluted seminiferous tubules during April to August was 171.03 µm, while during the remaining months of the year, the average diameter was found to be 110.02 µm. In non-adults, the tubular diameter averaged at 43.98 µm with no significant changes throughout the year.

A total of 5 models were tested, each using a different combination of body length (*M1_cm*), day of the year (*yday*) and maturity class (*maturity_class*) and age derived from GLG (*age_glg*). A model including a tensor smooth of *yday* (cyclic smoother) and *M1_cm* (thin plate smoother) was identified as the best model (m4) (Table 2). While model m5 technically yielded a better overall score, the inclusion of the by variable introduced an artificial prior classification of data. Model m4 performed slightly worse, but represented a purely numerical solution not requiring prior clustering of data and was therefore preferred over m5. Using model m4, the diameter of the convoluted seminiferous tubules across a range of a whole year was predicted (with Julian days spanning 1–366) and across ages ranging between 0 and 12 years of age. The maximum predicted age was restricted to 12 years as 75% of all samples were identified by the GLG as 12 years of age or younger. Using the average predicted tubular diameter for adult specimens (≥4 years), the predicted average tubular diameter is above the average of specimen classified as adults between early April and late July and always above the average of all non-adult specimens. Peak tubular diameters are predicted for mid-May to late June (Fig. 8).

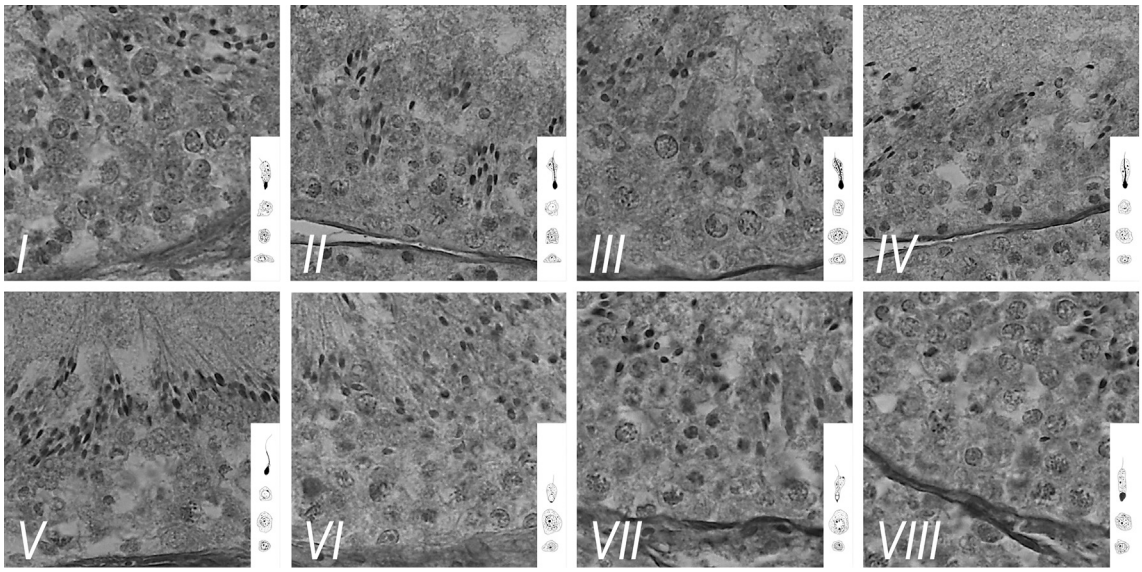

Fig. 5. Staging of the seminiferous epithelium as featured by morphology (staining: PAS, primary magnification: X 40).

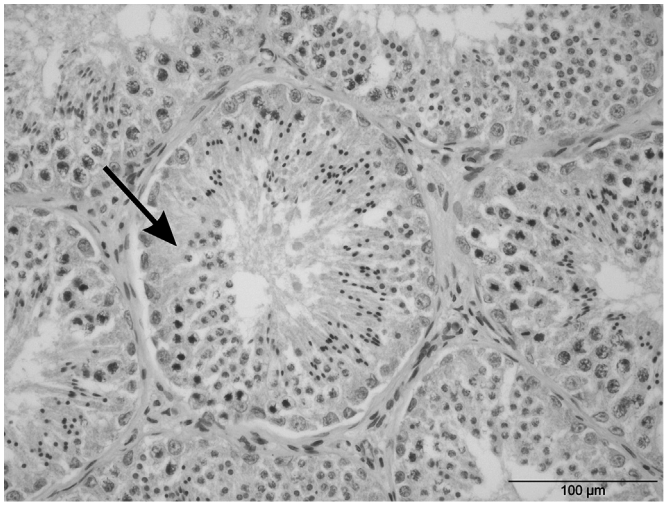

Fig. 7. Cross section through the seminiferous epithelium of an adult harbour porpoise found in July. The arrow indicates an area of missing germ cells. Staining: H&E, primary magnification: X 20.

