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REVIEW

# Prospects for captive breeding of poorly known small cetacean species

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ABSTRACT: Because of the precarious condition of small cetacean species and subpopulations listed as Endangered or Critically Endangered by the IUCN, use of captive breeding for conservation has been suggested for some of them, and will likely be suggested for others. 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 small cetaceans and discuss assisted reproductive technologies (ART) that have been used to enhance captive breeding efforts for other small cetaceans. We conclude that the techniques required for successful captive breeding of most Endangered or Critically Endangered small cetacean species have not been sufficiently developed. Development of these techniques should begin before a species or population is Critically Endangered. In particular, ARTs tend to be species specific, necessitating considerable time, money, and research to develop for each species of concern. Critically Endangered populations cannot afford to lose the individuals needed for technique development. The fairly large captive population sizes necessary (to avoid loss of genetic diversity, inbreeding, and genetic adaptation to captivity), limited space available in aquariums, and high costs 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 to prevent extinction of Critically Endangered small cetaceans is reduction or elimination of their primary threats, which are usually by-catch and habitat loss.

KEY WORDS: Breeding program  $\cdot$  *Ex situ* conservation  $\cdot$  Live capture  $\cdot$  Acclimation  $\cdot$  Assisted reproductive technologies  $\cdot$  ART  $\cdot$  Artificial insemination  $\cdot$  Dolphin  $\cdot$  Conservation threats

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## INTRODUCTION

Captive breeding and reintroduction have played a pivotal role in the recovery of some terrestrial species such as the Arabian oryx *Oryx leucoryx* (Stanley Price 1989), the golden lion tamarin *Leontopithecus rosalia* (Kleiman & Rylands 2002), the California condor *Gymnogyps californianus* (Ralls & Ballou 2004), and the black-footed ferret *Mustela nigripes* (Wisely et al. 2003). However, many attempts to develop captive breeding programs for other terrestrial species have failed because the species has not survived or reproduced well in captivity (Lees & Wilcken 2009).

The impending extinction of the Chinese river dolphin, 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 implemented. Captive breeding proved difficult, and multiple anthropogenic impacts soon led to the extinction of this species (Dudgeon 2005, Reeves & Gales 2006, Wang et al. 2006, 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 it has contributed to the recovery of some terrestrial species.

Although captive breeding has not played a major role in the conservation of any cetacean, there have been numerous captive births of the species most commonly kept in captivity (e.g. common bottlenose dolphins Tursiops truncatus, Indo-Pacific bottlenose dolphins T. aduncus, and killer whales Orcinus orca), and attempts are being made at captive propagation of less frequently held small cetaceans such as the Yangtze finless porpoise Neophocaena asiaeorientalis asiaeorientalis (Wang et al. 2005, 2010, Wang 2009, Jefferson & Wang 2011).

Assisted reproductive technology (ART) for enhancement of captive 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 and improved management of captive populations (Robeck et al. 1994, 2008, O'Brien & Robeck 2010a).

In recent decades the magnitude and complexities of human impacts on ecosystem function have emerged as a major challenge to scientists and conservationists working to sustain biodiversity and habitat on local and global scales (Margules & Pressey 2000, Hooper et al. 2005, Knight et al. 2006). It is becoming evident that conservation in situ will require ongoing spatial management in the form of parks and reserves and rigorous habitat protection (Lee & Jetz 2008, Visconti et al. 2010). In addition, there is increasing recognition that zoological institutions have limited animal-carrying capacity and propagation ex situ must be much more closely managed (Russello & Amato 2007, Conway 2010). Snyder et al. (1996, 1997) provided a convincing rationale (including the possibility of disease among the founders of the population, prohibitively high costs of long-term maintenance, and the difficulties of reintroduction) that captive breeding can play a legitimate role in the recovery of only a limited number of endangered species and should be adopted only when feasible alternatives are unavailable.

In general, a captive 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 technical guidelines for management of ex situ populations as a method of conservation (IUCN 2012). These provide practical guidance for determining when ex situ management is warranted for a given taxon and for assessing the potential feasibility of ex situ conservation as well as the risks to the wild population. The IUCN guidelines emphasize the need for overall strategic planning for a species to be undertaken as early as possible.

