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PROSPECTS FOR BREEDING POORLY-KNOWN SPECIES OF SMALL CETACEANS IN CAPTIVITY

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ABSTRACT

Ex-situ or captive breeding was suggested for baiji, *Lipotes vexillifer*, but such a program was not successfully implemented and the species is now extinct. Given the precarious condition of the other small cetacean species listed as endangered or critically endangered by IUCN, use of captive breeding as a means of conservation is likely to be suggested for some of them in the future. A successful captive breeding program for a new species cannot be implemented until reliable capture and husbandry techniques have been developed. Techniques for assisted reproduction and reintroduction may also be needed. We review attempts to capture, maintain, and breed poorly-known species of small cetaceans in captivity and discuss the assisted reproductive technologies (ART) that have been used to enhance captive breeding efforts for other small cetaceans. We conclude that the techniques required to enable successful captive breeding of the small cetaceans listed by the IUCN as endangered or critically endangered have not been sufficiently developed. Development of these techniques should begin before a population is critically endangered. In particular, assisted reproductive technologies tend to be species-specific, necessitating considerable time and research to develop them for each species of concern. Furthermore, critically endangered populations cannot afford to lose the individuals needed for *ex-situ* technique development. The fairly large captive population sizes necessary to avoid inbreeding and adaptation to captivity, the limited space available in aquariums, and the high cost of captive breeding and reintroduction programs make it unlikely that captive breeding will play a major role in the conservation of most small cetaceans. The substantive conservation measures needed for critically endangered small cetaceans are habitat preservation and the elimination of by-catch. Captive-bred small cetaceans should not be reintroduced into an area until these needs have been met.

INTRODUCTION

Ex-situ or captive breeding can contribute to species conservation, but has not been successful with many taxa (Lees and Wilcken, 2009). The impending extinction of the baiji, *Lipotes vexillifer*, prompted an examination of the usefulness of captive or semi-captive breeding in a reserve as a means of conserving it (Perrin *et al.*, 1989; Ralls, 1989, Ridgway *et al.*, 1989; Braulik *et al.*, 2005), but such a program was not successfully implemented. Captive breeding proved difficult and multiple factors led to the extinction of this species (Reeves and Gales, 2005; Wang *et al.*, 2006; Dudgeon, 2005; Turvey *et al.*, 2007, Turvey, 2008). The suggestion to use captive breeding as a means of conserving other endangered or critically endangered small cetacean populations is nonetheless likely to emerge repeatedly, because this technique has contributed to the recovery of some vertebrate taxa, such as the Arabian oryx, *Oryx leucoryx* (Stanley Price, 1989), the golden lion tamarin, *Leontopithecus rosalia* (Kleiman and Rylands, 2002), the California condor, *Gymnogyps californianus* (Ralls and Ballou, 2004), and the black-footed ferret, *Mustela nigripes* (Wisely *et al.*, 2003).

Although captive breeding has not played a major role in the conservation of any cetacean, there have been numerous captive births of the cetaceans most commonly kept in captivity (*e.g.* bottlenose dolphins, *Tursiops truncatus*, and killer whales, *Orcinus orca*) and attempts are being made at captive propagation of less frequently held species such as the finless porpoise, *Neophocaena phocaenoides* (Wang, 2009; Wang *et al.*, 2005, 2010).

Assisted reproductive technology (ART) for enhancement of *ex-situ* breeding in cetaceans has been developed for several commonly held species, and refinement of the techniques involved has contributed significantly to scientific knowledge of cetacean reproductive physiology (Robeck *et al.,* 1994; O'Brien and Robeck, 2010a). O'Brien and Robeck (2010a) reviewed the use of ART in cetaceans, discussing the potential benefits of the research involved to conservation management of free-ranging populations and the potential value of *ex-situ* breeding for species conservation.

However, an *ex-situ* breeding program should not be undertaken for conservation of a wild population if numbers of free-ranging individuals are insufficient for the population as a whole to withstand the removal of some individuals. The International

Union for the Conservation of Nature, IUCN, has broad technical guidelines for management of *ex-situ* populations as a method of conservation (IUCN, 2002), stating that "Ex situ conservation should be initiated only when an understanding of the target taxon's biology and ex situ management and storage needs are at a level where there is a reasonable probability that successful enhancement of species conservation can be achieved; or where the development of such protocols could be achieved within the time frame of the taxon's required conservation management, ideally before the taxa becomes threatened in the wild."

In addition, we suggest that a captive breeding program for a new species cannot be implemented successfully until reliable techniques have been developed for capture and husbandry of that species. Although there have been many advancements in methodologies for the husbandry, maintenance, and medical care of small cetacean species in recent decades (Brando, 2010; Houser *et al.*, 2010; Joseph and Antrim, 2010), risk is inherent to the process of bringing poorly-known cetacean species into captivity. There is often a learning process for the housing institution, that may come at the price of compromised health and/or mortality of new captives and some species apparently may not acclimate to the captive environment (Walker, 1975). Technologies for assisted reproduction and techniques for release or reintroduction into the wild may also need to be developed.

Here, we review the history of attempts to capture, maintain, and breed poorly-known small cetacean species in captivity and discuss the assisted reproductive technologies that have been applied to other small cetaceans. We then examine the prospects for using captive breeding to help save endangered or critically endangered small cetaceans, such as the Asian Ganges and Indus river dolphins, *Platanista* species, the vaquita, *Phocoena sinus*, and the river-dwelling subpopulations of the Irrawaddy dolphin, *Orcaella brevirostris*, (Table 1).

CAPTURE AND TRANSPORT OF SMALL CETACEANS

Although capture and transport of cetaceans are known to be high risk activities, few descriptions of the difficulties of these activities, and the often high injury and mortality rates of the animals involved, appear in the scientific literature. Improvements in capture and transport techniques (*e.g.* those discussed by Braulik *et al.*, 2005, Bonar *et al.,* 2007, and used by Wells *et al.*, 2004) have developed over decades of experience among cetacean researchers, veterinarians and zoological institutions. Nevertheless, drowning, trauma, capture wounds (from nets or other capture gear) and bacterial and other infections related to capture damage and transport conditions are a major cause of death among cetaceans during and soon after capture (Walker, 1975; Ridgway *et al.*, 1989; Wang *et al.*, 2000; Fisher and Reeves, 2005). Van Waerebeek *et al.* (2008) also have documented the potentially disastrous effects of ill-conceived live capture endeavors.

Small cetacean species such as the harbor porpoise, *Phocoena phocoena,* Dall's porpoise, *Phocoenoides dalli*, common dolphins belonging to the genus *Delphinus*, and the northern right whale dolphin, *Lissodelphis borealis*, have had relatively high mortality rates and short survival times after capture (Walker, 1975; Reeves and Mead, 1999; Dima and Gache, 2004). In some instances, this mortality may have been related to stress during capture (Curry, 1999; Cowan and Curry, 2008),

Capture and transport conditions vary widely depending on capture location, climate, and characteristics of transportation (vessel, vehicle, aircraft, and available climate control). Duration of transport can last many hours, and conditions of transport are likely to be more difficult in the remote or underdeveloped regions of many poorly-known small cetacean species (*e.g.* Tas'an and Leatherwood, 1984; Sylvestre, 1985; Caldwell *et al.*, 1989; Boede *et al.*, 1998; Bonar *et al.*, 2007).