Table 2
Model diagnostics of the generalized additive modelling analysis. Model: identifier of the model as used throughout the text; covariates: combination of smoother functions and covariates as supplied to the gam; N: sample size available used in the analysis (numbers vary due to missing values in some covariate combinations); dev: explained deviance; GCV: generalized cross validation score. Chosen model in bold (m4).

Model	covariates	N	dev	GCV
m5	yday, M1_cm, by: maturity_class	63	0.9123	0.05
m4	**yday, M1_cm**	**63**	**0.8846**	**0.07**
m3	yday, age_glg	19	0.9216	0.09
m2	yday, by: maturity_class	68	0.2152	0.34
m1	yday	68	0.1405	0.38

4. Discussion

4.1. Testicular maturation and staging of the seminiferous epithelium

Our study provides the first detailed histological description of the seminiferous epithelial cycle in adult male harbour porpoises. The observation of a various number of spermatogenetic stages within one seminiferous tubule confirms the findings of former investigations in cetaceans performed by other authors [24–26,48,49] and is called multi-stage-arrangement [50]. Although other authors that examined spermatogenesis did not mention a clear definition for a spermatogenic stage, they gave hints for the presence of this phenomenon [23–26,48,51].

Our results are similar to findings in testes from new world monkeys (*Platyrrhini*), old world monkeys (*Cercopithecoidea*) and humans (*Homo sapiens*) [50,52]. Until now, this phenomenon was unique to these species [50,52]. In humans, 6 stages of spermatogenesis are identified and have been described in detail [50,53]. The appearance, number and abundance of the different morphological stages depend on the mammalian species. Other mammals show species-specific numbers of stages (Dog: VIII stages, Cat: VIII, Mouse: XV, Rat: XIV stages, Horse: VIII stages, Cow VIII stages) [54–59] according to the association of distinct germ cells defined by the differentiation of the spermatid acrosome. Regarding the number of stages of spermatogenesis, the harbour porpoise can be compared with dogs and bovines, which show also eight stages of spermatogenesis. In the case of cattle, this might link to the phylogenetic relation of odontocetes with artiodactyls.

While in peak breeding season in summer, all generations of germ cells and thus all stages of spermatogenesis can be detected; only residual germ cells are observed in the autumn and winter, which conforms to findings in other waters [24–26,35]. This can be affiliated to the fact that as season progresses, more missing generations of germ cells are found in the cross sections resulting in the level of spermatozoa as the last remaining germ cells of the season. Our results show that re-initiation of spermatogenesis starts in May, while in August and September the spermatogenic processes are ceasing. This seasonal cycle, which is enforced mainly by photoperiod, supports energy balance in terms of seasonal foraging pattern, is a common regulatory mode in mammalian species [60–62]. In wildlife species, a seasonal focus in sperm production is suspected to support sperm competition. This mechanism targets the female ovulation period as a strategy for successful mating and may support behavioural mating strategies in different ways [20,63–65]. The fact that missing germ cell generations occurred already during the peak breeding season could be documented both as a physiological or pathological process. In other mammalian species, evidence is given that missing generations are a sign of reduced fertility [66] due to environmental disturbances. Whether this fact is transferable to harbour porpoises or whether it might be a physiological phenomenon as a sign of low efficiency has to be proven in further studies that are supported by a larger sample size.

4.2. Morphometrical analysis and age groups

Based on GSI measurements and histological features of the testes, no seasonal shift can be found in animals classified as non-adults, which coincided with a maximum age >4 years and thus no sexual activity can be assumed for these individuals. Specimens classified as adults were consistently older than 4 years and showed a seasonal variation in the GSI and in histological features. Based on these observations, we conclude a sexual maturity in individuals older than 4 years.