We argue that a captive breeding program for a new species cannot be successfully implemented 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 & Antrim 2010), risk is inherent to 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 or individuals may not acclimate well to the captive environment (Walker 1975, Small & De Master 1995a). Technologies for assisted reproduction and techniques for release or reintroduction into the wild may also need to be developed.

To examine the prospects for using captive breeding to help conserve Endangered or Critically Endangered small cetaceans such as the Asian Ganges and Indus river dolphins (Platanista gangetica gangetica and P. g. minor, respectively), the vaguita Phocoena sinus, and the river-dwelling and marine subpopulations of the Irrawaddy dolphin Orcaella brevirostris (Table 1), we review attempts to capture, maintain, and breed poorly known small cetaceans in captivity. We then discuss the current state of ART for captive cetaceans and the prospects for applying these techniques to other poorly known small cetaceans. Finally, we outline the minimum objectives that would be necessary to initiate a captive breeding program for such species. We hope this paper will provide a starting point for identifying and addressing weaknesses in the potential for successful captive breeding of poorly known small cetaceans, prompting scientists and conservationists to more carefully examine and evaluate the logistical realities and risks of creating captive breeding programs for individual taxa of concern.

Table 1. Some of the poorly known small cetacean populations listed by the International Union for the Conservation of Nature (IUCN) as Critically Endangered, and the Endangered Ganges and Indus river dolphins whose populations are severely fragmented

| Species<br>Subpopulation                            | IUCN status              | Native country                             | Population<br>estimate | Source   |  |  |
|---|--------------------------|--|------------------------|--|--|--|
| South Asian river dolphin                           | Platanista gangetica     |  |                        |  |  |  |
| Ganges river dolphin P. g. gangetica                | Endangered               | India, Bangladesh,<br>Nepal (poss. Bhutan) | <2000-4000             | Mohan et al. (1997),<br>Smith et al. (2004a)         |  |  |
| Indus river dolphin<br><i>P. g. minor</i>           | Endangered               | Pakistan                                   | 965                    | Braulik et al. (2004)                                |  |  |
| Vaquita <i>Phocoena sinus</i>                       |                          |  |                        |  |  |  |
| Species level: subpop.<br>not applicable            | Critically<br>Endangered | Mexico                                     | 245                    | Gerrodette et al. (2011)                             |  |  |
| Irrawaddy river dolphin O                           | rcaella brevirostris     |  |                        |  |  |  |
| Ayeyarwady River                                    | Critically<br>Endangered | Myanmar                                    | 59                     | Smith et al. (1997),<br>Smith (2004)                 |  |  |
| Mahakam River                                       | Critically<br>Endangered | Indonesia                                  | 67-70                  | Kreb & Syachraini (2007),<br>Jefferson et al. (2008) |  |  |
| Malampaya Sound                                     | Critically<br>Endangered | Philippines                                | 77                     | Smith et al. (2004b),<br>Smith & Beasley (2004a)     |  |  |
| Mekong River  | Critically<br>Endangered | Cambodia, Laos<br>PDR, Vietnam             | 69                     | Smith & Beasley (2004b)                              |  |  |
| Songkhla Lake                                       | Critically<br>Endangered | Thailand                                   | Unknown <sup>a</sup>   | Beasley et al. (2002),<br>Smith & Beasley (2004c)    |  |  |
| <sup>a</sup> Estimated to be <50 mature individuals |                          |  |                        |  |  |  |

#### CAPTURE AND TRANSPORT OF SMALL CETACEANS

Although capture and transport of cetaceans are known to be high-risk procedures, especially for poorly known species, 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. Small cetaceans 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 & Mead 1999, Dima & Gache 2004). In some instances, this mortality may have been related to stress during capture (Curry 1999, Cowan & Curry 2008). Van Waerebeek et al. (2008) also have documented the potentially disastrous effects of ill-conceived live-capture endeavors.