From the early 1950s to 1970s, Amazon River dolphins, *Inia geoffrensis*, were captured, mostly with nets, transported over several days, and kept captive in the United States, Europe, and Japan (Brownell, 1984; Sylvestre, 1985; Caldwell *et al.*, 1989; Tobayama and Kamiya, 1989). Mortalities often occurred during transport (Caldwell *et al.*, 1989). During the 1970s to 1990s, this species was also captured and held in Brazil (da Silva, 1994), and was transported, for up to 12 hours, to be held at the Valencia Aquarium,Venezuela (Boede *et al.*, 1998).

A few small cetacean species have been captured and transported to aquaria in Japan since the 1930s, with records dating to 1963. At least, one hundred and fifty three finless porpoises were captured there between 1963 and 1984 (Kasuya *et al.*, 1984).

These porpoises were obtained from incidental catches before 1972, but beginning in 1973 Toba Aquarium captured them directly using seine nets (Kasuya *et al.*, 1984). At least 78 finless porpoises were directly captured for display between 1973 and 1993 (Kasuya *et al.*, 1984; Reeves *et al.*, 1997). Mortality during capture has not been commonly reported. Survival rate in captivity is not well known. Aquariums in Japan can replace dead individuals with new ones because it is relatively easy to obtain them from the wild. Live porpoises are captured mainly in Ise Bay and the Inland Sea of Japan, with a few having come from the Pacific Coast, south of Sendai Bay, Japan (Kasuya *et al.*, 1984; Miyashita *et al.*, 2005).

There are known difficulties with capture and transport of Irrawaddy dolphins, especially in riverine habitat. Beasley (2007) suggested that translocation of Irrawaddy dolphins, from one part of their range in the Mekong River to reinforce the population size in critical habitat in another part of their range, is not a viable option for conservation management. Mortality during capture and transport is likely, and removal could disrupt the structure of the Irrawaddy dolphin's small, stable groups (Whitehead, 1997; Whitehead *et al.*, 2000; Kreb, 2004; Beasley, 2007; Beasley *et al.*, 2009). Also, in 1974, Tas'an *et al.* (1980) used drive capture to remove eight Irrawaddy dolphins from the Mahakam River, Indonesia, retaining six for transport to the Jaya Ancol Aquarium in Jakarta (Tas'an and Leatherwood, 1984). Three of these dolphins died from one to twenty days after capture (Table 2), with mortality attributed to stress of capture and transport. Seven Irrawaddy dolphins were apparently captured illegally from the Mahakam River during 1997 and 1998 (Beasley, 2007).

ACCLIMATION TO CAPTIVITY

The acclimation period subsequent to capture and transport is critical as the individual may be recovering from capture stress, and must adjust to many factors including new surroundings, water conditions, interactions with other individuals, as well as, feeding and other human interactions.

Small and DeMaster (1995a) noted that a better understanding of the time needed for a newly introduced captive to acclimate to captivity may allow for comparison of husbandry practices among institutions, which could in turn allow for improvement of marine mammal care. Similarly, Walker (1975) suggested that investigations of the causes of mortality during acclimation could provide insight to the health concerns encountered during capture and acclimation of small cetacean species.

Bottlenose dolphins, are the cetacean species most commonly held in captivity, and perhaps most tolerant of captive conditions. Small and DeMaster (1995a) estimated an acclimation period (during which the likelihood of mortality was higher than afterwards) of 35 days for a bottlenose dolphin brought to captivity from the wild. Small and DeMaster (1995a) evaluated acclimation periods for wild born (n=1,270), captive born (n=332), and captive-transferred (n=911) bottlenose dolphins, along with those for wild born (n=1,650), captive born (n=992), and captive-transferred (n=336) California sea lions, *Zalophus californianus*, and found a 60 day period of relatively high mortality for newly caught or captive-transferred marine mammals. During this period there is a need for close monitoring (*e.g.* behavior, nutrition, water quality, and medical care; Antrim and Joseph, 2010), as well as minimization of potential stressors.

River dolphins have experienced high rates of mortality during acclimation post-capture and transport. Bonar *et al.* (2007) examined pathology records from 123 of the 147 dolphins taken into captivity. Mortality was highest in the first two months post-capture and transport (32 of 123 animals for which records were available). A high incidence of pneumonia, which was identified as the cause of mortality within the first month of capture, and was attributed to stress of capture and transport (and see Caldwell *et al.*, 1989). In some cases a predisposition to infection due to parasite load (a high percentage of new captives with pneumonia had pulmonary trematodes) may have existed (Bonar *et al.* 2007). Amazon river dolphins also experienced a high incidence of bacterial disease (septicemia; without obvious symptoms) that led to sudden death in captivity. Bonar *et al.* (2007) noted that these problems relate, in part, to capture and transport, and suggested that improvements such as sling design, temperature control and sanitary water quality, in addition to prophylactic anthelminthic treatment combined with broad spectrum antimicrobial therapy could be used to address some of the problems that have been encountered in transport of this species.

Mortality post-capture and transport was also high for baiji (Chen and Liu, 1989). Of six baiji captured between 1981 and 1986, three died between 17 days and four months of capture, and a fourth died within nine months (Chen and Liu, 1989). Table 3.

The South American fransiscana, *Pontoporia blainvillei*, survived only days in captivity during the 1950s (Ridgway and Harrison, 1989, Reeves and Mead, 1999).

Three Indus River dolphins, *P. g. minor*, were captured and imported to California from Pakistan in the late 1960's, but the "rigors of capture and transport were such that none of them survived for long" (McCosker, 2007; and see Reeves and Brownell, 1989).

In 2004, nine finless porpoises (five male, four female) were live-captured in Ise Bay, Japan, using six purse seine vessels (Miyashita *et al.*, 2005). One male died two weeks after capture due to bacterial infection (methicillin-resistant *Staphylococcus aureus*, MRSA, strain of micrococcus; Miyashita *et al.*, 2005, and see Morris *et al.*, 2010).

The river-dwelling subspecies, the Yangtze River finless porpoise, *N. p. asiaorientalis*, has been taken into captivity in China since the 1960s (Liu *et al.*, 2002; Wang, 2009). Most of the porpoises captured during early attempts at captivity in China (from the mid-1960s to mid-1990s) died in less than one year (Liu *et al.*, 2002; Wang, 2009). Table 4.

MAINTAINING SMALL CETACEANS IN CAPTIVITY

In general, zoos and aquariums have most success maintaining and breeding species whose management needs are similar to those of domestic animals or other species with which zoos have had extensive experience. For example, zoos were able to maintain and breed California condors, without much difficulty because they had years of experience breeding closely related Andean condors, *Vultur gryphus* (Ralls and Ballou, 1992). For species with which there is little prior experience, maintenance and breeding success may initially be poor until suitable husbandry techniques are developed (Ralls and Meadows, 2001; Kleiman *et al.*, 2010). Because husbandry techniques are often species-specific, new captive breeding programs generally require substantial research development regarding behavior, reproductive physiology, nutrition, genetics and disease (Ralls and Meadows, 2001; Kleiman *et al.*, 2010).

