Multiple insights into the reproductive function of harbour porpoises (*Phocoena phocoena*): An ongoing study.

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ABSTRACT

The harbour porpoises kept at the Fjord&Bælt since April 1997 offer a unique opportunity to gain a better understanding of the reproductive function in harbour porpoises, especially in terms of physiological cycle and concomitant behavioural traits. A study was initiated in 1997 with the following aims: 1) characterising the annual reproductive cycle in terms of behaviour and endocrine activity; 2) finding the most suitable techniques for a longitudinal investigation of the reproductive function, in particular with respect of the small size of the species; 3) ensuring a precise monitoring of the reproductive state of the Fjord&Bælt porpoises; 4) evaluating the best techniques for a vertical assessment of the reproductive state in wild harbour porpoises; 5) providing comparative basis for toxicological studies.

Three harbour porpoises have participated in the study: a male and a female estimated 1-2 years old at their arrival at the Centre in 1997, and a one-year old female. The different methods for investigating their reproductive function include techniques not previously used with harbour porpoises, such as behavioural observation, measurement of sexual hormones in blood and other matrices, vaginal cytology, body temperature, and ultrasound scanning of testes and ovaries. These methods are discussed in terms of practicality and invasiveness. Selected examples of the preliminary results obtained are reported.

Projects have concentrated on the sexual behaviour of the adult male and female (frequency, initiative, courtship behaviours) and their hormonal correlates, as well as on the interaction of the juvenile with the 2 adult animals. Behavioural sexual activity is very seasonal (peaking at the end of July and August), as is the testosterone cycle (levels increasing from less than 1ng/ml to 30ng/ml in May) and the development of the testis (peaking in July-August). Progesterone and oestrogen levels vary between less than 1 to 17ng/ml and less than 0.1 to 1.8 ng/ml respectively, but infrequent blood sampling precluded obtaining a detailed picture of the ovarian cycle. We are attempting to measure sexual hormones in saliva and eye secretion. Successful matings have been confirmed by the presence of sperm on vaginal smears in 4 consecutive summers, but no pregnancy has occurred yet.

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INTRODUCTION

Studies on reproduction in harbour porpoises have long been limited to point sampling on dead animals (*e.g.* for Danish strandings: Møhl-Hansen 1954, Andersen *et al.*1992, Sørensen and Kinze 1994, Lockyer and Kinze 2003). Physiological cycles have not been investigated and techniques like vaginal cytology, sperm investigation and ultrasound diagnostics have not been used. Hormonal monitoring in cetaceans started being commonly used in the 1980s. To our knowledge, however, hormonal values have not been reported for harbour porpoises, and are available only for one phocoenid species, the Dall's porpoise *Phocoenoides dalli* (Temte and Spielvogel 1985, Subramanian *et al.* 1987, Temte 1990).

Serious concerns have been expressed that toxins may cause reproductive failure in harbour porpoises, and may be contributing to the apparent decline of some populations (Anon. 1997, Reijnders *et al.* 1999). However, the effect of toxins on reproductive capabilities is very difficult to investigate without a thorough understanding of the reproductive events; physiological, anatomical as well as behavioural.

The harbour porpoises kept at the Fjord&Bælt offer a unique opportunity to gain a better understanding of the reproduction in this species, especially in terms of the physiological cycle and concomitant behavioural traits. Only a few animals are available and the interest of the project resides in the long-term monitoring of individuals, including transition to adulthood.

A project was therefore initiated in 1997 to study the reproductive function in live harbour porpoises (Desportes 1999). The source of material was two-fold: the long-term monitoring of the porpoises kept at the Fjord&Bælt and sampling of porpoises bycaught in Danish pound nets and included in a Danish satellite tagging project (Teilmann *et al.* MS 1999a, b, 2001).

The main objectives of the project are the following:

i) To characterise the annual reproductive cycle of male and female harbour porpoises, both in terms of behaviour (visual and acoustic) and gonad activity;
ii) To find the most suitable methods for a longitudinal investigation of the reproductive function, with regard to the small size of the species and considering the degree of invasiveness, the precision and predictive value of the results obtained and the effort required for sample collection;
iii) To ensure a precise monitoring of the reproductive state of the harbour porpoises at the Fjord&Bælt;
iv) To evaluate the best collection of methods for a vertical assessment of the reproductive state in wild porpoises (live bycatch), knowing that sampling has to be minimally invasive, quickly performed, and feasible from a small boat;
v) To provide multiple long-term reproductive data in relation to known blood contaminant levels and known ingestion of contaminants as a comparative basis for toxicological studies.

This paper describes the methods used and their feasibility. It presents the progress made and reports selected examples of the preliminary results obtained in the framework of this ongoing study.