Capture and transport conditions vary widely depending on capture location, climate, and characteristics of transportation (vessel, vehicle, or aircraft; ambient temperature control). Duration of transport can be many hours, and conditions are likely to be more difficult in remote or underdeveloped regions where many poorly known small cetacean species occur (e.g. Tas'an & 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 USA, Europe, and Japan (Brownell 1984, Sylvestre 1985, Caldwell et al. 1989, Tobayama & Kamiya 1989). No data exist for mortality at the time of capture, but deaths often occurred during transport over long time periods like to the USA (Caldwell et al. 1989). During the 1970s to 1990s, this species was captured and held in the Brazilian Amazon (da Silva 1994) and was also captured in the Orinoco River system and transported, for up to 12 h, 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, but good records start in 1963. At least 153 narrowridged finless porpoises Neophocaena asiaeorientalis 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 rates during capture have not been commonly reported, and 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 capAuthor copy

There are known difficulties with capture and transport of Irrawaddy dolphins, especially in riverine habitat. The drive method was used to capture 20 Irrawaddy dolphins from the Mahakam River, Indonesia, in 1974 and 1978 for transport (12 h) to the Jaya Ancol Oceanarium in Jakarta, and 6 more dolphins were captured in 1984 (Tas'an et al. 1980, Tas'an & Leatherwood 1984, Wirawan 1989). These captures involved mortality attributed to the stress of capture and transport (Table 2). In addition, Beasley (2002) reported that in 1999, during preparation for intra-aquarium transport activities (from Oasis Sea World, Chantaburi Province, Thailand, to Underwater World, Singapore), 1 of 4 Irrawaddy dolphins died, and the remaining 3 were considered to be 'unstable for transport,' leading to the cancellation of intended transport activities.

Twenty-seven (7 in 2008 and 20 in 2011) Irrawaddy dolphins were recently captured from the coastal waters off Kien Giang (Gulf of Thailand) near Hon Chong, Kien Giang Province, by the Vietnam-Russia Tropical Center for use in 'scientific research and circus performances' (Nguyen et al. 2010, 2012a). Dolphins were kept in a sea-pen and were subsequently transported via helicopter and airplane. Mortality was not reported, but disposition was reported for only 3 animals (Nguyen et al. 2010, 2012b) -2 adults and 1 immature dolphin were reported to be housed at Dai Nam Wonderland, Binh Duong Province. In June 2011, 4 Irrawaddy dolphins, in addition to the 3 in Binh Duong Province, were widely advertised to have been transported to Vinpearl Land, Nha Trang, Khanh Hoa Province.<sup>1</sup>

There have been improvements in capture and transport techniques (e.g. those discussed by Braulik et al. 2005 and Bonar et al. 2007, and used by Wells et al. 2004). These techniques were developed through

Table 2. Orcaella brevirostris. Twenty-six Irrawaddy dolphins captured between 1974 and 1984 from the Mahakam River, Indonesia, for captivity at Jaya Ancol Oceanarium (Tas'an et al. 1980, Wirawan 1989). Date of death and survival time are included if known. Note: Six of 16 individuals with unknown survival times were alive in 1985; 2 remained alive in 1995 (Tas'an et al. 1980, Tas'an & Leatherwood 1984, Stacey & Leatherwood 1997, Stacey & Arnold 1999)