Our results regarding the tubular diameter findings in adult specimens are in accordance to findings in harbour porpoises from other regions. Karakosta et al., 1999 [24] estimate the average tubular diameter in adult harbour porpoises from the Coasts of England and Wales at 117.5 µm (SD: 38.0). This value is slightly lower compared to our study (125.27 µm (±10.17), but is within confidence limits. Neimanis et al., 2000 [25] investigated harbour porpoises from the bay of Fundy and Gulf of Maine and reported a mean diameter of the convoluted seminiferous tubules of 161.6 µm (SD: 4.07) at the end of July and a maximum diameter of convoluted seminiferous tubules (225 µm) in an individual found in early July. This higher value might be the result of a different sample composition, since the study of Neimanis et al., 2000 includes individuals from June to December. These months include the peak breeding season (June-July [25]) but only a part of the low-breeding season. Generally, there are some factors that might have an influence on these values like sample size, sample handling and processing as well as the software used to measure the values. Our data predict a peak in the diameter of the convoluted seminiferous tubules in mid-May to June, while our data including macroscopic (GSI) and microscopic findings (stages of spermatogenesis) indicate a breeding season from late June to July. Additionally, both methods document a peak of spermatogenesis including the macroskopic analysis and the microscopic morphology in July. Although the majority of our findings comply with this season, some outliers like mentioned in 3.2. and Fig. 3 might indicate a shift in seasonality depending on the area the animal originates from and the subpopulation it belongs to. We cannot exclude that specimens found in German and Dutch areas might have migrated from other areas