THE CENTRE AND THE PORPOISES

The Fjord&Bælt porpoise project

In 1998, the Danish government established an action plan for reducing incidental bycatches of harbour porpoises (Jepsen 1998). As a contribution to that, the centre obtained permission from the Danish Ministry of Environment and Energy to keep 4 porpoises in human care for a period of 8 years for research purposes and public information and awareness. The research was to focus on understanding the process of entanglement and the ways of reducing bycatch. (Kristensen and Desportes 2001).

The facilities

The porpoises are kept in an outdoor enclosure of about 1900 m^3, bordered on one side by a 25 m long tunnel with underwater viewing. It is spanned by nets and benefits from a natural,

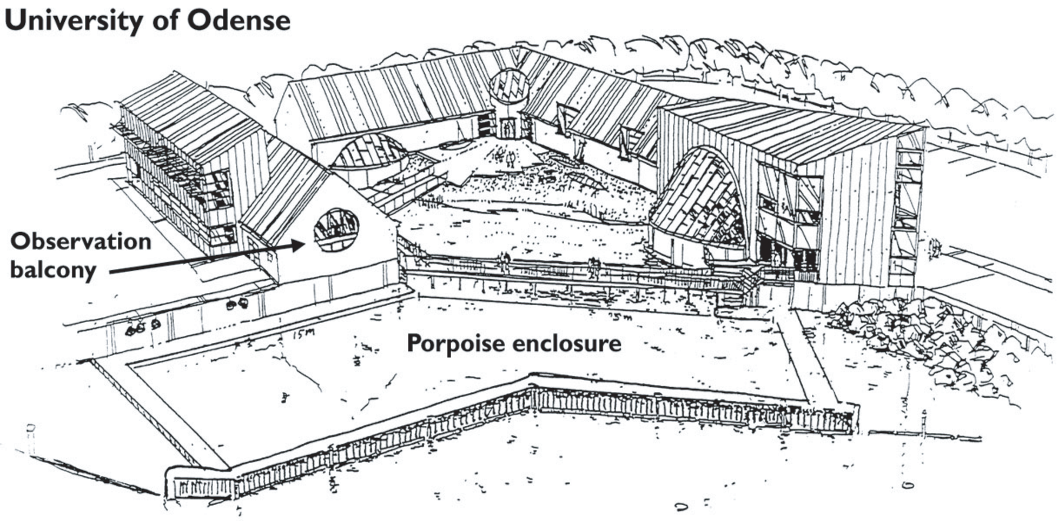


Fig. 1. Plan of the porpoise enclosure and general drawing of the Fjord&Bælt. The Centre for Aquatic Biology from the University of Southern Denmark can be seen on the left, with the balcony from which behavioural observations are made.

tidal water flow all year round (Fig. 1). The animals are thus exposed to natural environmental conditions of water quality, flow, tidal currents, temperature and light, as well as diurnal and seasonal variations in flora and fauna.

The animals
The 2 first harbour porpoises, a male and a female, arrived at the centre in April 1997, after being rescued from the same pound net, and have always been kept together since. The male, Eigil, weighed 37.5 kg and was 130 cm long, and the female, Freja, weighed 40.5 kg and was 127 cm long. They were considered to be in good health, 1 to 2 years of age and immature. A juvenile female, Nuka, arrived in April 1999. She was 115 cm long and weighed 31 kg and was likely born the previous year. Unfortunately, she died in February 2000 from a septicaemia due to the bacteria *Staphylococcus aureus* (Siebert *et al.* 2002).

METHODS

Besides shedding light on different aspects of the reproductive function, the different investigation methods available have varying degrees of accuracy, predictive value, practicality and invasiveness. In the framework of a long-term monitoring, invasiveness and induced stress are factors particularly important to consider. For some techniques these can, however, be reduced by training the animals in voluntary husbandry behaviours (VHB), *i.e.*, training them to co-operate so sampling can be performed without physical restraint. In the case of aquatic animals, this implies eliminating an important stress factor, the removal from the water, by collecting the samples at pool-side, with the animal remaining in the water at all times. Monitoring of cortisol levels has shown that the Fjord&Bælt porpoises have habituated very little to being taken out of the water over a 4-year period, although the procedure has been kept fairly constant. Also cortisol levels in blood collected at pool-side are about 4 times less than in blood collected on land during the same period (Buholzer *et al.* MS 2001, Desportes *et al.* MS 2001b).