| Identification   | Sex                  | Date<br>captured | Date of<br>death | Survival<br>time | Reported condition/pathologies                 |  |
|--|----------------------|------------------|------------------|------------------|--|--|
| 1 individual   | Female               | 15 Oct 1974      | Unknown          | Unknown          | Pregnant — released after capture              |  |
| 1 individual   | Unknown              | 15 Oct 1974      | Unknown          | Unknown          | 'Not fit for transport'—released after capture |  |
| 74GSA16mOb1  | Female <sup>a</sup>  | 15 Oct 1974      | Deceased         | Unknown          | Unknown  |  |
| 74GSAl7mOb2  | Male                 | 15 Oct 1974      | 4 Nov 1974       | 20 d             | Gastrointestinal ulcers, stress                |  |
| 74GSA18mOb3  | Female               | 15 Oct 1974      | 23 Oct 1974      | 10 d             | Gastrointestinal ulcers, stress                |  |
| 74GSA18mOb4  | Male                 | 15 Oct 1974      | Deceased         | Unknown          | Unknown  |  |
| 74GSA20mOb5  | Male                 | 15 Oct 1974      | 2 Jul 1978       | 3 yr 261 d       | Pulmonary infection                            |  |
| 74GSA21mOb6  | Female               | 15 Oct 1974      | 16 Oct 1974      | 1 d              | Stress   |  |
| 1 individual   | Unknown              | 24 Sep 1978      | Unknown          | Unknown          | Unknown—not transported                        |  |
| 1 individual   | Unknown              | 24 Sep 1978      | Unknown          | Unknown          | Unknown—not transported                        |  |
| 74GSA94mOb7  | Male                 | 24 Sep 1978      | Deceased         | Unknown          | Unknown  |  |
| 78GSA95mOb8  | Male                 | 24 Sep 1978      | 17 Jan 1979      | 115 d            | 'Constitutional heart weakness'                |  |
| 78GSA96mOb9  | Male                 | 24 Sep 1978      | Deceased         | Unknown          | Unknown  |  |
| 78GSA97mOb10   | Male                 | 24 Sep 1978      | Deceased         | Unknown          | Unknown  |  |
| 78GSA98mOb11   | Female               | 24 Sep 1978      | Deceased         | Unknown          | Unknown  |  |
| 78GSA99mOb12   | Male                 | 24 Sep 1978      | Deceased         | Unknown          | Unknown  |  |
| 78GSA100mOb13  | Male                 | 24 Sep 1978      | Deceased         | Unknown          | Unknown  |  |
| 78GSA111mOb14  | Male                 | 24 Sep 1978      | Deceased         | Unknown          | Unknown  |  |
| 78GSA112mOb15  | Male                 | 24 Sep 1978      | Deceased         | Unknown          | Unknown  |  |
| 78GSA113mOb16  | Male                 | 24 Sep 1978      | 24 Oct 1978      | 30 d             | Pneumonia, liver cirrhosis                     |  |
| 79GSA125Ob18   | Unknown <sup>b</sup> | 11 Dec 1979      | 11 Dec 1979      | Died at birth    | Unknown  |  |
| Six individuals  | Unknown              | 1984             | Unknown          | Unknown          | Unknown  |  |
| <sup>a</sup> Mature female—gave successful birth to captive-born female (see Table 5)<br><sup>b</sup> Captive birth—conception, parentage not reported |                      |                  |                  |                  |  |  |

<sup>&</sup>lt;sup>1</sup>Disclosed on multiple Internet websites (e.g. www.travelblog.org/Asia/Vietnam/blog-624450.html, http://bachhoa24.com/ dua-ca-heo-ong-su-vao-phuc-vu-du-lich-n-6156.html)

decades of experience among cetacean researchers, veterinarians as well as zoological and other institutions, and have been applied primarily to common bottlenose dolphins. Carefully designed captures, conducted under suitable environmental conditions with ample experienced personnel and equipment, can be successful. R. S. Wells and colleagues have been conducting a comprehensive research study on a population of common bottlenose dolphins in Sarasota, Florida, since 1970. Their highly successful dolphin health assessment program has been ongoing for >2 decades and involves capture and an approximately 1 h health examination (including standardized measurements, ultrasound, temperature probe, and a suite of collected samples). Capture occurs in shallow, sheltered bay waters that are typically 1 to 4 m deep, providing relatively easy access, but may be up to 10 m deep. Capture operations are intensive, meticulous, highly coordinated, and rely on extremely experienced personnel, including vessel operators, researchers experienced in capture-release fishery operations and dolphin handling, as well as expert veterinary personnel (Scott et al. 1990, Wells et al. 2004, 2005, B. E. Curry pers. obs.). These animals are not transported, but are transferred from the capture net to foam pads and examined under shaded cover on a vessel (Wells et al. 2004). Total capture time ranges from 1 to 4 h. More than 180 individuals have been successfully captured and released (Wells et al. 2004). In addition, the United States Navy Marine Mammal Program routinely transports captive bottlenose dolphins for military purposes (e.g. Olds 2003). Transport procedures and equipment have evolved since 1959 when the navy first began working with marine mammals, and today dolphins are safely transported (Reddy 1991, Houser et al. 2010), sometimes up to a day, by vehicle, vessel, and aircraft (DON 2009). Such transports can be extremely costly, relying on advanced military aircraft, for example, and are labor intensive, requiring experienced veterinary personnel and cetacean handlers.

