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Controlled Unassisted Breeding of Captive Indo-Pacific Bottlenose Dolphins, *Tursiops aduncus*, Using Ultrasonography

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Abstract

Ultrasonographic monitoring of folliculogenesis and prediction of ovulation was used for ten years to control breeding in a group of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) at Ocean Park, Hong Kong, where male and female dolphins were housed separately from 1991. When folliculogenesis was identified in a selected female, the cycle was monitored until ovulation was predicted within the next 12 to 24 h; a male was selected and then placed with the female overnight.

Ultrasonography was repeated the next morning to determine ovulation. If ovulation had occurred, the animals were separated; if not, the pair remained together until ovulation was confirmed. Ultrasonography was subsequently used to monitor the development of the corpus luteum and to identify pregnancy. This procedure was conducted 11 times between 1993 and 2003.

In nine cases, ovulation was predicted accurately and occurred within 24 hours. In two cases, ovulation occurred within two to four days of the predicted date. In ten cases, pregnancy ensued. To date, nine calves have been born live after gestation lengths of 349-382 days.

Key Words: Bottlenose dolphin, *Tursiops aduncus*, ovarian cycle, controlled breeding, ultrasonography

Introduction

Bottlenose dolphins have been maintained in zoological parks and oceanaria since at least the 1930s, usually in small groups. Dolphins are reproductively successful in captivity, and many calves have been born (Asper et al., 1992; Cornell et al., 1987; Duffield et al., 1995). Calf mortality remains high (Joseph et al., 2000), however, and some animals have not reproduced due either to inappropriate management or possible fertility problems (Cornell et al., 1987; Joseph et al., 2000).

The self-sustainability and genetic health of small, enclosed animal populations pose concerns and should be managed carefully to "preserve genetic diversity and retain reproductive fitness" (Montgomery et al., 1997). Inbreeding reduces reproductive fitness and health and impacts wild marine mammal populations (Acevedo-Whitehouse et al., 2003); however, little attention has been given to genetic management and controlled breeding in captive dolphins. Recently, the captive population of bottlenose dolphins was described as "limited," and it was stated that their management should be improved (Lacy, 2000).

This need is now acknowledged, and there is increasing interest among marine mammal curators to plan and manage breeding. Of the more than 40 facilities represented at an international Dolphin Breeding Workshop in San Diego in 1999 (Duffield & Robeck, 2000), however, only 15 (36%) returned a completed questionnaire giving details of dolphin reproduction. Of the 15, 11 reported they were "intentionally managing" a breeding program for their dolphins, and 14 facilities intended to manage this over the next ten years; however, there was no information given about the management strategies used or proposed. In addition, seven facilities reported they were "actively discouraging" reproduction, but unplanned, incidental reproduction reportedly still occurred in at least 20% of facilities (Joseph et al., 2000).

The role of advanced reproductive technology, such as artificial insemination (AI), in reproductive management of dolphins has been investigated for some time (Robeck et al., 1994; Schroeder, 1990) and successful AI in the killer whale (*Orcinus orca*) and the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) is reported (Brook & Kinoshita, 2004; Kinoshita et al., 2004; Robeck, 2001; Robeck et al., 2001; Robeck et al., 2004). The advantages of AI in small, enclosed populations are obvious, and AI clearly is needed, among other tools, to manage captive stocks of dolphins in the long term. Transporting genetic material, rather than live animals, between institutions is a safe and more cost-effective option; however, AI in dolphins is still in its infancy—the success rate is low and few facilities have the resources or expertise to manage their dolphin breeding programs using this method. At this time, we believe AI is not required immediately by every facility in which dolphins are maintained.