In recent decades, many advances have been made in the husbandry and maintenance of small cetaceans in the captive environment including improvements in health care, nutrition, water quality, space requirements, and behavioral stimulation (Brando, 2010; Joseph and Antrim, 2010). However, zoos and aquariums have had extensive, long-term experience and success with only a few small cetaceans other than bottlenose dolphins and killer whales (*e.g.* Pacific whitesided dolphins, *Lagenorhynchus obliquidens*, beluga whales, *Delphinapterus leucas*, and Commerson's dolphins, *Cephalorhynchus commersonii*; Asper *et al.*, 1990). In addition, relatively few reports of longitudinal data regarding mortality and survival of individual cetacean species in captivity are available for comparison among species and institutions (DeMaster and Drevenak, 1988; Duffield and Wells, 1991; Reeves *et al.*, 1994).

The infrastructure, funding level, and extent of cumulative experience at individual institutions are likely to have a strong influence on small cetacean survival rates as DeMaster and Drevenak (1988) examined zoological records (dates of captures, births, deaths), and found significant differences in survival rates of captive bottlenose dolphin among institutions. They also found that for captive-born bottlenose dolphin calves (birth to one year) the survival rate was significantly lower than that of older captive animals. The authors concluded that mortality may be highest from captive birth to one year of age, and for individuals in their first year of captivity (DeMaster and Drevenak,1988; and see Bigg and Wolman 1975, Greenwood and Taylor, 1985). Male killer whales had lower survival rates in captivity than females, but sex-specific survival rates were similar for both bottlenose dolphins and beluga whales (DeMaster and Drevenak, 1988). Small and DeMaster (1995b) found that survival of captive bottlenose dolphin and killer whale calves was significantly lower than in wild populations.

Small cetaceans that live in rivers have proved difficult to maintain. Amazon River dolphins, baiji, and south Asian Ganges and Indus river dolphins, *Platanista gangetica*, have all had poor survival rates in captivity (Caldwell *et al.*, 1989; Chen and Liu, 1989; Reeves and Brownell, 1989). However, survival rates have not been calculated controlling for the amount of experience in capturing various species; for instance, comparing survival rates for the first 20 Amazon River dolphins brought into captivity with those for the first 20 bottlenose dolphins brought into captivity. Such a standardized analysis would enable researchers to distinguish between the possibility that poor survival rates in Amazon River dolphins are due to inexperience in capturing and husbandry of this species from the possibility this species is more difficult to capture and maintain successfully than bottlenose dolphins due to some biological difference between the species.

Bonar *et al.* (2007), examined 97 of 147 post-mortem records for captive Amazon River dolphins, and found that, as in bottlenose dolphins (DeMaster and Drevenak, 1988), mortality was highest in the first year of captivity. Only nine of the 97 river dolphins remained alive after 10 years in captivity. Longevity in captivity is10 to 26 years (Best and da Silva, 1993). Based on their survey of pathology in captive individuals, Bonar *et al.* (2007) suggested that control of microbiological water quality is vital to maintenance, and that a robust preventative medical program aimed at preventing bacterial infection could promote the longevity of Amazon River dolphins in captivity (and see Caldwell *et al.,* 1989).

The longest surviving baiji, the only one of six captives to live more than three years, was rehabilitated from injuries and lived 22 years (Chen and Liu, 1989; Braulik *et al.,* 2005; Table 3).

In 1970, five (one male, four female) Ganges River dolphins, *P. g. gangetica*, survived in captivity at Kamogawa Sea World from 74 to 299 days (Tobayama and Kamiya, 1989, Reeves and Mead, 1999).

There are currently two main holding areas for Yangtze River finless porpoises in China (Wang, 2009). One is a semi-natural reserve (Tian'e-Zhou Oxbow of the Yangtze River) where animals are able to interact freely, and into which 49 Yangtze finless porpoises have been introduced since 1990 (Wang *et al.*, 2000; Wang, 2009). Approximately 30 porpoises occupied the reserve in 2010. Another small reserve, currently occupied by ten porpoises, was established in Tongling, Anhui Provence in 1994 (Wang *et al.,* 2010). Females are producing calves annually in these reserves (Wang, 2009; Wang *et al.,* 2010). The second main holding area is an aquarium at the Institute of Hydrobiology, IHB, in Wuhan, which was maintaining five Yangtze River finless porpoises in 2009 (Table 4), including one captive born male (Wang, 2009). The IHB maintained six porpoises (three male, three female) in 2010 (Wang *et al.*, 2010).

Irrawaddy dolphins have been kept in captivity in Indonesia, Thailand and Japan, but mortality rates have been relatively high and there have been significant problems with water quality and feeding at institutions in both Indonesia and Thailand (Tas'an *et al.*, 1980; Perrin *et al.*, 1996; Stacey and Arnold, 1999). Twenty two Irrawaddy dolphins were taken from the Mahakam River to be maintained for public display in Indonesia (Table 2; Tas'an *et al.* 1980, Tas'an and Leatherwood 1984, Stacey and Leatherwood, 1997; Wirawan, 1989).

There is no known live-capture for display purposes from other freshwater Irrawaddy dolphin populations, but coastal Irrawaddy dolphins have been captured in Thai and Cambodian waters (Stacey, 1996; Perrin *et al.* 2005; Beasley, 2007). Irrawaddy dolphins are housed in two facilities in Thailand (Oasis Sea World and Safari World aquariums; Stacey, 1996; Stacey and Leatherwood, 1997; Beasley, 2007). In 1994, eight Irrawaddy dolphins were caught using nets in the coastal waters of Cambodia and taken into captivity at Safari World, Thailand (Stacey and Leatherwood, 1997). In 1995, two Irrawaddy dolphins were exported by Safari World, and imported to Japan by Marine World Uminonakamichi, Fukuoka City, Japan, but both have since died (T. K. Yamada pers. comm., 16 Sept. 2009; Stacey, 1996; Stacey and Leatherwood, 1997).

BREEDING SMALL CETACEANS IN CAPTIVITY

Many wild-caught animals fail to breed in captivity (Lees and Wilcken, 2009). As noted above, this failure is often due to behavioral problems caused by inadequate husbandry techniques (Ralls and Meadows, 2001). For example, social grouping is often critical for breeding success (*e.g.* avoiding possible suppression of spermatogenesis in subordinate male bottlenose dolphins; O'Brien and Robeck, 2010a), as well as to health and longevity, of small cetaceans in captivity. Unsuccessful breeding and short lifespan of Amazon River dolphins in captivity were attributed, in part, to a lack of knowledge regarding aggressive behavior in social groupings (in particular, larger males needed to be separated from other animals), and to stress-related diseases associated with transport and housing (Sylvestre, 1985; Caldwell *et al.*, 1989; Best and da Silva, 1993). Bonar *et al.* (2007) examined pool size and number of individuals that had been housed among 13 institutions and found a statistically significant correlation between dolphin survival and volume of water, noting that the correlation may have related to water quality and space requirements among individuals. Some of the problems involving behavior, space requirements and disease in these dolphins have been addressed over time, but not before losing individuals to mortality caused by lack of knowledge.