#### ACCLIMATION TO CAPTIVITY

The acclimation period subsequent to 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 conspecifics, as well as feeding and other human interactions. An understanding of the time needed for newly introduced individuals of a species to acclimate to captivity allows for improved marine mammal husbandry practices. The estimated acclimation period (during which the likelihood of mortality is higher than afterwards) for a common bottlenose dolphin *Tursiops truncatus* brought to captivity from the wild is 35 d (Small & DeMaster 1995a). However, Small & DeMaster (1995a) evaluated acclimation periods for wild-born (n = 1270), captive-born (n = 332), and captive-transferred (n = 911) bottlenose dolphins, along with those for wildborn (n = 1650), captive-born (n = 992), and captive-transferred (n = 336) California sea lions *Zalophus californianus* and estimated a 60 d period of relatively high mortality for newly caught or captive-transferred marine mammals.

River dolphins have experienced high rates of mortality during acclimation post-capture and transport. There were 147 Amazon river dolphins Inia geoffrensis taken into captivity from 1956 to 2006 (Bonar et al. 2007). Analysis of pathology records for 123 of the 147 captive individuals indicated that mortality was highest in the first 2 mo post-capture and transport (32 of the 123 deaths; Bonar et al. 2007). A high incidence of pneumonia, which was identified as a cause of mortality within the first month of capture, was attributed to stress of capture and transport (Caldwell et al. 1989, Bonar et al. 2007). In about 25% of these cases, a predisposition to infection due to parasite load (pulmonary trematodes) may have existed. 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).

Mortality post-capture and transport was also high for the baiji *Lipotes vexillifer* (Chen & Liu 1989). Of 6 baiji captured between 1981 and 1986, 3 died between 17 d and 4 mo of capture, and a fourth died within 9 mo (Table 3; Chen & Liu 1989).

South American fransiscanas *Pontoporia blainvillei* have only been held captive a few times, under poor conditions, in Uruguay. Two individuals survived only days in captivity during the 1950s (Monzón & Corcuera 1991, Reeves & Mead 1999), and 1 lived for several days held captive in the early 1970s (Brownell 1989).

Four female Indus river dolphins *Platanista gangetica minor* were captured and imported to the Steinhart Aquarium in San Francisco, California, from Pakistan in 1968 and 1970, but the 'rigors of capture and transport' were such that they survived for only 24, 38, and 44 d (McCosker 2007, Reeves & Brownell 1989). Two died of pneumonia and the third had complications from a pre-existing injury to the lower jaw (Reeves & Brownell 1989). At least 7 Indus river

| Identification         | Sex            | Length (cm) | Date captured              | Date of death       | Survival time      | Institution  |
|------------------------|----------------|-------------|----------------------------|---------------------|--------------------|--|
| Qi Qi                  | Male           | 143         | 12 Jan 1980                | 14 Jul 2002         | 22 yr 190 d        | Institute of Hydrobiology                              |
| Su Su                  | Female         | 182         | 3 Mar 1981                 | 20 Mar 1981         | 17 d               | Nanjing Normal University                              |
| Rong Rong              | Male           | 151         | 22 Apr 1981                | 3 Feb 1982          | 228 d              | Institute of Hydrobiology                              |
| Jiang Jiang            | Male           | 174         | 7 Dec 1981                 | 16 Apr 1982         | 129 d              | Nanjing Fisheries Research<br>Institute                |
| Lian Lian<br>Zhen Zhen | Male<br>Female | 203<br>152  | 31 Mar 1986<br>31 Mar 1986 | 14 Jun 1986<br>1988 | 76 d<br>2 yr 182 d | Institute of Hydrobiology<br>Institute of Hydrobiology |

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

dolphins were captured—2 in December 1969 (Pilleri 1970), 1 in March 1972 (Pilleri 1972), and 4 in December 1972 (Pilleri et al. 1976)—for transport to the Brain Anatomy Institute in Berne, Switzerland (Reeves & Brownell 1989).