Good husbandry and changes in the management of unassisted breeding may be extremely effective (Lasley & Anderson, 1991), making AI unnecessary in the short term for some facilities with adequate numbers of animals. Controlled unassisted breeding of dolphins could be used to preserve genetic diversity by enabling all individuals to reproduce. If the timing of insemination is predicted, all mating pairs and pregnancies can be planned and controlled. This control allows curators to minimize inbreeding and ensure all capable individuals produce offspring, thus maximizing the genetic diversity of the next generation. For AI to attain the same results, all captive males must be trained for voluntary semen collection, semen must be evaluated and preserved effectively, all reproductively fit males must be represented in AI procedures, and insemination techniques need to become more reliable. This is unlikely to be accomplished soon.

Ultrasonography is a simple, noninvasive, and cost-effective imaging modality that allows detailed scrutiny of the reproductive organs in many species. Since 1990, ultrasonography has been used to investigate and monitor reproductive events in a group of Indo-Pacific bottlenose dolphins maintained at Ocean Park, Hong Kong. These studies showed that ultrasonographic monitoring of ovarian function allows accurate identification of folliculogenesis and prediction of ovulation in individual dolphins (Brook, 1997, 2000, 2001; Brook et al., 2001). This technique also is effective in other cetacean species, including killer whales, false killer whales (*Pseudorca crassidens*), belugas (*Delphinapterus leucas*), and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) (Robeck et al., 2001).

Prior to 1993, Ocean Park was not successful in breeding dolphins. Only six pregnancies had been recorded in three *T. aduncus* over a period of almost 20 years. Of these, three (50%) ended in abortion or stillbirth, two live-born calves died from trauma, and one calf died at two years of age from gastrointestinal problems. Whether the poor survival rate was due in part to females being first-time mothers or to the inappropriate management of pregnancy and parturition is not clear (Kinoshita et al., 2000).

Another factor to consider at Ocean Park is the weather. There are annual monsoon and typhoon seasons in Hong Kong, when weather conditions can be severe and exposure to certain pathogens, such as *Pseudomonas pseudomallei*, may be increased (Hicks et al., 2000). These conditions may affect calf survival, and it is necessary to avoid parturition at these times.

In 1993, a carefully planned reproduction and breeding program was set up with the objectives of using controlled breeding to ensure a self-sustaining population of *T. aduncus*, as well as further collection of scientific data about the species (Kinoshita et al., 2000).

Materials and Methods

In 1993, Ocean Park had four reproductively mature *T. aduncus* females (F1-F4) and five mature males in a small group. Three females and one male were from Taiwan; the rest were from Indonesia. The other two females later included in the program (F5 & F6) arrived from Indonesia in 1997. Although detailed DNA studies had not been conducted, they had been caught at different times and places, and it is unlikely that any are closely related. Males were separated from females, although often in adjoining pools, which allowed auditory and visual contact. Diet and pool dimensions for this population are reported in Brook (2000, 2001).

Ultrasonography had been used to determine pre-ovulatory follicular size in all females and has been used since 1992 in this facility to assess ovarian activity weekly. Maturity of males was determined by assessing testicular size and appearance using ultrasonography (Brook, 2000) and by the presence of sperm in ejaculates collected using voluntary behavior. Ultrasound examinations were performed under voluntary behavior with the animals in the water. Aloka SSD 630, 900, and 1700 units [Aloka Co. Ltd., Mitakashi, Tokyo] were used, with a 3.5 MHz linear or 3.5-5.0 MHz curvilinear transducer.

When a female selected for breeding was determined to be ovulating, the growth of the follicle was monitored carefully until 12 to 24 h before ovulation was predicted. She then was placed in a separate pool and a selected male was placed with her overnight. The animals were observed intermittently and copulations noted. Ultrasound examination the next morning was used to determine ovulation. If ovulation had occurred, the animals were separated and returned to their respective social groups; if not, the pair was left together until ovulation was confirmed. Ultrasonography was used to follow development of the corpus luteum and to identify pregnancy. This standardized procedure was conducted 11 times between 1993 and 2003.