Invasive techniques
Blood collection for hormonal analysis
Blood collection is the most invasive technique used in this program. To reduce stress on the animals we have emphasised training the animals to enable blood collection at pool-side (Fig. 2). The blood of the porpoises has been collected once to twice a month for medical

Fig. 2. Taking a blood sample from a harbour porpoise at poolside, Fjord&Bælt, Kerteminde, Denmark. (Photo: Fjord&Bælt).

monitoring since the porpoises arrived at the centre. In the reproductive season, blood is sampled up to 3 times a month. It is drawn from the dorsal veins of the flukes in the morning before the first feed if the animals must be taken out of the water or at the first morning feed for poolside sampling. Blood for plasma is collected in NH_4-Heparin tubes and processed using standard techniques. Plasma steroid levels are measured in duplicate by enzyme-immunoassays using an Elisa kit. Plasma oestrogen, progesterone and testosterone levels are monitored. Other hormones (*e.g.* prolactin and gonadotropins) will also be measured if possible.

Less invasive techniques

The following techniques are minimally invasive, especially if they can be performed under VHB, with the animals remaining in the water. Collection of data can then become routine husbandry and be performed regularly and frequently.

Body temperature

Rectal temperature is measured with a digital thermometer (Ellab medical precision thermometer, DM 852), inserting the probe 20 cm into the rectum. It can now be measured at poolside on Eigil and Freja.

Ultra sound examination of ovaries, uterus and testes

The growth and seasonal development of the testes was monitored between May 1998 and August 2000, by ultrasound imaging (Sonoscop 20, General Electric, 5 MHz convex probe and Toshiba, Just vision, 6-8 MHz, micro-convex probe). The length (L) and maximum diameter (d) were measured for both testes and the testicular volume was then approximated to be $\pi(0.5d)^3L$. The animals are currently trained so that a several-minute long ultrasound examination can be performed at poolside. We hope in the future to be able to follow closely testicular development and the ovarian and endometrium cycle, and check for and follow pregnancy if it does occur.

Collection of eye secretion and saliva

Blood samples taken from fluke presentation are a valuable source of information, but blood cannot be taken on a regular basis from small cetaceans. Frequent insertion of needles into the skin and the very fragile blood vessels would cause damage. Also, because the vessels lie deep under the surface, and the animals are free swimming, catheters cannot be used. The feasibility of using alternative media to obtain hormone levels, that can be collected non-invasively and frequently (*e.g.*, urine, faeces, eye secretion, saliva and milk), is presently being tested.

Training for collection of eye secretion and saliva involves mainly desensitisation and was rapid. The time taken to condition urination on command is much longer, and the animal must first urinate spontaneously under the observation of a trainer, so the behaviour can be captured. To collect a pure sample, the whale has to be out of the water, *e.g.*, beached on a platform. Urination on command is not trained yet, but beaching is, and it is hoped that the training can progress.

Eye secretion has been collected when the porpoises were taken out of the water for medical examinations, but could also be collected at pool-side. Saliva was collected by voluntary husbandry behaviour from poolside, 3 times a week for the female and twice a week for the male from spring to autumn 2000 and 2001.

Vaginal and prepucial smears
The physiological phase of females can be monitored in many species by looking at cytological changes in the vaginal sphere, which can be examined on vaginal smears (*e.g.*, D'Souza 1978). Prepucial smears can be used to monitor the release of spermatozoids, the end product of testicular exocrine activity. In addition mating can be confirmed by the presence of sperm on vaginal smears.

Genital smears are collected by inserting a swab in the genital slit and then smearing it on an object glass. The slides are then stained with the Wright-Giemsa coloration (Haema-Schnell-Färbesatz, Heiland, Germany).

Genital swabs were taken monthly from Freja and Eigil between November 1997 and June 1999. From June 1999, vaginal swabs could be obtained from poolside and were collected once a week and every time a successful mating was suspected. Since spring 2000, they have been collected 3 times per week and every time a successful mating was suspected from spring to autumn, otherwise once a week. Prepucial swabs have been collected once a week. With Nuka, swabs could be collected about every 2 weeks from July 21, *i.e.*, about 5 weeks after she had been released with the 2 adult animals.

Semen collection
Semen collection, which should allow for comparison of semen production and testosterone levels and provide data on semen quality, requires the training/co-operation of the male. The training, which can only be performed in spring and summer in such a seasonal animal, is not completed, although progressing. No sperm collection has been performed yet.

Non-invasive techniques
Behavioural observations
Behavioural observations are non-invasive, but time-consuming and require some consistency in observers. In semi-natural conditions such as those at the Fjord&Bælt, external conditions such as weather and water visibility might impair the quality of observations.

An ethogram was adapted from previous studies (Andersen and Dziedzic 1964, Andersen 1969, Amundin and Amundin 1971, 1974) and completed through preliminary opportunistic observations. Two types of systematic observations are carried out following Altmann (1974), Martin and Bateson (1993) and Mann (1999):

i) Dedicated observations, where the behaviour of the porpoises is recorded by focal sampling on a Psion palmtop computer, using behaviour study software (The Observer 3.0, Noldus Information Technology), from a balcony overlooking the enclosure (Fig. 1). Observations are mostly conducted over 2 to 3 hr evening sessions, but whole-night sessions and rolling 2 to 3 hr sessions through 17:00 to 09:00 are also carried out.
ii) Recording of sexual behaviour during feeding and training sessions by the trainers, using the one-zero sampling technique. Such recording has been done since the animals arrived at the centre.