Acclimation of narrow-ridged finless porpoises *Neophocaena asiaeorientalis* is not well documented. In one account from 2004, 9 finless porpoises (5 male, 4 female) were live-captured in Ise Bay, Japan, using 6 purse seine vessels (Miyashita et al. 2005). One male died 2 wk after capture due to bacterial infection (methicillin-resistant *Staphylococcus aureus* [MRSA] strain of micrococcus; Miyashita et al. 2005, Morris et al. 2011).

One newborn vaquita *Phocoena sinus* stranded near Puerto Peñasco, Sonora, Mexico, on 13 May 1994, and was held captive at the Intercultural Center for the Study of Deserts and Oceans (CEDO), but only survived for a few hours (P. Turk Boyer pers. comm., 24 February 2012). No other specimens have been held in captivity.

Sixteen of the 20 Irrawaddy river dolphins *Orcaella brevirostris* captured from the Mahakam in 1974 and 1978 were held for approximately 1 mo periods in a net holding pen erected in the Pela tributary. The dolphins were transitioned from feeding on live to dead fish during these periods. Three of 6 dolphins died during this period in 1974, and 1 died at 30 d in 1978 (Ta'san et al. 1980; Table 2). Water in the holding pen was 'dirty and polluted,' and surviving individuals were administered antibiotics prior to transport (Ta'san et al. 1980).

An analysis of odontocete cetaceans that stranded alive in California waters from 1977 to 2002 illuminated the difficulties of stranded cetaceans in acclimating to captivity post-handling and transport (Zagzebski et al. 2006). The authors suggested that stress or pathologies related to the physical act of stranding and subsequent capture/transport procedures likely impeded successful rehabilitation of livestranded cetaceans.

Systematic investigation of the causes of mortality during acclimation could provide insight to the health concerns encountered during capture and acclimation of small cetacean species (Walker 1975). Bacterial infections, for example, are likely to relate, at least in part, to capture and transport, and may be more of a problem for freshwater species than for marine species. Improvements to sling design, climate control, and sanitary water quality, in addition to prophylactic anthelminthic treatment combined with broad-spectrum antimicrobial therapy, have been suggested to address some of the problems that have been encountered in the transport of Amazon river dolphins (Bonar et al. 2007) and would likely benefit other species, especially freshwater species. During a wide window of time (minimum 60 d) surrounding the acclimation period, there is a need for close monitoring (e.g. of behavior, nutrition, water quality, and medical care; Joseph & Antrim 2010), as well as minimization of potential stressors.

## MAINTAINING SMALL CETACEANS IN CAPTIVITY

In general, zoos and aquariums have the 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 with the closely related Andean condors *Vultur gryphus* (Ralls & 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 & Meadows 2001, Kleiman et al. 2010, Ralls & Ballou 2013).

Zoos and aquariums have had extensive, long-term experience and success with only a few small cetaceans other than bottlenose dolphins *Tursiops*  spp. and killer whales *Orcinus orca* (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 longitudinal data regarding mortality and survival of individual cetacean species in captivity are available for comparison among species and institutions (DeMaster & Drevenak 1988, Duffield & Wells 1991, Reeves et al. 1994). Today, with the International Species Information System (ISIS) and the advent of the Zoological Information Management System (ZIMS) as a repository for standardized collections data, better comparisons may become possible for a wider variety of species and institutions.

Even so, the infrastructure, funding level, and extent of cumulative experience at individual institutions are likely to have a strong influence on small cetacean survival rates. Comparison of available zoological records (dates of captures, births, deaths) has shown significant differences in survival rates of captive common bottlenose dolphin among institutions (DeMaster & Drevenak 1988). For killer whales, mortality has been found to be highest from captive birth to 1 yr of age, and for individuals in their first year of captivity (DeMaster & Drevenak 1988, see also Bigg & Wolman 1975, Greenwood & 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 & Drevenak 1988). Survival of captive bottlenose dolphin and killer whale calves was significantly lower than in wild populations (Small & DeMaster 1995b). Innes et al. (2005) compared institutional records from 1973 to 2003, and documented a slight but significant increase in annual survival rates for bottlenose dolphins over time. Increased survival rate was attributed to improvements in husbandry and veterinary care, as well as institutional efforts to improve care for this species.