Once pregnancy was confirmed, a detailed protocol for managing pregnant females and parturition was followed (Kinoshita et al., 2000) and special attention was given to maintaining husbandry behaviors so that maternal and fetal well-being could be monitored throughout gestation.

Results

In nine of the 11 cases, ovulation was predicted accurately and occurred within 24 h. In the remaining two cases, ovulation occurred within two to four days of the predicted date (Table 1).

Pregnancy occurred in ten cases. To date, nine calves have been born live after gestation lengths of 349-382 days.

In Case 9, the female fetus had appeared morphologically normal on prenatal ultrasound examination, but fetal growth parameters and reduced amniotic fluid volume indicated a problem. The pregnancy went to term (368 days), but the calf was stillborn.

In Case 10, ovulation appeared to occur normally, followed by the expected rise in serum progesterone levels. Mating was observed, but no pregnancy ensued.

Of the nine live-born calves, one (Case 2) was born after a gestation of only 349 days and was suspected to be premature. A routine ultrasound examination one week before birth showed hyperechoic patches in the left lung, consistent with consolidation/infection. The calf demonstrated obvious respiratory distress and difficulty in swimming and died three days later. Necropsy confirmed pulmonary infection and extensive consolidation.

One calf (Case 4) was traumatized by its first-time mother and died within hours. The remaining seven calves survived (Table 1).

On each occasion, males were paired with a different female so that no resulting calves would be full siblings. This increases the number of possible breeding pairs in this population (Figure 1).

Discussion

Ocean Park recognized that, for the breeding program to be successful, teamwork and husbandry practices had to change, particularly the need to separate and resocialize male and female dolphins. It appears that separating males and females improved maternal well-being and calf survival. Although not studied in detail, there is no obvious evidence that the absence or reintroduction of males affects or alters breeding activity in this group.

Table 1. Details of 11 controlled, unassisted breeding procedures in *Tursiops aduncus* conducted at Ocean Park, 1993-2003; I=dolphins from Indonesia, T=dolphins from Taiwan, O=ovulation, Dcd=deceased.

Case number	Dates of mating/ ovulation	Female (estimated age in yrs)	Previous known births	Male (estimated age in yrs)	Previous sire	Estimated date of delivery	Date of delivery	Outcome
1	23/4/93	F1 [T] (18+)	2	M1 [T] (20+)	Yes	18/4/94	2/5/94	Live male, healthy
2	23/6/93	F2 [T] (23+)	1	M2 [I] (23+)	Yes	2/7/94	7/6/94	Live male, respiratory distress [Dcd. Day 3]
3	27-28/7/94	F3 [I] (10+)	1	M3 [I] (10+)	No	21-22/7/95	19/7/95	Live male, healthy
4	30-31/3/95	F4 [T] (11+)	0	M4 [I] (12+)	No	8/4/96	3/4/96	Live male, trauma by dam [Dcd. Day 1]
5	12/4/96	F1 [T] (21+)	3	M3 [I] (12+)	Yes	17/4/97	1/5/97	Live male, healthy
6	5-9/9/97 [O: 7-9/9/97]	F4 [T] (12+)	2	M5 [I] (30+)	No	16-18/9/98	17/9/98	Live male, healthy
7	27-28/4/98	F3 [I] (14+)	2	M5 [I] (31+)	No	20-21/4/99	1/5/99	Live male, healthy
8	29/12/99-3/1/00 [O: 2-3/1/00]	F5 [I] (15+)	0	M4 [I] (16+)	Yes	24/12/00 - 7/1/01	5/1/01	Stillborn female
9	21-22/5/00	F1 [T] (25+)	4	M4 [I] (17+)	Yes	25/5/01 - 8/6/01	3/6/01	Live female, healthy
10	28-30/8/03 [O: 29-30/8/03]	F6 [I] (15+)	0	M4 [I] (20+)	Yes	No pregnancy	-	-
11	25-27/10/03 [O: 26-27/10/03]	F3 [I] (19+)	3	M4 [I] (20+)	Yes	19/10/04 - 6/11/04	28/10/04	Live male, healthy