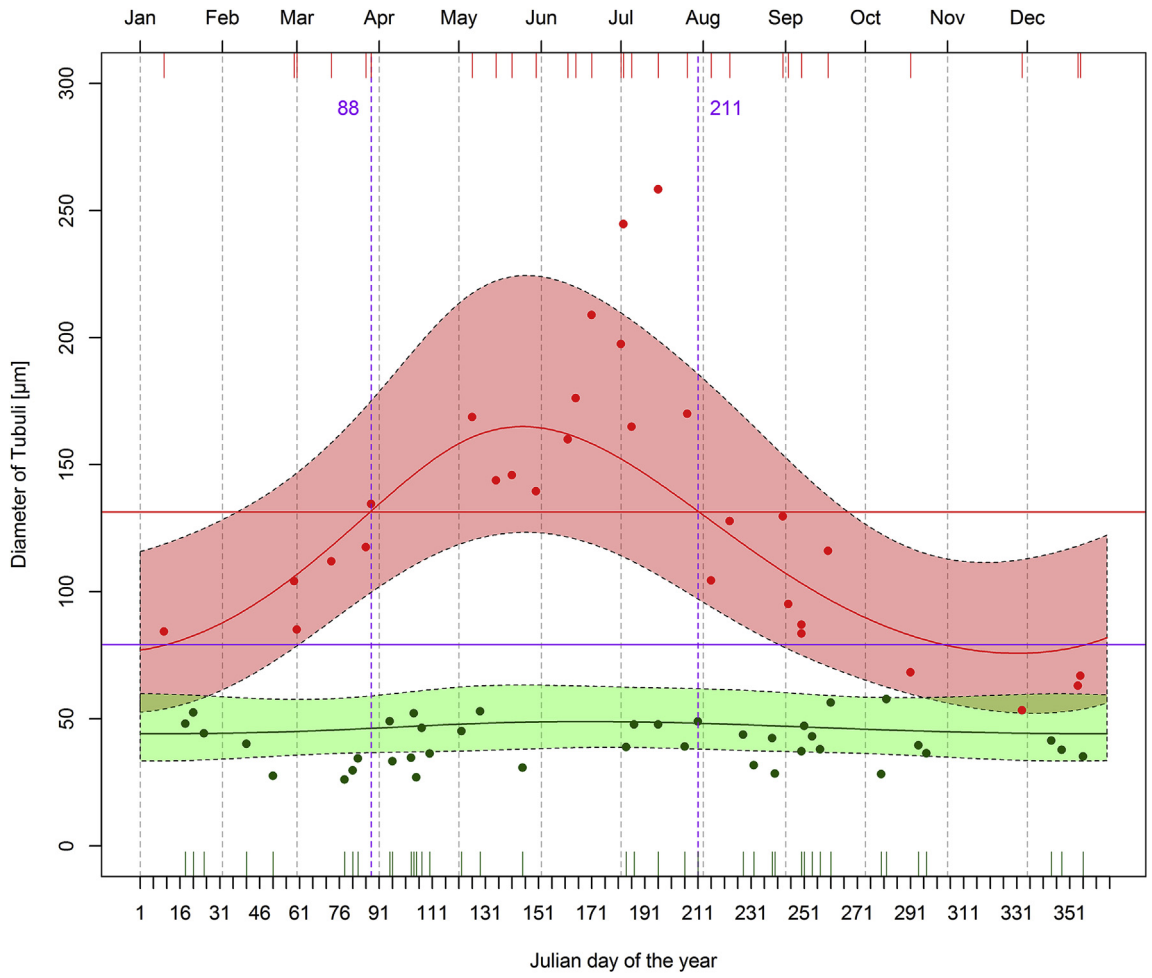

Fig. 8. Predicted diameter of tubules per maturity class based on model m4 from generalized additive modelling. The light green area indicates the prediction for non-adult specimens; the horizontal solid purple line indicates the average tubules diameter of all specimens across the full year; the horizontal thick red line indicates the average diameter of the convoluted seminiferous tubules of adult specimens across the full year. The light red area indicates the predicted the diameter the convoluted seminiferous tubules of adult specimen across the full year; red and green dots mark the dataset of non-adult and adult specimens, respectively. The dotted vertical lines indicate the time of year with predicted the convoluted seminiferous tubules diameter above the mean the convoluted seminiferous tubules diameter (in all animals; corresponding Julian days next to respective lines).

that show different seasonal pattern. Another possible reason for a shift in seasonality would be changes in the endocrine system due to various reasons like pathological disorders or endocrine disruption we just can suggest at this point of the analysis and with this small sample size. These issues could be further investigated in the future using a larger dataset including information about blood hormone levels and genetics.

Our observations suggest that adult male harbour porpoises synchronise their spermatogenesis with the ovulation time of females, thus maximising their chance at reproductive success which supports the hypothesis of sperm competition [65].

For other wildlife mammals it is known, that they synchronise their energetically demanding sperm production to the oestrus of female individuals and thus to the most favourable time of the year [67]. Environmental factors regulate spermatogenesis through the hypothalamic–pituitary–gonadal axis and trigger the onset of testicular function in spring and the testicular regression in autumn [67]. Since the exact point of time when juvenile males step into puberty cannot be observed macroscopically, the histological analysis of the testes would give additional evidence on the developmental stage of the individual. A combination of assessing the existing germ cell types with measuring the diameter of the seminiferous tubules draws a reliable picture on the sexual status of a juvenile/pubertal animal. As a next step, more samples with morphological records are required to study the transition period between immature and mature individuals in detail. This would complement a recent analysis of female harbour porpoises of the area [20]. In addition, more studies are needed to assess the correlation between the variability in testes diameter observed in this study and morphometric features such as body length, body weight and indicators of the endocrine system.

Due to the fact, that our study provides first evidence of the stages of spermatogenesis with a small number of samples, further analyses on this topic are strongly required to confirm our findings. Using more samples of good quality, various other parameters that improve the understanding of male reproduction like efficiency of spermatogenesis or duration of the stages can be described on a higher level. Furthermore, immunohistochemistry could reveal information about proliferation and apoptosis within the spermatogenic stages, as well as knowledge about the cellular mechanisms during puberty.