River dolphins have proved difficult to maintain. Amazon river dolphins *Inia geoffrensis*, baiji *Lipotes vexillifer*, and South Asian river dolphins *Platanista gangetica* have all had poor survival rates in captivity (Caldwell et al. 1989, Chen & Liu 1989, Reeves & Brownell 1989). However, survival rates have not been calculated controlling for level of experience in capturing different species; for instance, comparing survival rates for the first 20 Amazon River dolphins brought into captivity with those for the first 20 bot-tlenose 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, and the possibility that this species is more difficult to capture and maintain successfully than bottlenose dolphins due to some biological differences between the species. This specific analysis cannot be conducted because there are no data on the survival of the first 20 bottlenose dolphins taken into captivity.

Examination of post-mortem records for captive Amazon river dolphins (97 of 147) indicated that, as in bottlenose dolphins (DeMaster & Drevenak 1988), mortality was highest in the first year of captivity (Bonar et al. 2007). Only 9 of 97 river dolphins remained alive after 10 yr in captivity. Longevity in captivity is 10 to 26 yr (Best & da Silva 1993). Control of microbiological water quality is considered vital to maintenance, and a robust preventative medical program aimed at preventing bacterial infection may promote the longevity of Amazon river dolphins in captivity (Caldwell et al. 1989, Bonar et al. 2007).

The longest surviving baiji, the only 1 of 6 captives to live >3 yr, was rehabilitated from injuries and lived 22 yr (Chen & Liu 1989, Braulik et al. 2005). The next longest surviving captive baiji lived for 2 yr 182 d (Chen & Liu 1989, Braulik et al. 2005; Table 3). One additional individual was held in semi-natural conditions from December 1996 to June 1997, surviving 187 d in the Shishou Reserve, Hubei Province, China (Liu et al. 2002).

Some of the 7 Indus river dolphins *Platinista* gangetica minor known to have been captured and transported to the Brain Anatomy Institute are thought to have survived for several years (Reeves & Brownell 1989). One Indus river dolphin female was maintained at the Steinhart Aquarium from May 1970 to July 1971 (Reeves & Brownell 1989). In 1970, 5 (1 male, 4 female) Ganges river dolphins *P. g. gangetica* survived in captivity at Kamogawa Sea World from 64 to 299 d (Reeves & Brownell 1989). Indus river dolphins have survived for up to 5 yr in captivity (Reeves & Mead 1999, Collet 1984).

Most individuals captured during early attempts to maintain the Yangtze finless porpoise *Neophocaena asiaeorientalis asiaeorientalis* in China died in <1 yr (Liu et al. 2002, Wang 2009). There are currently 3 main holding areas for these porpoises in China (Wang 2009). One is the semi-natural Shishou Reserve (in the 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). Author copy

Approximately 30 porpoises occupied the reserve in 2010. Another small reserve, currently occupied by 10 porpoises, was established in Tongling, Anhui Province, in 1994 (Wang et al. 2010). Females produce calves annually in these reserves (Wang 2009, Wang et al. 2010). The third main holding area is an aquarium at the Institute of Hydrobiology (IHB) in Wuhan, which was maintaining 5 Yangtze finless porpoises in 2009, including 1 captive-born male (Table 4; Wang 2009). The IHB maintained 6 porpoises (3 male, 3 female) in 2010 (Wang et al. 2010, Zhang et al. 2012).

Both subspecies of narrow-ridged finless porpoise *Neophocaena asiaeorientalis* — the Yangtze finless porpoise *N. a. asiaeorientalis* and the East Asian finless porpoise *N. a. sunameri* — from coastal marine waters are currently housed at institutions in China (Zhang et al. 2012). Six of these are the Yangtze finless porpoises at the IHB (Wang et al. 2010); the remaining 9 (3 males, 6 females) are individuals of both subspecies at other institutions (see Zhang et al. 2012).