Environmental conditions (tidal state, *i.e.* current direction and speed, wind force and direction, water temperature and clarity) and human activities around the enclosure are recorded during the observations.

During some of the observations, the activity of the porpoises is recorded on a fixed colour surveillance camera. Acoustic activity is then picked up with hydrophones, transformed to the human audible range by means of click detectors (custom made; Loughborough University), and recorded on the audio channels of the VCR's tapes.

Behavioural observation have mostly been/are conducted by students in the framework of BSc and MSc programs, each of them focussing on a different aspect. The methodology and the general ethogram are common to all projects. The configuration for data entry and the behaviours recorded vary, however, from project to project:

i. BSc University of Southern Denmark (Kristensen 1999): Changes in plasma testosterone level and behaviour in a male during sexual maturation - observations conducted in the summer and fall of 1998.
ii. MSc University of Bangor (Benham 1999): Preliminary results of an ongoing investigation into the socio-sexual behaviour of an adult female harbour porpoise in captivity - observations conducted in June - September 1999.
iii. MSc University of Bangor (Wilson 1999): Investigation into the behaviour of a juvenile harbour porpoise in captivity - observations conducted in June - September 1999.
iv. MSc University of Southern Denmark (Jepsen 2002): The sexual behaviour of a female harbour porpoise in relation to the concentration of sexual hormones, measured in blood and other secretions - observations conducted in March 2000 - February 2001.

PRELIMINARY RESULTS AND DISCUSSION

The project is providing long-term physiological data never obtained before on harbour porpoises, as well as interesting behavioural parallels. Also behaviours not reported before for porpoises have been described (Benham 1999, Wilson 1999, Jepsen 2002). Preliminary results have been presented at conferences (Desportes *et al.* MS 1999a,b,c, Kristensen *et al.* MS 1999, Benham *et al.* MS 2001, MS 2002, Desportes *et al.* MS 2001a, Jepsen *et al.* MS 2001, MS 2002a,b, Wilson *et al.* MS 2001, MS 2002), and selected examples of the results obtained are presented below.

Body temperature
The rectal temperature varied between 34.2° C and 36.8° C in the male, 35.4° C and 36.9° C in the older female and 36.3° C and 36.6° C in the juvenile female. Until now, rectal temperature has mainly been taken during medical checks, on a monthly basis, and the variation observed could not be related to any particular factors. Frequent and regular measurements at pool-side may allow detection of cyclical variations.

Testis development
Ultrasound examination of the testes was performed for the first time on May 25, 1998, when Eigil was 135 cm in body length and weighed 42.7 kg. A testis length/diameter of 140 x 35 mm was recorded for both testes. There are few other reports of testis size in harbour porpoises. Sørensen and Kinze (1994) examined 102 testis pairs from porpoises stranded on the Danish coasts, and reported that immature testes were less than 80 mm long and were found in porpoises less than 131 cm long (n=69). Bandomir and Siebert (unpublished data) gave 77 x 33 mm and 71 x 33 mm as the size of the largest immature testis pair, while the smallest mature testis pair measured 113 x 55 mm and 133 x 57 mm for an animal stranded in February. Seasonal variation in size and weight is only observed in mature testes (Desportes *et al.* 1993, Read and Hohn 1995). In May 1998, Eigil's testes were larger than the maximum size reported for immature testes, suggesting that, at a body length of 135 cm, he had become sexually mature.

A length of 135 cm at sexual maturity is also in agreement with previous results (for a review, see Lockyer 2003). Sørensen and Kinze (1994) estimated 130.8 cm as the median length of males at sexual maturity. Lockyer (1995) estimated that males from British waters attain sexual maturity when body length was greater than 130 cm. Karakosta *et al.* (1999) reported that mature males from British waters always exceeded 140 cm in length and 40 kg. Using body weight as criteria for sexual maturity should, however, be done with caution because of the dramatic seasonal variation in weight (Lockyer *et al.* 2003). As an example, in 1998 when Eigil became mature, his body weight varied from 44.75 kg in January to 37.4 kg in October.

Testes size varied considerably throughout the year. The smallest testis size was recorded in January (95 x 35 mm) and the largest one in June (201 x 86 mm). The sharp increase in testicular volume observed from June to August in the year 1999 and 2000 (Fig. 3) fits well with the June-August sharp increase in testis mass described by Sørensen and Kinze (1994) for Danish porpoises.