5. Conclusions

Prior studies on testicular activity of harbour porpoises of the German North and Baltic Sea were restricted to small sample sizes

and short periods of time. We here present long-term dataset on reproductive parameters in male harbour porpoises in accordance to age classes and across seasons. With the detailed description of spermatogenesis and stages of spermatogenesis, a baseline is given for further studies on the reproductive biology of male harbour porpoises and can be used to develop an indicator for the effects of environmental impacts on the reproductive system such as impaired spermatogenesis due to various factors. It has been observed in other domesticated and wildlife mammals that stress (influence of toxicants) has a negative impact on spermatogenesis [67,68] as well as a wide variety of endocrine disruptors that are capable of compromising male infertility [69–72]. Studies on cetaceans in human care may not reveal representative results for wild individuals and therefore a long-term stranding monitoring programme is a favourable method in order to achieve a sufficient sample size as well as a seasonal average of specimen. With that, information on the reproductive system of harbour porpoises in the North and Baltic Sea could be provided and build an important basis for management plans for endangered species that are no longer capable of reaching the Good Environmental Status like suggested by the Marine Strategy Framework Directive of the European Union. This would be essential for the evaluation of genetic and evolutionary studies and thus essential for conservation biology.

Acknowledgements

We would like to thank all personnel helping during the necropsies at the Institute for terrestrial and aquatic wildlife research (ITAW Büsum) Büsum. Special thanks go to our lab technicians at the Institute for terrestrial and aquatic wildlife research (ITAW Büsum) and the Institute of Anatomy from the University of Veterinary Medicine Hanover, Germany as well as at the Department of Pathology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands, who helped us tremendously with the acquisition of samples, their handling and age determination. We are especially grateful to our lab technicians at the Institute of Anatomy, University of Veterinary Medicine Hanover, Germany and at the Institute of Veterinary Anatomy, Histology and Embryology, University of Giessen, Germany for professional histology. We also thank Christina Lockyer for her great effort to help us with the age determination technique. We thank Abbo Van Neer for proof-reading the article. Furthermore, we would like to thank everyone participating in the stranding network who regularly collect, report and transfer specimen to the ITAW. Special thanks belong to Caren Imme von Stemm, Institute of Anatomy from the University of Veterinary Medicine Hanover, Germany, for her depictions of germ cell stages. This project was partly funded by the Ministry of Energy, Agriculture, the Environment and Rural Areas Schleswig Holstein (MELUR) and the state centre for coastal protection, national park and ocean protection Schleswig - Holstein (LKN).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2018.11.031.