Success with captive breeding of dolphins that live in rivers has been extremely limited (Table 5; Reeves and Mead, 1999). Amazon River dolphins were widely held in captivity from the early 1950s to the late 1970s, as noted above, but Caldwell *et al.* (1989) reported only two live captive births. Both calves died shortly after birth; one after minutes, and one after approximately two weeks (Huffman, 1970; Caldwell and Caldwell, 1972). In 2005 there was a live birth of a male calf conceived in captivity at the Valencia Aquarium (Boede, 2005; Bonar *et al.*, 2007). Two subsequent captive births occurred at the Valencia Aquarium; both have died (Pelaez, 2010; Rojas 2010, 2011).

In 2011, four of six Amazon River dolphins maintained at the Valencia Aquarium (including a captive-born female born in 2009) died in a four month period (Rojas, 2011). Table 6.

There has been success breeding captive finless porpoises (*N. phocaenoides*) in Japan, which have been bred to the third generation beginning in the mid-1970s (Furuta *et al.*, 1976; Wang *et al.*, 2000; Miyashita *et al.*, 2005).

Captive breeding of Yangtze River finless porpoises in China has taken decades to organize and has included considerable trial and error (Perrin *et al.*, 1989; Wang *et al.*, 2000; Wang *et al.*, 2006; Wang, 2009). Currently, captive breeding is considered to be an integrated part of an overall conservation effort for this subspecies in China including *in-situ* protection measures such as the establishment of reserve areas for the wild population (Wang, 2009). In recent years, improved science and husbandry, international collaboration and specialized staff training have contributed to some success in captive breeding. In 2005, after nine years in captivity, one female produced the first captive-born individual of this subspecies (Wang *et al.*, 2005), and the male offspring was reported to remain healthy (Wang *et al.*, 2010)[1]. The same female gave birth to a second calf in June 2007 and died 39 days later, followed by the death of the calf after 11 days.

[1] This captive born male and a rehabilitated individual captured after sustaining injuries in 2008, were reported to have been released into the Tian'e-Zhou Oxbow in April 2011 (wwfcn.panda.org/?3440/).

In July 2008, the second female gave birth to a calf, but did not lactate, and the calf died after five days (Wang, 2009). Table 5.

As a part of captive breeding in China, reproductive husbandry protocols were developed and applied to captive Yangtze finless porpoises, and research evaluating reproductive physiology of the subspecies preceded the 2005 birth (Liu *et al.*, 2002; Wang, 2009). Blood samples of captive porpoises were collected monthly (as part of an overall evaluation of physical condition), with fecal, saliva, and blowhole secretion samples collected daily in an attempt to monitor reproductive hormones (Wang, 2009). Behavioral observations were undertaken to monitor mating activities (Wei *et al.*, 2004). In addition, Chen *et al.* (2006) monitored levels of serum testosterone in one of the two captive males over an approximately six year period from 1997 to 2003 (and see Wu *et al.*, 2010), and opportunistic endocrine monitoring of 66 (41 male, 25 female) free-ranging finless porpoises provided preliminary information on serum gonadotropins and steroid hormones (Hao *et al.*, 2007).

A few live births of Irrawaddy dolphins, conceived in captivity, have occurred in two aquariums: two healthy dolphins were born at the Jaya Ancol Aquarium (Tas'an *et al.*, 1980; Tas'an and Leatherwood, 1984; Stacey, 1996; Tables 2 and 6), and an unknown number of births have occurred at Oasis Sea World, Thailand (Perrin *et al.*, 1996; Stacey, 1996; Stacey and Arnold, 1999).

ASSISTED REPRODUCTIVE TECHNOLOGIES

Assisted reproductive technologies can be useful to enhance captive breeding programs for the conservation of endangered species. The benefits of using artificial insemination (AI) and associated procedures such as the synchronization of estrus include improved genetic management of propagation (facilitating use of one sire to several females by extending semen; allowing more efficient breeding amongst institutions without animal transport) and a potentially shorter interval between generations (Andrabi and Maxwell, 2007; Thomassen and Farstad, 2009). O'Brien and Robeck (2010a) outlined the development and use of these technologies including AI, estrus synchronization, sperm preservation, and sperm sexing in cetaceans. These techniques can be useful for efficient genetic management of captive cetacean populations (whether for public display or *ex-situ* conservation). However, Wildt *et al.* (1993) noted that the preservation of a species requires routine and efficient production of progeny, and although there have been successes applying ART in large-scale captive breeding with a few species, such as the peregrine falcon, *Falco peregrinus* (Cade, 1988), many applications of ART have been limited or one-time events. Many failed attempts at assisted reproduction go unreported (Wildt *et al.*, 1993).

Assisted reproductive technologies are species-specific, and some aspects of these technologies are inefficient for many endangered species because of insufficient knowledge on basic reproduction such as structural anatomy, estrous cycle, seasonality, gamete physiology and site for semen deposition (Wildt, *et al.*, 1986; Wildt, 1989; Comizzoli *et al.*, 2000; Andrabi and Maxwell, 2007). In addition, there are at least two initial criteria that must be met for artificial insemination and associated reproductive technologies to benefit endangered species. First, the *ex-situ* population should be breeding successfully. Artificial insemination is best achieved when applied to populations that are currently reproducing successfully, not as a substitute for reproductive viability, but as a tool for improving the efficiency of breeding management (Lasley and Anderson, 1991; Robeck *et al.*, 1994). Second, to successfully use artificial insemination and other ART, it is essential to have a fundamental understanding of reproductive anatomy and detailed knowledge of reproductive physiology for both males and females of the species concerned (Wildt, 1989; Wildt, *et al.*, 1986; Robeck *et al.*, 1994, 2004, 2005b).

There have been successes in the artificial insemination of five cetacean species. However, these occurred in only a few institutions, after decades of species-specific research combined with refined clinical experience. Results of the first successful AI trials included the live births of three bottlenose dolphins (Robeck *et al.* 2005b), two killer whales, (Robeck *et al.*, 2004), one beluga whale, (O'Brien *et al.*, 2008), and five Pacific whitesided dolphins (Robeck *et al.*, 2009). O'Brien and Robeck (2010a) recounted collaboration amongst 36 researchers and institutions, and that an additional 18 bottlenose dolphins, two killer whales, three belugas, and one Indo-Pacific bottlenose dolphin, *Tursiops aduncus*, were produced from artificial insemination (six of these were reported as still in utero from AI procedures conducted during 2010). This work marks a significant achievement for the genetic management of the captive specimens of the species involved, and if AI is proven replicable (as it has been for bottlenose dolphins), will allow for careful planning and selection of breeding combinations without requiring transportation of individuals amongst facilities. In addition, the research may contribute to the goal of sharing the gene pool amongst zoological institutions worldwide, thereby potentially enhancing the captive populations (Ballou *et al.*, 2010), and diminishing the impetus to collect individuals from the wild (Robeck *et al.*, 1994).

The research that was conducted to achieve the successful AI procedures noted above has contributed greatly to the scientific knowledge of reproduction in these species, providing the potential for future use in applied wildlife conservation *in-situ* (Wildt *et al.*, 1992; O'Brien and Robeck, 2010a). However, although the application of ART for a variety of mammalian species is becoming increasingly successful, it is often the case that the most significant contribution of the research required to develop ART is better scientific knowledge of the species involved (*e.g.* Howard and Wildt, 2009). Because of the intensive level of investigation required to develop species-specific reproductive techniques and the logistical constraints of their application, the outcome of research efforts often is a deeper understanding of the unique adaptive traits and physiological mechanisms that define a particular species, rather than large-scale assisted breeding or even the production of numerous offspring (Wildt *et al.*, 1992; Wildt and Wemmer, 1999; Goodrowe *et al.*, 2000; Andrabi and Maxwell, 2007).