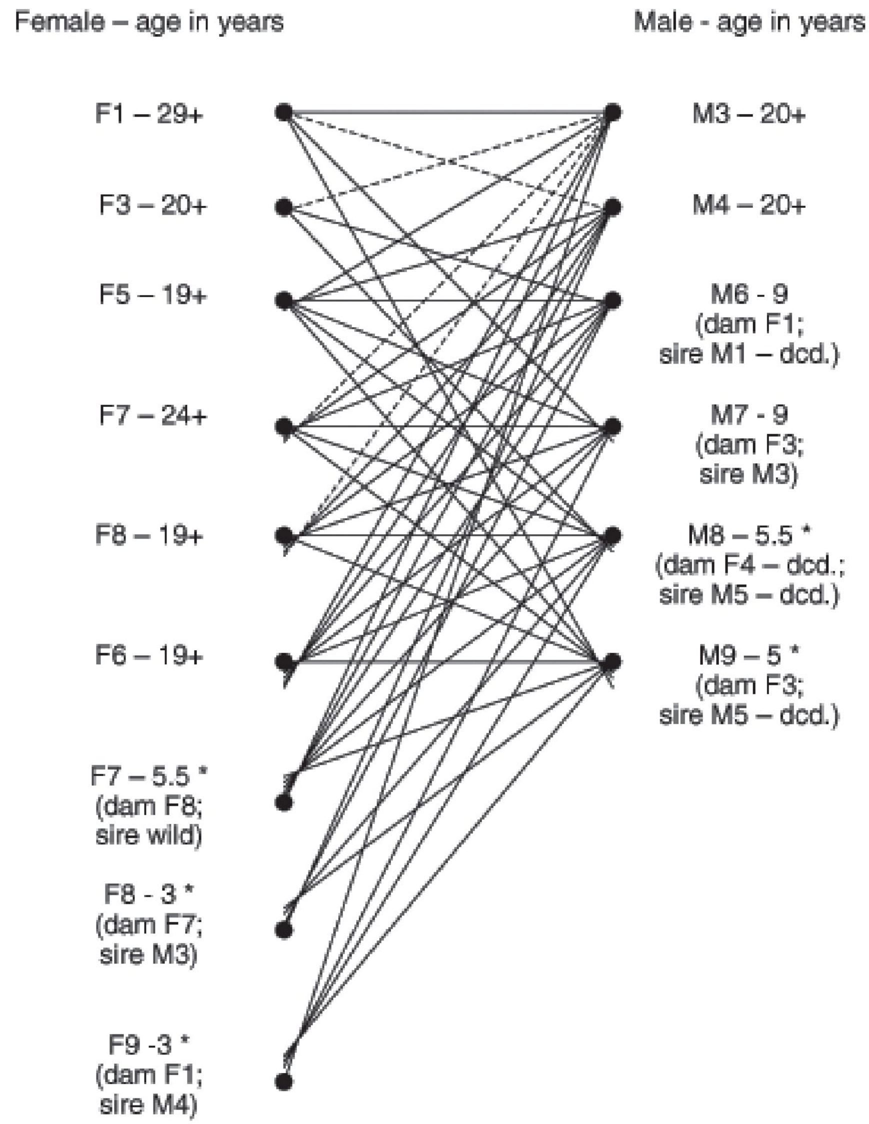

Figure 1. Schematic showing 40 further breeding options in 15 *Tursiops aduncus*, which would not produce any full siblings in the next generation of calves at Ocean Park; * denotes sexually immature animal, and ----indicates a pair which have already produced a surviving calf.