References

[1] Kinze CC. On the harbour porpoise in Danish waters: distribution, breeding areas, and threats. Fauna Flora Stock 1990;85:3–4.
[2] Benke H, Bräger S, Dähne M, Gallus A, Hansen S, Honnef CG, et al. Baltic Sea harbour porpoise populations: status and conservation needs derived from recent survey results. Mar Ecol Prog Ser 2014;495:275–90. https://doi.org/10.3354/meps10538.
[3] Addink MJ, Sørensen TB, García Hartmann M. Aspects of reproduction and seasonality in the harbour porpoise from Dutch waters. Dev Mar Biol 1995: 459–64. https://doi.org/10.1016/S0163-6995(06)80046-6.
[4] Heide-Jørgensen MP, Iversen M, Nielsen NH, Lockyer C, Stern H, Ribergaard MH. Harbour porpoises respond to climate change. Ecol Evol 2011;1:579–85. https://doi.org/10.1002/ece3.51.
[5] Dähne M, Adler S, Krügel K, Siebert U, Gilles A, Lucke K, et al. Effects of pile-driving on harbour porpoises (Phocoena phocoena) at the first offshore wind farm in Germany. Environ Res Lett 2013;8, 025002. https://doi.org/10.1088/1748-9326/8/2/025002.
[6] Nabe-Nielsen J, Sibly RM, Tougaard J, Teilmann J, Sveegaard S. Effects of noise and by-catch on a Danish harbour porpoise population. Ecol Model 2014;272: 242–51. https://doi.org/10.1016/j.ecolmodel.2013.09.025.
[7] Murphy S, Pierce GJ, Law RJ, Bersuder P, Jepson PD, Learmonth JA, et al. Assessing the effect of persistent organic pollutants on reproductive activity in common dolphins and harbour porpoises. J Northwest Atl Fish Sci 2010;42: 153–73. https://doi.org/10.2960/J.v42.m658.
[8] von Benda-Beckmann AM, Aarts G, Sertlek HÖ, Lucke K, Verboom WC, Kastelein RA, et al. Assessing the impact of underwater clearance of unexploded ordnance on harbour porpoises (Phocoena phocoena) in the Southern North Sea. Aquat Mamm 2015;41:503–23. https://doi.org/10.1578/AM.41.4.2015.503.
[9] Siebert U, Joiris CR, Holsbeek L, Benke H, Failing K, Frese K, et al. Potential relation between mercury concentrations and necropsy findings in cetaceans from German waters of the North and Baltic Seas. Mar Pollut Bull 1999;38: 285–95. https://doi.org/10.1016/S0025-326X(98)00147-7.
[10] Beineke A, Siebert U, McLachlan M, Bruhn R, Thron K, Failing K, et al. Investigations of the potential influence of environmental contaminants on the thymus and spleen of harbor porpoises (Phocoena phocoena). Environ Sci Technol 2005;39:3933–8. https://doi.org/10.1021/es048709j.
[11] Das K, Siebert U, Fontaine M, Jauniaux T, Holsbeek L, Bouquegneau J-M. Ecological and pathological factors related to trace metal concentrations in harbour porpoises Phocoena phocoena from the North Sea and adjacent areas. Mar Ecol Prog Ser 2004;281:283–95. https://doi.org/10.3354/meps281283.
[12] Mruk DD, Cheng CY. Environmental contaminants: is male reproductive health at risk? Spermatogenesis 2011;1:283–90. https://doi.org/10.4161/spmg.1.4.18328.
[13] Berggren P, Wade PR, Carlström J, Read AJ. Potential limits to anthropogenic mortality for harbour porpoises in the Baltic region. Biol Conserv 2002;103: 313–22. https://doi.org/10.1016/S0006-3207(01)00142-2.
[14] Bennett PM, Jepson PD, Law RJ, Jones BR, Kuiken T, Baker JR, et al. Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. Environ Pollut 2001;112:33–40. https://doi.org/10.1016/S0269-7491(00)00105-6.
[15] Jepson PD, Deaville R, Barber JL, Aguilar À, Borrell A, Murphy S, et al. PCB pollution continues to impact populations of orcas and other dolphins in European waters. Sci Rep 2016:1–17. https://doi.org/10.1038/srep18573.
[16] Borgå K, Fisk AT, Hoekstra PE, Muir DCG. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in Arctic marine food webs. Environ Toxicol Chem 2004;23:2367–85. https://doi.org/10.1897/03-518.
[17] Wolkers J, Burkow IC, Monshouwer M, Lydersen C, Dahle S, Witkamp RF. Cytochrome P450-mediated enzyme activities and polychlorinated biphenyl accumulation in harp seal (Phoca groenlandica). Mar Environ Res 1999;48: 59–72. https://doi.org/10.1016/S0141-1136(99)00032-X.
[18] Siebert U, Wünschmann A, Weiss R, Frank H, Benke H, Frese K. Post-mortem findings in harbour porpoises (phocoena phocoena) from the German North and Baltic Seas. J Comp Pathol 2001;124:102–14. https://doi.org/10.1053/jcpa.2000.0436.
[19] Wiemann A, Andersen LW, Berggren P, Siebert U, Benke H, Teilmann J, et al. Mitochondrial Control Region and microsatellite analyses on harbour porpoise (Phocoena phocoena) unravel population differentiation in the Baltic Sea and adjacent waters. Conserv Genet 2010;11:195–211. https://doi.org/10.1007/s10592-009-0023-x.
[20] Kesselring T, Viquerat S, Brehm R, Siebert U. Correction: coming of age: - do female harbour porpoises (Phocoena phocoena) from the North Sea and Baltic Sea have sufficient time to reproduce in a human influenced environment? PloS One 2017;12(10):e0186951. https://doi.org/10.1371/journal.pone.0186951. PLoS One 2018;13:1–14. doi:10.1371/journal.pone.0199633.
[21] Henriksen K, Hakovirta H, Parvinen M. Testosterone inhibits and induces apoptosis in rat seminiferous tubules in a stage-specific manner: in situ quantification in squash preparations after administration of ethane dimethane sulfonate. Endocrinology 1995;136:3285–91. https://doi.org/10.1210/endo.136.8.7628362.
[22] Li D, Han X. Evaluation of toxicity of methyl tert-butyl ether (MTBE) on mouse spermatogenic cells in vitro. Toxicol Ind Health 2006;22:291–9. https://doi.org/10.1177/0748233706070310.
[23] Holt WV, Waller J, Moore A, Jepson PD, Deaville R, Bennett PM. Smooth muscle actin and vimentin as markers of testis development in the harbour porpoise (Phocoena phocoena). J Anat 2004;205:201–11. https://doi.org/10.1111/j.0021-8782.2004.00328.x.
[24] Karakosta CV, Jepson PD, Ohira H, Moore A, Bennett PM, Holt WV. Testicular and ovarian development in the harbour porpoise (Phocoena phocoena). J Zool London 1999;249:111–21.
[25] Neimanis AS, Read AJ, Foster R a, Gaskin DE. Seasonal regression in testicular size and histology in harbour porpoises (Phocoena phocoena, L.) from the Bay