Robeck *et al.* (2004) emphasized that some of the failures encountered during trials of artificial insemination in captive cetaceans have occurred at the methodological development stage. Robeck *et al.*, (2005b) noted that their success with bottlenose dolphins highlights the necessity of "strategic and systematic investigation into the basic reproductive physiology of a wildlife species to allow the development of assisted reproductive technologies." Because the bottlenose dolphin has been most widely maintained in captivity, research on reproduction in this species has been particularly thorough (Schroeder, 1990; Schroeder and Keller, 1990; Robeck *et al.*, 1994; 2005b). Some of the earliest reports detailed reproductive behavior (*e.g.* Tavolga and Essapian, 1957) and anatomy (Harrison, 1969; Harrison *et al.*, 1972; Harrison and McBreaty, 1977; Bryden and Harrison, 1986). Also, scientific knowledge of bottlenose dolphin (and other cetacean species) reproductive biology has been greatly enhanced by opportunistic investigation of stranded, by-caught, and exploited specimens (*e.g.* Perrin and Reilly, 1984). In the late 1970s and 80s, Cornell *et al.* (1977, 1987) reported results of research focused on one multi-facility breeding colony (beginning with work in the early 1960s), and established plans for future captive breeding and the potential use of ART (Robeck *et al.*, 1994).

Research on bottlenose dolphins determined basic information regarding reproductive biology, including timing of ovulation and the estrous cycle, as well as aspects of the seasonality of reproduction in females (Sawyer-Steffan *et al.*, 1983; Kirby and Ridgway 1984; Kirby, 1990; Schroeder, 1990; Urian *et al.*, 1996), and variation in testosterone levels among age classes and seasons in males (Harrison and Ridgway, 1971; Kirby, 1990; Schroeder and Keller, 1989). In addition, long-term, systematic evaluation of endocrine changes elucidating ovarian activity, and collection of these data in conjunction with ultrasound evaluation of ovarian changes and male reproductive characteristics (Brook *et al.*, 2000; Brook, 2001), were necessary to achieve success with artificial insemination in the bottlenose dolphin. Likewise, intricate details of reproductive endocrine physiology in male and female killer whales, beluga whales, and Pacific whitesided dolphins were necessary to achieve the first successful artificial insemination in these species (O'Brien and Robeck, 2010a). Table 7 provides a general summary of some of the recent investigations, including the sampling intervals and duration of sampling required, that have contributed to the advancement of reproductive knowledge and artificial insemination techniques in these species.

Detailed knowledge of sperm characteristics and production (Fleming *et al.*, 1981; Miller *et al.*, 2002; Robeck and Monfort, 2006; O'Brien *et al.*, 2008), as well as semen preservation (Robeck and O'Brien, 2004; O'Brien *et al.*, 2008; Robeck *et al.*, 2009; O'Brien and Robeck, 2010a), has been fundamental to the success of AI for each of these species. Semen collection methodology is particularly important, and must be entirely voluntary on the part of the donor (which can be achieved through a series of training procedures) to yield an acceptable level of sperm quality (Robeck and Monfort, 2006, Yuen *et al.*, 2009). Semen preservation is imperative; currently multiple cryopreservation methods are being tested and developed for use in some cetacean species (O'Brien and

Robeck 2010b). Recent advances in reproductive technology and research regarding bottlenose dolphin sperm characteristics have allowed for refinement of sperm sexing and pre-selection of sex in this species (O'Brien and Robeck, 2006; 2010a).

Development of biological resource banking and organized banking of spermatozoa may be of future value for conservation of small cetacean species (O'Brien and Robeck, 2010a).

LOGISTICAL REQUIREMENTS FOR *EX-SITU* BREEDING

(In addition to the species-specific research that will be required to develop reproductive knowledge for the application of assisted reproductive technologies to captive propagation, many practical objectives must be met before a captive breeding population can be established for a new species of small cetacean, and ART can be used safely and successfully.) Joseph and Antrim (2010) described the requirements of suitable housing, nutrition, behavioral stimulation, and preventive medicine for marine mammals. The authors also remarked that while governmental and institutional organizations sometimes develop guidelines for the care of marine mammals in the captive environment, special considerations are necessary to the maintenance of these animals, and that, realistically, zoological institutions must exceed the minimum requirements to achieve success (Joseph and Antrim, 2010). Currently, there are several international organizations that may provide a useful baseline of standards for regulating zoos and aquariums aspiring to maintain cetaceans for captive breeding purposes. For example, the World Association of Zoos and Aquariums, WAZA, and Association of Zoos and Aquariums, AZA, have established codes of ethics, and the AZA has established an Accreditation Commission intended to uphold rigorous standards of animal management and care, as well as an extensive collection or "acquisition - disposition policy," and guidelines for "developing an institutional program animal policy." [2]

IUCN guidelines state that *ex-situ* breeding programs as a means of species conservation should not be instituted ad-hoc, but should be systematically developed to

[2] Information regarding WAZA code of ethics, and AZA code of ethics, policies, institutional accreditation, and taxon advisory groups is accessible at www.waza.org/en/site/home and www.aza.org.

integrate potential solutions to multiple risk factors. A captive breeding program should be undertaken only when there is a long-term commitment of the substantial financial resources required and buy-in from the local and national government agencies. In addition, the guidelines suggest that a captive breeding program should integrate the goals of public awareness, population management of threatened taxa, reinforcement or reintroduction and other support to wild populations (including habitat restoration and management), long-term banking of biomaterial, scientific research, and fundraising (IUCN, 2002).

Captive populations require careful demographic and genetic management, with sufficient numbers of reproductively viable, sexually mature males and females available in the founder population as well as an eventual captive population large enough to avoid excessive loss of genetic diversity and a high risk of extinction (*e.g.* Ralls, 1989, Ralls and Ballou, 1992; Ralls and Meadows, 2001; Ballou *et al.*, 2010). Hence a major difficulty for successful captive breeding of cetaceans is finding sufficient *ex-situ* habitat of adequate quality in semi-natural reserves or aquariums.

The overall infrastructure of a captive breeding program for small cetaceans must include experienced personnel (handlers, trainers, nutritionists, veterinarians, scientists), and facilities (as described by Joseph and Antrim, 2010) meeting or exceeding international standards (as noted above) for housing sufficient numbers of individuals. Husbandry and veterinary facilities including those for nutritional and medical care as well as specialized technological equipment for both routine and emergency healthcare will also be necessary. Use of ART requires a fully equipped laboratory for work including endocrine monitoring, microscopy for semen and potentially other analyses, ultrasonographic and endoscopic equipment, along with standardized liquid nitrogen and freezer storage for cryopreservation (Robeck and O'Brien, 2004; Robeck *et al.*, 2004, 2005a, b, 2009; Robeck and Monfort, 2006; O'Brien *et al.*, 2008, Yuen *et al.*, 2009, O'Brien and Robeck , 2010a).