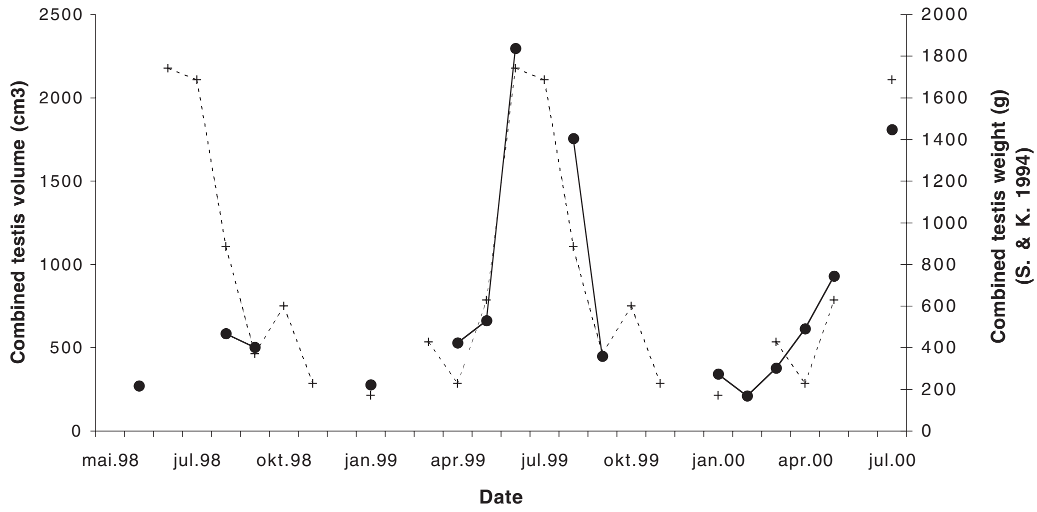


Fig. 3. *Seasonal changes in the combined size of Eigil's testes, expressed as their volume to. The variation in testis mass, given by Sørensen and Kinze (1994) for Danish porpoises is indicated for comparison as a dashed line.*

Plasma testosterone concentrations in 1997-98

The testosterone profile of Eigil in 1997-98 is presented in Fig. 4. Peaks were observed in May-June both in 1997 and 1998, with the levels sharply increasing from less than 1 ng/ml to 12 ng/ml and 30 ng/ml respectively. The timing of increase and decrease in the testosterone levels was similar in both years, though the peak was much higher in the second year. If the testosterone and testicular volume profiles were similar in 1998 and 1999, then the hormonal increase would have occurred early in the season, preceding by about a month the June-August peak in testis mass.

The maximum testosterone concentration observed in Eigil is somewhat higher than the maximum observed in mature male Dall's porpoises, reported as 20 ng/ml by Temte (1990). It is also higher than those reported for mature bottlenose dolphins and long-finned pilot whales, but lower than those observed in mature spinner dolphins (for a review, see Desportes *et al.* 1994).

Plasma oestrogen and progesterone concentration in 1997-2000

In the period April 1997 – March 2001, Freja showed progesterone and oestrogen levels varying between less than 1 to 17 ng/ml and less than 50 to 4,504 pg/ml respectively. The absence of sustained high levels of progesterone in the

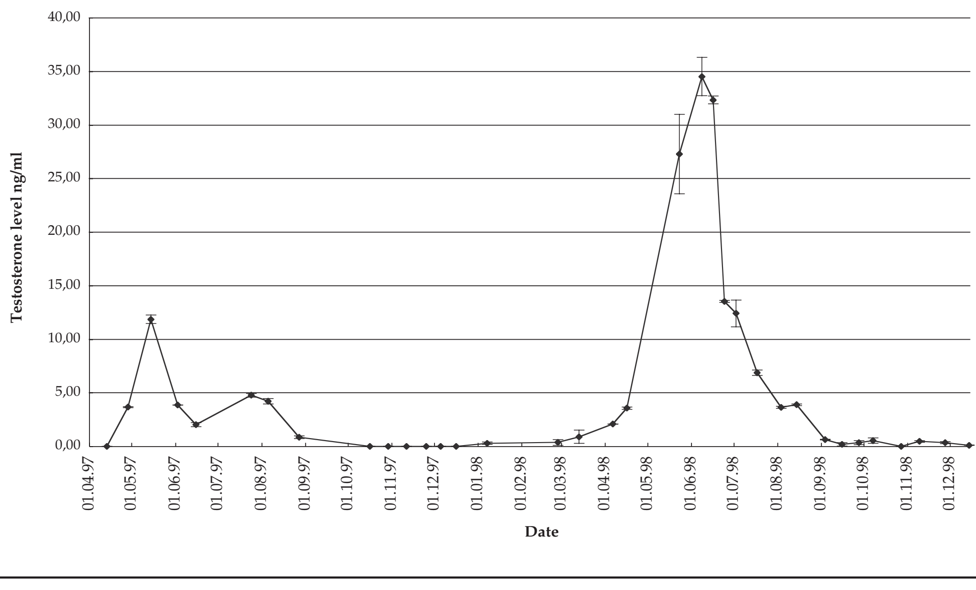


Fig. 4. *Eigil's testosterone profile between April 1997 and December 1998.*

autumn periods confirmed that Freja had not become pregnant. A much higher level of progesterone (42 ng/ml) was observed in a wild female accompanied by a calf in April 1999 (Desportes, unpubl. data).