We did not understand why Case 10 did not succeed when all others resulted in pregnancy. The male (M4) is a proven sire. The female (F6) arrived in Ocean Park in 1997; her reproductive history is unknown. She is known to have had parasitic mastitis, but this is not thought to affect fertility. This female was treated with a synthetic oral progestagen (Regu-mate, Hoechst Roussel Vet, Melbourne, Australia) from 30 April to 29 May 2003. A 1.0-cm follicle was observed on 25 June, however, and this developed into a 4.7 x 4.2 cm, thick-walled cyst and no ovulation occurred. The cyst persisted for several weeks before resolving. Female F6 cycled again in September when the controlled unassisted breeding was conducted. Ovulation (29-30 August 2003) appeared to be normal, but no pregnancy occurred. We have encountered similar problems with the use of

Regu-mate before this case. Further research is required to evaluate the effects of synthetic oral progestagens on ovulation and fertility in dolphins.

This controlled breeding program allowed all mature males to participate in breeding and most to be represented in the next generation, which may not have been achieved without such control. Previous studies showed that, even in relatively large groups of captive dolphins, uncontrolled breeding may result in only a few males, or even just one dominant animal, siring calves (Duffield, 2001).

Since 1993, all sexually mature males at Ocean Park have had an opportunity to reproduce. This is particularly important since three of the founder males in the breeding program have died, two at advanced ages; two males (M1 and M5) are represented in three living offspring. The third male (M2) sired four calves in his lifetime; however they all were small/weak. One was anencephalic (Brook, 1994) and none survived. Although M2 was a founder male, it was considered too much of a risk at that time to use him as a sire again, and he was excluded from the breeding program in 1994. There are anecdotal reports of similar losses of multiple calves from a single sire in other facilities. It may be that some dolphins carry deleterious genes that render them reproductively ineffective; this is recognized in other species (Montgomery et al., 1997). If curators rely on a single, reproductively unfit male for breeding, the implications for the next generation and/or impact on calf survivorship can be severe.

Identifying folliculogenesis and thereby controlling breeding in captive groups allows physical interaction between males and females, while avoiding the risk of pregnancy. This allows reproductively sound females to breed only with reproductively sound males.

Notwithstanding his suspect history, semen from M2 has been cryopreserved for further evaluation and possible future use. Semen from M5 also has been cryopreserved for future AI procedures and may be used to produce more offspring. Two further males born at Ocean Park (M6 and M7) have now attained sexual maturity and will be included in the breeding program in the near future.

A *Bottlenose Dolphin North American Studbook* is kept to record *Tursiops truncatus* births in the United States (Dudley et al., 1999). The report of 1999 lists 661 captive births. The same 16 males sired almost 20% of these calves, and fewer than 70 male *T. truncatus* are recorded as having produced offspring between 1953 and 1998. Some managers obviously have relied on only one mature male for many years, even transferring the same male between several facilities. This greatly exacerbates the problem of inbreeding, as these males will be overrepresented in subsequent generations (Barber, 2000). Also, multiple calves have been born to the same male/female pairs, further increasing inbreeding. Of the hundreds of *T. truncatus* in captivity in North America, many animals have not had the opportunity to reproduce and much genetic diversity has been lost from this population over the past fifty years. This is also likely in other facilities in other regions. With the technology and techniques now available, it is our opinion that this approach to breeding management can be changed.

Artificial insemination of cetaceans has been accomplished (Brook & Kinoshita, 2004; Kinoshita et al., 2004; Robeck et al., 2001, 2004) and will allow the necessary control of breeding in the total captive dolphin population by introducing new genetic material into closed groups; however, known success rates of AI in cetaceans are only 33% (Brook & Kinoshita, 2004) and 38% (Robeck et al., 2004) and further research is required to improve the application of this technique in these animals. The technology and equipment for successful preservation of semen may not be widely or quickly available, and it is likely that the genetic diversity of many animals will be lost before they have reproduced. Ultrasonographic assessment of the ovaries can be used to reliably identify and monitor the reproductive status of live female dolphins and to predict ovulation (Brook, 1997, 2000, 2001; Brook et al., 2001), allowing unassisted breeding to be planned and controlled.