Although the quality of husbandry and medical care for all captive species should meet, and indeed often exceed, the highest prescribed standards (*e.g.* Joseph and Antrim, 2010), this is not always the case. Ridgway *et al.* (1989) asserted that the best husbandry methods for small cetaceans are those that require the least amount of restraint and attain the maximum cooperation from the animal. This approach is strongly evident in the research that has resulted in successful artificial insemination in small cetaceans, and is in fact needed for collection of suitable quality urine and semen samples (Robeck *et al.*, 2004, 2005a, b, 2009; O'Brien *et al.*, 2008). To carry out research and implement ART in cetaceans (and for the routine husbandry associated with captivity and captive breeding), captive individuals must be trained and conditioned to allow safe, voluntary (unrestrained) handling and husbandry procedures including voluntary urine, blood and semen donation, as well as vaginal manipulation (Keller, 1986; Lenzi, 2000; Surovik *et al.*, 2001; Fripp *et al.*, 2005; Robeck and Monfort, 2006; O'Brien and Robeck, 2010a). Training animals for these procedures requires experienced trainers and may be best achieved with continuity between trainers and dolphins over extended periods of time. Implementation of these routine husbandry procedures will require the physical presence of a qualified, experienced veterinarian and implementation of ART will require the presence of a veterinarian with expertise in that field and ongoing participation of other qualified, experienced veterinarians and scientists.

DIFFICULTIES WITH REINTRODUCTIONS OF CAPTIVE-BRED ANIMALS

Even if a species can be bred routinely in captivity, use of captive-bred individuals to reinforce the wild populations or reintroductions of captive-bred animals to form a new population in suitable ecological habitat can be difficult, complex, expensive and costly (Kleiman 1996; Seddon *et al.*, 2007; Earnhardt, 2010). While there have been successful species reintroductions, and there may be circumstances when reinforcement or reintroduction is the best advisable conservation measure, there have been more failures (Jule *et al.,* 2008; Bowkett, 2009). Reintroduction of captive-bred cetaceans has not yet been accomplished. The IUCN Reintroduction Specialist Group has provided guideline for reintroductions, and is a source of advice for those planning to reintroduce captive-bred taxa to the wild (www.iucnsscrsg.org). For example, IUCN advises that the problems that caused the original wild population to go extinct should be greatly reduced or eliminated before captive-born individuals are reintroduced into an area.

CONCLUSIONS

We have described the difficulties that are encountered when attempting to breed small cetaceans in captivity, particularly those that have been less widely studied. Recently, IUCN has recognized the need for refinement of technical guidelines on the management of ex-situ populations for conservation (Traylor-Holzer *et al.,* 2011) to include considerations of the assessment of feasibility and risk. These guidelines, once fully established, may prove useful to developing strategies for the conservation of small cetacean species.

The techniques required to enable successful captive breeding of the small cetaceans listed by the IUCN as endangered or critically endangered have not been sufficiently developed. Development of these techniques should begin before a population is critically endangered. We emphasize that any effort to capture and transport small cetaceans, especially an unfamiliar species and those in an estuarine or riverine environment, involves a substantial risk of injury and mortality. Acclimation to captivity, which may be an especially acute challenge for river-dwelling species, including subpopulations of Irrawaddy dolphins, poses an additional risk of mortality.

The effects of removing individuals on the viability of the remaining wild population must be carefully weighed. This is a particularly important consideration when dealing with small subpopulations that are listed by IUCN as critically endangered, such as those of Irrawaddy dolphins. Removal of individuals from a cetacean population by live-capture is the biological equivalent of lethal removal, with captured animals no longer available for reproduction. Removal of individuals from any of the Irrawaddy dolphin subpopulations, for instance, would pose risk to a small population already susceptible to inbreeding depression, and could diminish the overall fitness and potential for reproductive success of the wild population that is already exposed to other potential threats such as loss of habitat and entanglement in fishing nets. Thus, it is advisable to begin a captive breeding program well before the wild population becomes critically endangered.

Finally, *ex-situ* conservation should incorporate *in-situ* efforts as recommended by the IUCN (2002), and should consist of a systematically developed breeding program that includes planning for collection and housing of breeding populations, best implemented with guidance from collaboration among expert authorities. A captive breeding program for any small cetacean not currently widely held in captivity is unlikely to be successful without a substantial research program combined with accredited facilities and staff. Even under the best of circumstances, developing a successful program takes many years of work. Assisted reproductive technologies, such as artificial insemination, are not a short-term solution to breeding enhancement. These techniques are species-specific and usually take years of research to develop for a particular species. Existing research on cetacean reproductive biology and the use of ART of a few extensively studied odontocete species can provide insight and experience for use in other cetacean species but species-specific research will still be required before ART can be used on any new species.

The development of successful techniques for the release of captive-bred individuals into the wild poses additional challenges. We conclude that captive breeding has the potential to contribute to the conservation of only a very limited number of carefully selected small cetacean species. The main conservation measures needed for critically endangered small cetaceans are habitat preservation and the elimination of by-catch (Beasley *et al.*, 2009; Kreb *et al.*, 2010; Gerrodette and Rojas-Bracho, 2011; Ross *et al.*, 2010, 2011). Captive-bred small cetaceans should not be reintroduced into an area until these needs have been met.

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Table 1. Small cetacean populations listed by IUCN as critically endangered.

SPECIES	SUBPOPULATON	IUCN STATUS	NATIVE COUNTRY	POPULATION ESTIMATE	SOURCE
South Asian river dolphin, ***Platanista gangetica***	Ganges River dolphin, *Platanista gangetica gangetica*	Endangered	India, Bangladesh, Nepal (possibly Bhutan)	< 2000 - 4000	Mohan *et al.*, 1997; Smith *et al.*, 2004
	Indus River dolphin, *Platinista gangetica minor*	Endangered	Pakistan	965	Braulik *et al.*, 2004
Vaquita, ***Phocoena sinus***	species level	Critically Endangered	Mexico	567	Jaramillo-Legorreta *et al.*, 1999; Rojas-Bracho *et al.*, 2008
Irrawaddy River Dolphin ***Orcaella brevirostris***	Ayeyarwady River	Critically Endangered	Myanmar	59	Smith *et al.*, 1997; Smith, 2004
	Mahakam River	Critically Endangered	Indonesia	67-70	Kreb *et al.*, 2007; Jefferson *et al.*, 2008
	Malampaya Sound	Critically Endangered	Philipines	77	*Smith* et *al.*, 2004; Smith and Beasley, 2004a
	Mekong River	Critically Endangered	Cambodia, Lao People's Democratic Republic, Vietnam	69	Smith and Beasley, 2004b
	Songkhla Lake	Critically Endangered	Thailand	unknown*	Beasley *et al.*, 2003; Smith and Beasley, 2004c

* Estimated to be fewer than 50 mature individuals.

Table 2. Irrawaddy dolphins *Orcaella brevirostris* captured between 1974 and 1984 from the Mahakam River, Indonesia, for captivity at Jaya Ancol Aquarium. Date of death and survival time is included where known. Note: Only six of the 16 individuals with unknown survival times were alive in 1985; by 1995 only two were alive (Tas'an *et al.*, 1980; Stacey and Leatherwood, 1997; Tas'an and Leatherwood 1984; Stacey and Arnold, 1999).