These results provide the first range of values for oestrogen and progesterone levels in harbour porpoises. Peaks were clearly observed for both hormones, but did not exhibit any clear seasonal pattern (Jepsen 2002, Jepsen *et al.* MS 2001, MS 2002a,b). Blood was collected at a minimum interval of 10 days, which may have prevented capturing some of the relatively short term secretion peaks which characterise mammalian ovarian activity (*e.g.* Bronson 1989). Clearly, a detailed picture of the ovarian endocrine cycle requires more frequent sampling. We are presently investigating the feasibility of measuring levels of sexual hormones in saliva and eye secretion, which are much more easily collected.

Sexual and social behaviour

Opportunistic behavioural observations have been carried out since the animals arrived at the Centre in April 1997. Dedicated observations focussed on reproductive behaviour were carried out in the summer and fall of 1998 and 1999 and between March 2000 and March 2001.

Wilson (1999) concentrated her observations on the introduction of the one-year old female to the 2 older porpoises, estimated then to be 4 to 5 years old. She described how and how much the 3 animals interacted with one another, in pairs or as a trio, both in a social and a sexual context (defined as the presence or absence of an erection in the male). She also recorded the attempts of the male to mate with the juvenile female. The juvenile female predominantly preferred a solitary behaviour pattern. Social interactions with the adults were few, mostly instigated by them and characterised by their dominance. The activity of the juvenile changed over time, in parallel with age-related development, increased familiarity with the environment, a surrogate mother-calf relationship with the adult female and the breeding-season related activities of the adults. If these results can be generalised to the wild, they might partly explain the apparent vulnerability of wild juveniles to bycatch, which tends to be skewed towards year classes 0 and 1 (Wilson 1999, Wilson *et al.* MS 2001, MS 2002).

Benham (1999) focussed on the behaviour of the adult female, especially looking at her role in sexual activity and courtship behaviours. A marked reproductive seasonality was observed. The period when the female was most receptive (as indicated by behaviour and vaginal cytology) and when successful matings were observed, was the last 2 weeks of July and the first week of August. The female was not seen to initiate mating behaviour, but exhibited specific behaviours in response to the male‘s advances, which decided the outcome of the mating attempts (Benham 1999, Benham *et al.* MS 2001, MS 2002).

Kristensen (1999) focussed on the male's behaviour and its hormonal correlates. Infrequent erections had been observed since the arrival of the male at the Centre in April 1997. Mating attempts were first observed in October 1997, and continued throughout the winter of 1997-98, in spite of very low levels of testosterone (<1ng/ml). Sexual behaviour of the male (defined as erection or mating attempt) during training sessions (Fig. 5) was observed first in November 1997 and continued more or less throughout the entire winter until the end of August 1998. The frequency of occurrence varied during the observation period and peaked around January, mid-April, the end of May and the second half of July. During dedicated observations in summer and fall 1998 (evening or night), no mating attempts were observed before July (Fig. 6). They were frequent in July and August, and stopped during the last week of August. The cessation of sexual activity, observed both during training sessions and dedicated observations, coincided with very low testosterone levels. The frequent observation of sexual behaviour during training sessions and their absence during evening and day observations in June, may be due to specific body positions of the female during training that elicited a sexual response in the male. The female's immobility while stationed at the surface by the trainer may in particular have triggered mating attempts by the male. The peak in testosterone level preceded the period of highest frequency