The present study shows a success rate of 91% in controlled, unassisted breeding. As expected, this is much higher than AI. Controlled breeding of captive dolphins will ensure that all reproductively fit animals within one group are able to reproduce and can be used to minimize inbreeding, depending on the number of animals within the group. A combination of these two methods should ensure the continued genetic health and self-sustainability of the bottlenose dolphin in captivity, greatly reducing the need to take more animals from the wild.

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Literature Cited

Acevedo-Whitehouse, K., Gulland, F., Greig, D., & Amos, W. (2003). Disease susceptibility in California sea lions. *Nature*, *422*, 35.

Asper, E. D., Andrews, B. F., Antrim, J. E., & Young, W. G. (1992). *Establishing and maintaining successful breeding programs for whales and dolphins in a zoological environment* (International Marine Biological Research Institute Report No. 3). Kamagowa, Japan: International Marine Biological Research Institute.

Barber, R. C. (2000). Inbreeding and heritable diseases in captive populations. In D. A. Duffield & T. R. Robeck (Eds.), *Report from the Bottlenose Dolphin Breeding Workshop* (DBW184-189) (pp. 184-189). Silver Spring, MD: American Zoological Association Marine Mammal Taxon Advisory Group. 376 pp.

Brook, F. M. (1994). Ultrasound diagnosis of anencephaly in a bottlenose dolphin. *Journal of Zoo and Wildlife Medicine*, *25*(4), 123-128.

Brook, F. M. (1997). *The use of diagnostic ultrasound in assessment of the reproductive status of the bottlenose dolphin,* Tursiops truncatus aduncas, *in captivity and applications in management of a controlled breeding programme.* Ph.D. dissertation, The Hong Kong Polytechnic University, Hong Kong. 339 pp.

Brook, F. M. (2000). Sonographic testicular and ovarian assessment in the bottlenose dolphin, *Tursiops truncatus aduncus*. In D. A. Duffield & T. R. Robeck (Eds.), *Report from the Bottlenose Dolphin Breeding Workshop* (DBW184-189) (pp. 207-222). Silver Spring, MD: American Zoological Association Marine Mammal Taxon Advisory Group. 376 pp.

Brook, F. M. (2001). Sonographic imaging of the reproductive tract of the female bottlenose dolphin, *Tursiops truncatus aduncas. Reproduction*, *121*, 419-428.

Brook, F., & Kinoshita, R. E. (2004). Ultrasonographic assessment of the ovarian cycle in the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) at Ocean Park, Hong Kong and its use in controlled natural breeding and artificial insemination. In *Proceedings of the 35th Annual Meeting of the International Association of Aquatic Animal Medicine* (pp. 61-63).

Brook, F., Van Bonn, W., & Jensen, E. (2001). Ultrasonography. In L. Dierauf & F. Gulland (Eds.), *CRC handbook of marine mammal medicine* (pp. 593-620). Boca Raton, FL: CRC Press. 1,063 pp.

Brook, F., Rourke, N., Mauroo, N., Rayner, C., Kinoshita, R., Cheung, M., & Metreweli, C. (2002). Sonographic diagnosis of clinically silent parasitic mastitis in three Indonesian bottlenose dolphins (*Tursiops aduncus*). In *Proceedings of the 33rd Annual Meeting of the International Association of Aquatic Animal Medicine* (pp. 138-139).

Cornell, L. H., Asper, E. D., Antrim, J. E., Searles, S. S., Young, W. G., & Goff, T. (1987). Results of a long range captive breeding program for the bottlenose dolphin, *Tursiops truncatus* and *Tursiops truncatus gilli*. *Zoo Biology*, *6*, 41-53.

Dudley, M., Duffield, D., & Shell, D. (1999). *Bottlenose dolphin North American studbook report: Single population analysis & records keeping system (SPARKS), Vol 4.*

Duffield, D. A. (2001). Genetic and physiological research applications in marine mammal medicine. In L. Dierauf & F. Gulland (Eds.), *CRC handbook of marine mammal medicine* (pp. 371-381). Boca Raton, FL: CRC Press. 1,063 pp.