SEX	IDENTIFICATION	DATE CAPTURED	DATE OF DEATH	SURVIVAL TIME	REPORTED PATHOLOGIES
female*	74GSA16mOb1	15 October 1974	Deceased	Unknown	Unknown
male	74GSAl7mOb2	15 October 1974	4 November 1974	20 d	Gastrointestinal ulcers, stress
female	74GSA18mOb3	15 October 1974	23 October 1974	10 d	Gastrointestinal ulcers, stress
male	74GSA18mOb4	15 October 1974	Deceased	Unknown	Unknown
male	74GSA20mOb5	15 October 1974	2 July 1978	1,356 d	Pulmonary infection
female	74GSA21mOb6	15 October 1974	16 October 1974	1 d	Stress
male	74GSA94mOb7	24 September 1978	Deceased	Unknown	Unknown
male	78GSA95mOb8	24 September 1978	17 January 1979	115 d	Coronary pathology
male	78GSA96mOb9	24 September 1978	Deceased	Unknown	Unknown
male	78GSA97mOb10	24 September 1978	Deceased	Unknown	Unknown
female	78GSA98mOb11	24 September 1978	Deceased	Unknown	Unknown
male	78GSA99mOb12	24 September 1978	Deceased	Unknown	Unknown
male	78GSA100mOb13	24 September 1978	Deceased	Unknown	Unknown
male	78GSA111mOb14	24 September 1978	Deceased	Unknown	Unknown
male	78GSA112mOb15	24 September 1978	Deceased	Unknown	Unknown
male	78GSA113mOb16	24 September 1978	24 October 1978	30 d	Pneumonia, liver cirrhosis
unknown**	79GSA125Ob18	11 December 1979	11 December 1979	Died at Birth	Unknown
unknown	six individuals	1984	Unknown	Unknown	Unknown

* Mature female – gave successful birth to captive-born female (see Table 6).

** Captive birth – conception, parentage not reported.

Table 3. Survival times for Baji, *Lipotes vexillifer*, captured from the Yangtzee River and introduced into captivity. After Braulik *et al.* (2005), and see Chen and Liu, (1989).

IDENTIFICATION	SEX	LENGTH (cm)	DATE OF CAPTIVITY	DATE OF DEATH	SURVIVAL TIME	INSTITUTION
Qi Qi	male	143	12 January 1980	14 July 2002	22 yr 6 mo	Institute of Hydrobiology
Su Su	female	182	3 March 1981	20 March 1981	17 d	Nanjing Normal Univesrity
Rong Rong	male	151	22 April 1981	3 February 1982	228 d	Institute of Hydrobiology
Jiang Jiang	male	174	7 December 1981	16 April 1982	129 d	Nanjing Fisheries Research Institute
Lian Lian	male	203	31 March 1986	14 June 1986	76 d	Institute of Hydrobiology
Zhen Zhen	female	152	31 March 1986	1988	2 yr 6 mo	Institute of Hydrobiology

Table 4. Yangtzee River finless porpoise, *Neophcaena phocaenoides*, taken into captivity in China. After Liu *et al.* (2002) and Braulik *et al.* (2005).

INDIVIDUAL	YEAR CAPTURED	SURVIVAL TIME	INSTITUTION	SOURCE
-	1965	245 d	Quingdao Marine Museum	Liu *et al.*, 2002; Braulik et al., 2005; Wang 2009
multiple	During 1970-1980	~ 1 yr	Shanghai Zoo	Liu et al., 2002; Braulik *et al.*, 2005
-	1978	60 d	Institute of Hydrobiology	Liu et al., 2002; Braulik et al., 2005
-	1981	~ 1 yr	Institute of Hydrobiology	Liu et al., 2002; Braulik *et al.*, 2005
-	1985	180 d	Institute of Hydrobiology	Liu et al., 2002; Braulik *et al.*, 2005
-	1988	759 d	Nanjing Normal University	Liu et al., 2002; Braulik *et al.*, 2005
multiple	1992	Longest 1 yr 3 mo	Tongling Conservation Farm	Liu et al., 2002; Wang 2009
-	1992	180 d	China Aquarium of Shanghai	Liu et al., 2002; Braulik *et al.*, 2005
-	1993	1 yr 6 mo	Institute of Hydrobiology	Liu et al., 2002; Braulik *et al.*, 2005
1 male	1996	Alive in 2009	Institute of Hydrobiology	Liu *et al.*, 2002; Braulik et al., 2005; Wang 2009
1 female	1996	Alive in 2009	Institute of Hydrobiology	Liu *et al.*, 2002; Braulik et al., 2005; Wang 2009
-	1997	1 yr	Wuhan New Century Aquarium	Liu et al., 2002; Braulik *et al.*, 2005
multiple	1999-2001	1 yr	Wuhan New Century Aquarium	Liu et al., 2002; Braulik et al., 2005
1 female	1999	8 yr	Institute of Hydrobiology	Liu et al., 2002; Wang 2009
1 male	1999	Alive 2009	Institute of Hydrobiology	Liu et al., 2002; Wang 2009
1 male*	2004	Alive in 2009	Institute of Hydrobiology	Wang, 2009

* This male was taken from the Tian'e-Zhou Oxbow of the Yangtzee River.

Table 5. Reported live captive births for Amazon River dolphins, *Inia geoffrensis*, Yangtzee River finless porpoise, *Neophocoena phocaenoides asiaorientalis*, and Irrawaddy dolphins, *Orcaella brevirostris* (Mahakam River subpopulation) held in captivity between 1956 and 2011.

SPECIES	INSTITUTION	IDENTIFCATION	SEX	DATE OF BIRTH	DATE OF DEATH	SURVIVAL TIME	SOURCE
Inia geoffrensis	Fort Worth Zoological Park	-		1970	1970	Died at birth	Huffman, 1971; Caldwell*et al.*, 1989
	Marineland of Florida	-				15 d	Caldwell and Caldwell, 1972; Caldwell *et al.*, 1989
	Valencia Aquarium	Telemachus	female	November 2000	2005	Accident	Pelaez, 2010; Rojas, 2010
	Valencia Aquarium	Zeus	male	November 2005	Alive 2011		Boede, 2005
	Valencia Aquarium	Helena II	female	23 October 2009	14 April 2011	538 d	Pelaez, 2010; Rojas 2011
Neophocoena phocaenoides asiaorientalis	Institute of Hydrobiology	-	male	2005	Alive 2011		Wang*et al.*, 2005; Wang, 2009
	Institute of Hydrobiology	-	male	2007	2007	39 d	Wang, 2009
	Institute of Hydrobiology	-	male	2008	2008	5 d	Wang, 2009
Orcaella brevirostris	Jaya Ancol Aquarium	Isui (79GSA105mOb17)	female	4 July 1979	Deceased	Unknown - Alive in 1984	Tas'an*et al.*, 1980; Tas'an and Leatherwood, 1984; Beasley, 2007
Orcaella brevirostris	Jaya Ancol Aquarium	-	-	-	Deceased	Unknown - Alive in 1984	Tas'an*et al.*, 1980; Tas'an and Leatherwood, 1984; Beasley, 2007

Table 6. Amazon River dolphins, *Inia geoffrensis*, captured from 1975 to 1994 in the Orinoco River, Venezuela, and taken into captivity at the Valencia Aquarium as reported by Boede *et al.,* 1998. Survival times are reported where known.