of Fundy and Gulf of Maine. J Zool 2000;250:221–9. https://doi.org/10.1017/S0952836900002077.
[26] Plön S, Bernard R. Testis, spermatogenesis and testiculat cycles. In: Miller DL, editor. Reprod. Biol. Phylogeny cetacea whales, dolphins porpoises. first ed. Boca Raton, Fl: CRC Press; 2007. p. 116–35.
[27] Hess RA, De Franca LR. Spermatogenesis and cycle of the seminiferous epithelium. Adv Exp Med Biol 2008;636:1. https://doi.org/10.1007/978-0-387-09597-4_1.
[28] Meistrich ML, Hess RA. Assessment of spermatogenesis through staging of seminiferous tubules. In: Carrell DT, Aston KI, editors. Methods mol. Biol., vol. 927. Totowa, NJ: Humana Press; 2013. p. 299–307. https://doi.org/10.1007/978-1-62703-038-0_27.
[29] Hogarth CA, Evans E, Onken J, Kent T, Mitchell D, Petkovich M, et al. CYP26 enzymes are necessary within the postnatal seminiferous epithelium for normal murine Spermatogenesis1. Biol Reprod 2015;93:19. https://doi.org/10.1095/biolreprod.115.129718.
[30] Learmonth JA, Murphy S, Luque PL, Reid RJ, Patterson IAP, Brownlow A, et al. Life history of harbor porpoises (Phocoena phocoena) in Scottish (UK) waters. Mar Mamm Sci 2014;30:1427–55. https://doi.org/10.1111/mms.12130.
[31] Lockyer C, Heide-Jørgensen MP, Jensen J, Kinze CC, Buus Sørensen T. Age, length and reproductive parameters of harbour porpoises Phocoena phocoena (L.) from West Greenland. ICES J Mar Sci 2001;58:154–62. https://doi.org/10.1006/jmsc.2000.0998.
[32] Ólafsdóttir D, Víkingsson GA, Halldórsson SD, Sigurjónsson J. Growth and reproduction in harbour porpoises (Phocoena phocoena) in Icelandic waters. NAMMCO Sci Publ 2003;5:195–210. https://doi.org/10.7557/3.2747.
[33] Sørensen TB, Kinze CC. Reproduction and reproductive seasonality in Danish harbour porpoises, Phocoena Phocoena. Ophelia 1994;39:159–76. https://doi.org/10.1080/00785326.1994.10429541.
[34] Read AJ, Hohn AA. Life in the fast lane: the life history of horbor porpoises from the Gulf of Maine. Mar Mamm Sci 1995;11:423–40.
[35] Dardente H, Lomet D, Robert V, Decourt C, Beltramo M, Pellicer-Rubio MT. Seasonal breeding in mammals: from basic science to applications and back. Theriogenology 2016;86:324–32. https://doi.org/10.1016/j.theriogenology.2016.04.045.
[36] Benke H, Siebert U, Lick R, Bandomir B, Weiss R. The current status of harbour porpoises (Phocoena phocoena) in German waters. Arch Fish Mar Res 1998;46:97–123.
[37] Lockyer C, Kinze C. Status, ecology and life history of harbour porpoise (Phocoena phocoena), in Danish waters. NAMMCO Sci Publ 2003;5:143–75. https://doi.org/10.7557/3.2745.
[38] Lockyer C. Harbour porpoises (phocoena phocoena) in the North atlantic: biological parameters. NAMMCO Sci Publ 2003;5:71–89. https://doi.org/10.7557/3.2740.
[39] Hasselmeier I, Abt KF, Adelung D, Siebert U. Stranding patterns of harbour porpoises (Phocoena phocoena) in the German North and Baltic Seas : when does the birth period occur ? J Cetacean Res Manag 2004;6:259–63.
[40] Kuiken T, Garcia Hartmann M, European Cetacean Society. Proceedings of the first ECS workshop on cetacean Pathology : dissection techniques and tissue sampling : Leiden, The Netherlands, 13-14 September 1991. Proc. First ECS work. Cetacean Pathol.. Stralsund, Germany: European Cetacean Society; 1991. p. 1–39.
[41] Aescht E, Büchl-Zimmermann S, Burmester A, Dänhardt-Pfeiffer S, Desel C, Hamers C, et al. Romeis Mikroskopische Technik 2010. https://doi.org/10.1007/978-3-8274-2254-5.
[42] Russell LD, Ettlin RA. Mammalian spermatogenesis. In: Sinha Hikim AP, Clegg ED, editors. Histol. Histopathol. Eval. Testis. first ed. Clearwater, Fl: Cache River Press; 1990. p. 1–40.
[43] Barber BJ, Blake NJ. Chapter 6 reproductive physiology. Dev Aquacult Fish Sci 2006;35:357–416. https://doi.org/10.1016/S0167-9309(06)80033-5.
[44] R Core Team R. A Language and environment for Statistical Computing. Vienna, Austria. 2018.
[45] Wood SN, Pya N, Säfken B. Smoothing parameter and model selection for general smooth models. J Am Stat Assoc 2016;111:1548–63. https://doi.org/10.1080/01621459.2016.1180986.
[46] Wood SN. Thin-plate regression splines. J Roy Stat Soc 2003;65:95–114.
[47] Wood SN. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J Roy Stat Soc 2011;73:3–36.