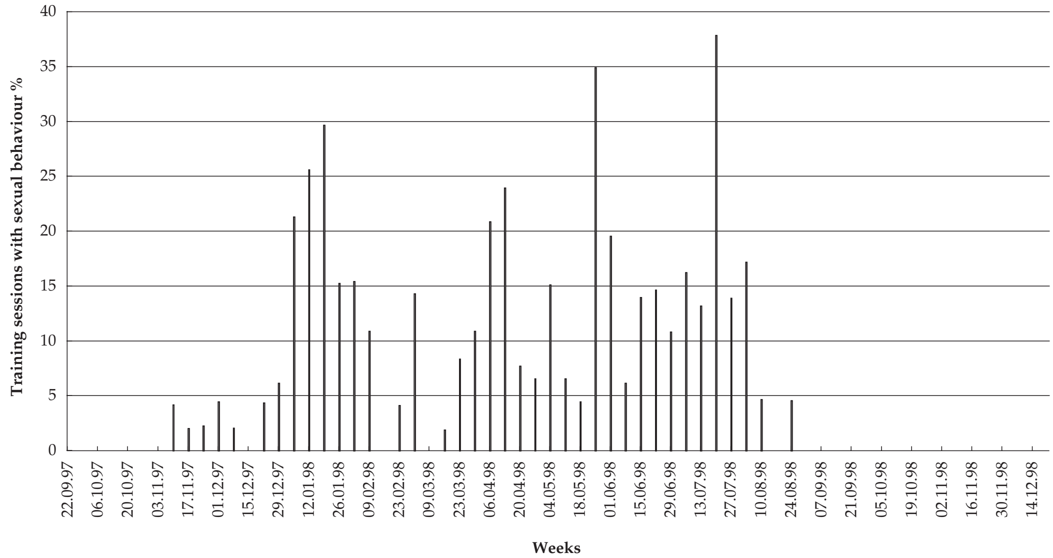
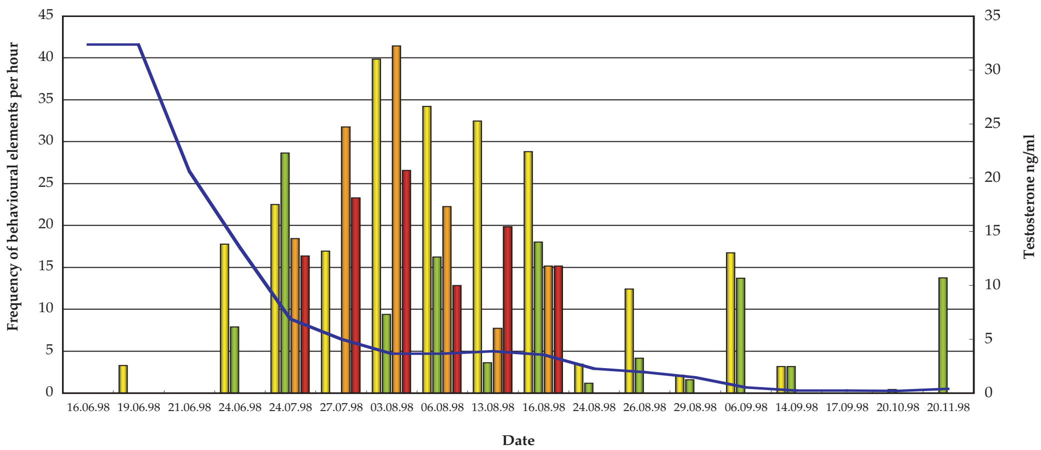


Fig. 5. *Occurrence of sexual behaviour during training sessions between September 1997 and December 1998, expressed as the weekly percentage of training sessions where sexual behaviour was observed.*

of sexual activity. It should be noted, however, that the majority of the dedicated behavioural observations were conducted after the occurrence of the testosterone peak (Kristensen 1999, Kristensen *et al.* MS 1999, Desportes *et al.* MS 1999a,b).

Testosterone level and testicular activity peaked dramatically in summer, fitting well with the clearly seasonal cycle described for wild porpoises (*e.g.* Møhl-Hansen 1954, Read 1990, Sørensen and Kinze 1994). In this perspective it is interesting that Eigil started showing signs

Fig. 6. *Frequency of interactions (yellow), non sexual contacts (green), erections (orange) and mating attempts (red) during dedicated observations (morning and evening observations combined) between July 1998 and December 1998. The frequency is expressed as the number of events per hour. Note that the date axis is not drawn to scale. Observations were conducted at all the dates mentioned, i.e., no bar indicates that the specific behaviour was not observed. The testosterone profile during the same period is indicated (blue line).*