SEX	IDENTIFICATION	DATE CAPTURED	DATE OF DEATH	SURVIVAL TIME	REPORTED PATHOLOGIES
5 male, 3 female	None	1975	Deceased	Not known	
1 male	Arquimides	1975	June 1987	~ 12 yrs	Gastrointestinal obstruction, ulcers
1 female	Nelly	1975	6 November 1993	~18 yrs	Warfarin poisoning (rodenticide)
1 female		10 July 1987	--	Released after capture	
1 male	Ulyses	10 July 1987	13 January 2011**	8,588 d (23 yr 6 mo)	Respiratory difficulties
1 female	Dalila	10 July 1987		Alive 2011 (23 yr)	
1 female	Penelope	20 April 1994	25 March 2011**	6,183 d (16 yr 11 mo)	
1 female	Helena I	20 April 1994	Deceased	Not known	
1 female*	Artemis	19 October 1994	5 February 2011**	5,953 d (16 yr 3 mo)	Hepatitis, pancreatitis, gastric ulcers

* Conceived in the wild.
** Rojas, 2011.

Table 7 . General summary of recent investigations contributing to the advancement of reproductive knowledge and artificial insemination techniques in small cetacean species. Sampling interval and duration, sample size, and number of individuals sampled are included.

SPECIES	RESEARCH INVESTIGATION	SAMPLING INTERVAL	INDIVIDUALS SAMPLED (n)	SAMPLING DURATION
Bottlenose Dolphin, *Tursiops truncatus*	**Female Reproduction**			
	Robeck et al., 2005b			
	Endocrine monitoring for characterization of estrus cycle (determination of total estrous cycle length and intra-estrus cycle phases). Urinary luteinizing hormone, estrogen conjugates.	Urine 1-3 daily	13 mature females	2 years
	Endocrine data were used in conjunction with ultrasound ovulatory data.	Ultrasound 1-3 daily		
	Administration of progesterone-analog (altrenogest; Regu-Mate). Monitoring of urinary luteinizing hormone, estrogen conjugates, progestins.	Urine 1-2 daily	12 mature females	
	Biancani *et al.*, 2009			
	Endocrine monitoring of ovarian activity, onset of sexual maturity. Fecal progestogens, estrogens.	Fecal 2 weekly	8 females (3 immature; 5 mature)	20 months
	Serum (progesterones, estrogens) and vaginal (cytological evaluation) samples were used for comparison.	Blood 1 monthly Vaginal smear 1-4 week		
	Male Reproduction			
	Robeck et al., 2005b			
	Semen collection, quality assessment, preservation.	Varible intervals (N=13 semen samples)	4 mature males	

Table 7 (cont.). General summary of recent investigations contributing to the advancement of reproductive knowledge and artificial insemination techniques in small cetacean species. Sampling interval and duration, sample size, and number of individuals sampled are included.

SPECIES	RESEARCH INVESTIGATION	SAMPLING INTERVAL	INDIVIDUALS SAMPLED (n)	SAMPLING DURATION
Killer Whale, *Orcinus orca*	**Female Reproduction**			
	Robeck et al., 1993			
	Endocrine monitoring for characterization of estrous cycle, seasonality of estrus. Urinary luteinizing hormone, follicle stimulating hormone, estrogen conjugates, pregnanediol-3-glucuronide.	Urine 1 daily	6 females	2 - 4 years
	Duffield et al., 1995			
	Endocrine monitoring for parameters of estrus cycle, mean gestation. S	Blood 2 monthly	18 females	10 years
	Robeck et al., 2004			
	Endocrine monitoring for determination of total estrous cycle length (and intra-estrus cycle phases). Serum progesterone. Urinary luteinizing hormone, estrogen conjugates.	Blood 1 monthly Urine 2 daily	3 mature females	1.5-2 years
	Endocrine data were used in conjunction with ultrasound ovulatory data.	Ultrasound 1-3 daily		
	Administration of progesterone-analog (altrenogest; Regu-Mate). Monitoring of urinary luteinizing hormone, estrogen conjugates, progestins.	Urine 1 daily		
	Male Reproduction			
	Robeck and Monfort, 2006			
	Endocrine monitoring of variation with reproductive maturity, season. S	Variable intervals (N=1388 serum samples)	10 males	1 - 12 years
	Semen production	Variable intervals (N=90 semen samples)		
	Endocrine/semen production data used in conjunction with morphologic data on age at maturity			

Table 7 (cont.). General summary of recent investigations contributing to the advancement of reproductive knowledge and artificial insemination techniques in small cetacean species. Sampling interval and duration, sample size, and number of individuals sampled are included.

SPECIES	RESEARCH INVESTIGATION	SAMPLING INTERVAL	INDIVIDUALS SAMPLED (n)	SAMPLING DURATION
Beluga Whale, *Delphinapterus leucas*	**Female Reproduction**			
	Robeck *et al.*, 2005a			
	Endocrine monitoring of seasonal ovarian activity; onset of sexual maturity. Serum progesterone.	Blood 1-2 weekly	17 females	2-15 years
	Male Reproduction			
	Robeck et al., 2005a			
	Endocrine monitoring. Serum testosterone.	Blood 2 weekly - 1quarterly	12 males	6 months - 15 years*
	O'Brien *et al.*, 2008			
	Endocrine monitoring. Serum testosterone.	Blood 2 monthly	1 male	21 months
	Semen production	Variable intervals		

Table 7 (cont.). General summary of recent investigations contributing to the advancement of reproductive knowledge and artificial insemination techniques in small cetacean species. Sampling interval and duration, sample size, and number of individuals sampled are included.

SPECIES	RESEARCH INVESTIGATION	SAMPLING INTERVAL	INDIVIDUALS SAMPLED (n)	SAMPLING DURATION
Pacific Whitesided Dolphin, *Lagenorhynchus obliquidens*	**Female Reproduction**			
	Robeck et al., 2009			
	Endocrine monitoring for characterization of estrus cycle, seasonality of estrus. Serum progesterone, Urinary luteinizing hormone, estrogen conjugates.	Blood 1- 2 monthly Urine 3 - 5 daily (N=3388 urine samples)	7 females	Blood 8 - 23 years
	Endocrine data were used in conjunction with ultrasound ovulatory data.	Ultrasound 1 - 3 daily	13 females	
	Administration of progesterone-analog (altrenogest; Regu-Mate). Monitoring of urinary luteinizing hormone, estrogen conjugates, progestins.	1 daily (20 - 30 day periods; N=76 treatments)	10 females	
	Male Reproduction			
	Robeck et al., 2009			
	Endocrine monitoring for seaonal variation of testosterone levels. Serum testosterone.	Blood 2 montly	1 male	7 years
	Endocrine data were used in conjunction with ultrasound testicular data.	Ultrasound 2 weekly - 1 monthly	1 male	1.5 years
	Semen production, seasonal variation in spermatozoa	Variable intervals (N=290 ssemen samples)	1 male	3 years
	Semen Cryoppreservation			

* Sample collection data for "Male 6" could not be ascertained due to typographical error ("collection dates of 09/90–03/11"; Robeck et al., 2005.