[48] Hohn AA, Read AJ, Fernandez S, Vidal O, Findley LT. Life history of the vaquita, phocoena sinus (phocoenidae, cetacea). J Zool 1996;239:235–51. https://doi.org/10.1111/j.1469-7998.1996.tb05450.x.
[49] Neuenhagen C, García Hartmann M, Greven H. Histology and morphometrics of testes of the white-sided dolphin (Lagenorhynchus acutus) in bycatch samples from the northeastern Atlantic. Mamm Biol 2007;72:283–98. https://doi.org/10.1016/j.mambio.2006.10.008.
[50] Bergmann M. Spermatogenese. Urologe 2005;44:1131–8. https://doi.org/10.1007/s00120-005-0909-2.
[51] Learmonth JA, Murphy S, Luque PL, Reid RJ, Patterson IAP, Brownlow A, et al. Life history of harbor porpoises (Phocoena phocoena) in Scottish (UK) waters. Mar Mamm Sci 2014;30:1427–55. https://doi.org/10.1111/mms.12130.
[52] Luetjens CM, Weinbauer GF, Wistuba J. Primate spermatogenesis: new insights into comparative testicular organisation, spermatogenic efficiency and endocrine control. Biol Rev Camb Philos Soc 2005;80:475–88. https://doi.org/10.1017/S1464793105006755.
[53] Clermont Y. The cycle of the seminiferous epithelium in man. Am J Anat 1963;112:35–51. https://doi.org/10.1002/aja.1001120103.
[54] Foote RH, Swierstra EE, Hunt WL. Spermatogenesis in the dog. Anat Rec 1972;173:341–51. https://doi.org/10.1002/ar.1091730309.
[55] Böhme G, Pier C. [Classification criteria for the epithelium cycle of the seminiferous tubule, based on studies in the cat (Felis catus)]. Berl Münchener Tierärztliche Wochenschr 1986;99:232–6.
[56] Oakberg EF. A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. Am J Anat 1956;99:391–413. https://doi.org/10.1002/aja.1000990303.
[57] Leblond CP, Clermont Y. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann N Y Acad Sci 1952;55:548–73. https://doi.org/10.1111/j.1749-6632.1952.tb26576.x.
[58] Johnson L, Kattansaid AF, Hardy VB, Scrutchfield WL. Isolation and staging of Horse seminiferous tubules by transillumination. J Reprod Fertil 1990;89:689–96. https://doi.org/10.1530/jrf.0.0890689.
[59] Ekstedt E, Söderquist L, Plöen L. Fine structure of spermatogenesis and Sertoli cells (epitheliocytus sustentans) in the. Bull. Anat Histol Embryol 1986;15:23–48. https://doi.org/10.1111/j.1439-0264.1986.tb00529.x.
[60] Takalhashi S, Turek FW. MRY. Circannual rhythms. Handb Behav Neurobiol 2001:509–28.
[61] Bronson FH. Climate change and seasonal reproduction in mammals. Philos Trans R Soc B Biol Sci 2009;364:3331–40. https://doi.org/10.1098/rstb.2009.0140.
[62] Vasantha I. Physiology of seasonal breeding: a review. J Vet Sci Technol 2015;07:1–4. https://doi.org/10.4172/2157-7579.1000331.
[63] Stockley P. Sperm competition in mammals. Hum Fertil 2004;7:91–7. https://doi.org/10.1080/14647270410001699054.
[64] Gomendio M, Roldan ERS. Mechanisms of sperm competition: linking physiology and behavioural ecology. Trends Ecol Evol 1993;8:95–100. https://doi.org/10.1016/0169-5347(93)90059-X.
[65] Fontaine PM, Barrette C. Megatestes - anatomical evidence for sperm competition in the harbor porpoise. Mammalia 1997;61:65–71.
[66] Gely-Pernot A, Raverdeau M, Teletin M, Vernet N, Féret B, Klopfenstein M, et al. Retinoic Acid receptors control spermatogonia cell-fate and induce expression of the SALL4A transcription factor. PLoS Genet 2015;11. https://doi.org/10.1371/journal.pgen.1005501. e1005501.
[67] Wingfield JC. Organization of vertebrate annual cycles: implications for control mechanisms. Philos Trans R Soc B Biol Sci 2008;363:425–41. https://doi.org/10.1098/rstb.2007.2149.
[68] Potemina TE. Impairment of spermatogenesis in male rats during stress. Bull Exp Biol Med 2008;145:700–2. https://doi.org/10.1007/s10517-008-0173-8.
[69] Reijnders PJH. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 1986;324:456–7. https://doi.org/10.1038/324456a0.
[70] Sedha S, Kumar S, Shukla S. Role of oxidative stress in male reproductive dysfunctions with reference to phthalate compounds. Urol J 2015;12:2304–16.
[71] Vos JG, Dybing E, Greim HA, Ladefoged O, Lambré C, Tarazona JV, et al. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit Rev Toxicol 2000;30:71–133. https://doi.org/10.1080/10408440091159176.
[72] Yeung BH, Wan HT, Law AY, Wong CK. Endocrine disrupting chemicals: multiple effects on testicular signaling and spermatogenesis. Spermatogenesis 2011;1:231–9. https://doi.org/10.4161/spmg.1.3.18019.