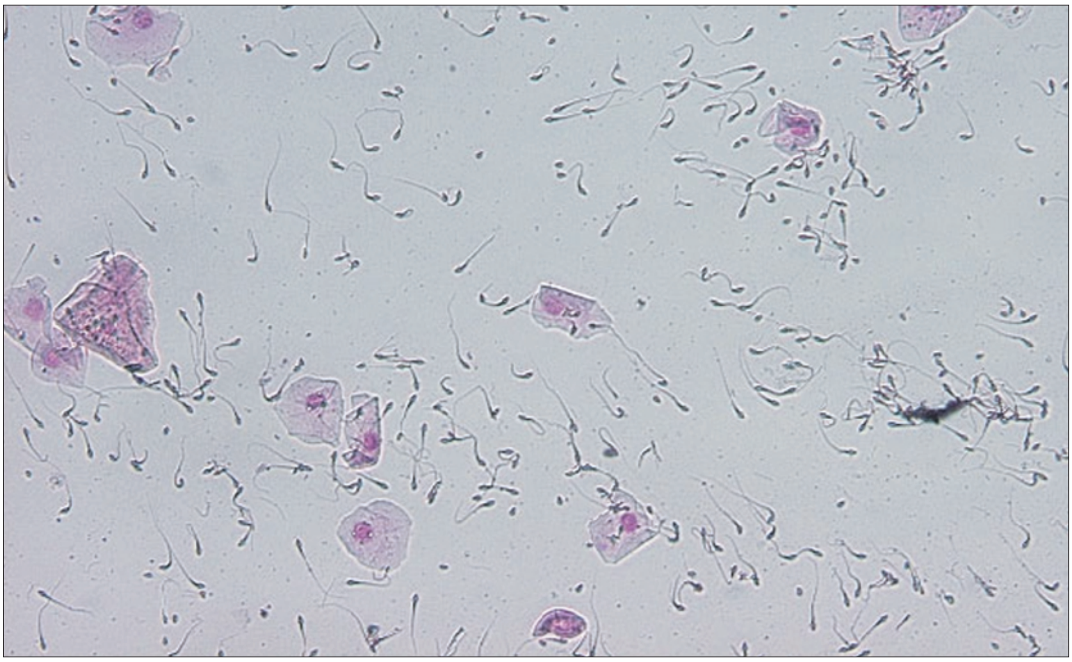


Fig. 7. *Freja's vaginal smear on August 3, 2000 (x 100, Wright-Giemsa coloration). Vaginal epithelial cells and spermatozoids can be seen.*

of sexual activity and attempted to mate for the first time in the fall of 1997. Mating attempts continued then throughout winter and spring despite low levels of testosterone. This could be interpreted as a captivity artefact, caused by the constant presence of the female, but it may also be a normal training "exercise" for a pubertal male. It is indeed noticeable that Eigil has not shown, apart from a few erections, any sign of sexual activity in the fall and winter of the following years, although grooming was regularly and frequently observed and the proximity with the female has remained constant. Also the behavioural evolution through time observed in the male's mating attempts tend to support this "training hypothesis". Mating attempts were at first characterised exclusively by sudden and very fast approaches by the male with an erection, with very short and "violent" contacts, not always even involving genital areas. These turned progressively into slower approaches with "softer" and better positioned contacts, involving genital areas.

Mating period

Dedicated behavioural observations offer only limited access to the overall reproductive activity of the porpoises, both because they are limited in time and because the occasional turbidity of the water and the configuration of the enclosure reduce the availability of the porpoises to the observers. It may also be difficult to judge whether a mating attempt has ended successfully. Vaginal swabs shed light on the success of mating attempts, besides providing information on the hormonal phase of the female (Fig. 7).

In 1998, when vaginal swabs where collected monthly, sperm was present on vaginal smears only in July and August. In 1999 when vaginal swabs were collected once a week and every time a successful mating was suspected, sperm was observed only in the first half of August. In 2000 and 2001 when vaginal smears were collected 2 to 3 times a week and every time a successful mating was suspected, sperm was present in July and definitely abundant in August (Jepsen 2002, MS 2002a, b). Although successful mating has been confirmed in 4 consecutive summers, pregnancy has not occurred, suggesting the possibility of some kind of dysfunction. The irregular oestrogen and progesterone cycles could point to hormonal anomalies in the female.

During the summer of 1999, Eigil tried to mate on numerous occasions with the juvenile female, although less frequently than with the mature female. Sperm was, however, never observed on the vaginal smears from the juvenile female.

CONCLUSION

The past 5 years of data collection have shed light on the timing of different physiological and behavioural reproductive events in harbour porpoises. It has generated a rough picture of the onset and the establishment of behavioural and physiological sexual activity and, for the first time, long-term profiles of testosterone, oestrogen and progesterone.

"…the initial use of several techniques and the subsequent construction of a reproductive cycle profile, will, in the end, prove more informative than results derived predominantly from concentration on a single method." (D'Souza 1978). An integrated approach to behavioural, morphological, histological and hormonal assessment of reproductive status is seldom possible in cetaceans, but offers the best tool for understanding and monitoring reproductive events. It is expected that the correlation of reproductive hormone fluctuations with ultrasound imaging of ovaries and testes, semen production and vaginal cytology will lead to a better understanding of the reproductive processes in harbour porpoises.

This approach should also provide the necessary basis to assess reproductive status in wild free-ranging porpoises, when only limited, opportunistically-collected samples are available. We hope it will generate comparative baseline data for toxicological studies since the natural ingestion of pollutants through the food and the level of contaminants in the blood can be monitored. In this regard, the ability to monitor semen quality and production might be particularly interesting.

ACKNOWLEDGEMENTS

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