Duffield, D. A., & Robeck, T. (2000). *Report from the Bottlenose Dolphin Reproductive Workshop* (DBW184-189). Silver Spring, MD: American Zoological Association Marine Mammal Taxon Advisory Group. 376 pp.

Duffield, D. A., Odell, D. K., McBain, J. F., & Andrews, B. (1995). Killer whale (*Orcinus orca*) reproduction at SeaWorld. *Zoo Biology*, *14*, 417-430.

Hicks, C. L., Kinoshita, R., & Ladds, P. W. (2000). Pathology of melioidosis in captive marine mammals. *Australian Veterinary Journal*, *78*, 193-195.

Joseph, B., Duffield, D., & Robeck, T. (2000). Summary data on reproduction of bottlenose dolphins in controlled environments. In D. A. Duffield & T. R. Robeck (Eds.), *Report from the Bottlenose Dolphin Breeding Workshop* (DBW184-189) (pp. 42-55). Silver Spring, MD: American Zoological Association Marine Mammal Taxon Advisory Group. 376 pp.

Kinoshita, R., Rayner, C., & Brook, F. (2000). In D. A. Duffield & T. R. Robeck (Eds.), *Report from the Bottlenose Dolphin Breeding Workshop* (DBW184-189) (pp. 17-25). Silver Spring, MD: American Zoological Association Marine Mammal Taxon Advisory Group. 376 pp.

Kinoshita, R., Spielman, D., Mauroo, N., Rourke, N., Rayner, C., & Brook, F. (2004). Artificial insemination in the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*). In *Proceedings of the 35th Annual Meeting of the International Association of Aquatic Animal Medicine* (pp. 71-72).

Lacy, R. C. (2000). Management of limited animal populations. In D. A. Duffield & T. R. Robeck (Eds.), *Report from the Bottlenose Dolphin Breeding Workshop* (DBW184-189) (pp. 75-93). Silver Spring, MD: American Zoological Association Marine Mammal Taxon Advisory Group. 376 pp.

Lasley, B. L., & Anderson, G. B. (1991). Where does biotechnology fit in captive breeding programmes? *Zoo Biology*, *10*, 195-196.

Montgomery, M. E., Ballou, J. D., Nurthen, R. K., England, P. R., Briscoe, D. A., & Frankham, R. (1997). Minimizing kinship in captive breeding programs. *Zoo Biology*, *16*, 377-389.

Robeck, T. R. (2001). Developing artificial insemination in cetaceans. In *Proceedings of the 32nd Annual Meeting of the International Association of Aquatic Animal Medicine* (pp. 153-154).

Robeck, T. R., Atkinson, S. K. C., & Brook, F. (2001). Reproduction. In L. Dierauf & F. Gulland (Eds.), *CRC handbook of marine mammal medicine* (pp. 193-236). Boca Raton, FL: CRC Press. 1,063 pp.

Robeck, T. R., Curry, B. E., McBain, J. F., & Kraemer, D. C. (1994). Reproductive biology of the bottlenose dolphin (*Tursiops truncatus*) and the potential application of advanced reproductive technologies. *Journal of Zoo & Wildlife Medicine*, *25*(3), 321-336.

Robeck, T. R., Steinman, K. J., Gearhart, S., Reidarson, T. R., McBain, J. F., & Montfort, S. L. (2004). Reproductive physiology and development of artificial insemination technology in killer whales (*Orcinus orca*). *Biology of Reproduction.* (DOI:10.1095/ biolreprod.104.027961)

Schroeder, J. P. (1990). Breeding bottlenose dolphins in captivity. In S. Leatherwood & R. R. Reeves (Eds.), *The bottlenose dolphin* (pp. 435-446). San Diego: Academic Press. 653 pp.