Irrawaddy dolphins *Orcaella brevirostris* have been maintained in captivity in Indonesia, Thailand, Japan, and Cambodia. Although mortality rates during capture and acclimation have been high for individuals of the Mahakam River subpopulation, captive survival rates once on public display in Indonesia are not well known (Tas'an et al. 1980, Tas'an & Leatherwood 1984, Stacey & Leatherwood 1997, Wirawan 1989). There has been 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 (Perrin et al. 1996, 2005, Stacey 1996, Beasley 2007, Beasley & Davidson 2007). The total numbers removed are unknown (Reeves & Fisher 2005). Irrawaddy dolphins have been housed in 2 facilities in Thailand (Oasis Sea World, Chantaburi Province, and Safari World, Bangkok aquariums; Stacey 1996, Stacey & Leatherwood 1997, Beasley 2007). Ten wild-caught Irrawaddy dolphins (4 males and 5 females taken in 1983, and 1 male taken in 1988) were reported to be housed at Oasis Sea World in 2002 (Beasley 2002).

In 1994, 8 Irrawaddy dolphins were caught using nets in the coastal waters of Cambodia and taken into captivity at Safari World, Bangkok (Stacey & Leatherwood 1997). In 1995, 2 Irrawaddy dolphins, thought to have been from the 1994 collection, were exported by Safari World to Marine World Uminonakamichi, Fukuoka City, Japan, but both have since died (T. K. Yamada pers. comm., 16 September 2009; Stacey 1996, Stacey & Leatherwood 1997). We could not find information regarding the length of captive survivorship for these individuals. Beasley (in Perrin et al. 2005) reported that at least 8 Irrawaddy dolphins were captured in Cambodian coastal waters in January 2002 and then transferred to the Koh Kong International Resort Hotel on the Thailand/Cambodia

Table 4. Neophocaena asiaeorientalis asiaeorientalis. Yangtze finless porpoise taken into captivity in China. After Liu et al. (2002) and Braulik et al. (2005). –: single individual, sex unknown

| Individual             | Year captured  | Survival time     | Institution                | Source   |  |  |  |
|------------------------|--|-------------------|----------------------------|--|--|--|--|
| _                      | 1965   | 245 d             | Quingdao Marine Museum     | Liu et al. (2002), Braulik et al. (2005),<br>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   | 2 yr 29 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),<br>Wang (2009) |  |  |  |
| 1 female               | 1996   | Alive in 2009     | Institute of Hydrobiology  | Liu et al. (2002), Braulik et al. (2005),<br>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 in 2009     | Institute of Hydrobiology  | Liu et al. (2002), Wang (2009)                           |  |  |  |
| 1 male <sup>a</sup>    | 2004   | Alive in 2009     | Institute of Hydrobiology  | Wang (2009)  |  |  |  |
| <sup>a</sup> This male | <sup>a</sup> This male was taken from the Tian'e-Zhou Oxbow of the Yangtze River |                   |                            |  |  |  |  |

border. They were thought to have died within 5 yr (Beasley & Davidson 2007). Significant problems with water quality and feeding have been reported for institutions in Indonesia and Thailand (Tas'an et al. 1980, Perrin et al. 1996, Stacey & Arnold 1999).

In recent decades, many advances have been made in 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 & Antrim 2010). However, because husbandry techniques are often species specific, new captive breeding programs may require substantial research development for captive care with regards to behavior, reproductive physiology, nutrition, and disease (Ralls & Meadows 2001, Kleiman et al. 2010, Ralls & Ballou 2013).

#### BREEDING SMALL CETACEANS IN CAPTIVITY

Many wild-caught animals fail to breed in captivity (Lees & Wilcken 2009). As noted above, this failure is often due to behavioral problems caused by inadequate husbandry techniques (Ralls & Meadows 2001, Ralls & Ballou 2013). Success with captive breeding of dolphins that live in rivers has been extremely limited (Table 5; Reeves & Mead 1999). Amazon river dolphins *Inia geoffrensis* were widely held in captivity from the early 1950s to the late 1970s, but Caldwell et al. (1989) reported only 2 live captive births. Both calves died shortly after birth: 1 after minutes and 1 after approximately 2 wk (Huffman 1970, Caldwell & Caldwell 1972). However, from 2000 to 2009, live births of 3 calves conceived in captivity occurred at the Valencia Aquarium (Boede 2005, Bonar et al. 2007, Pelaez 2010, Rojas 2010). Two of these have died (see Table 5).

In 2011, 4 of the 6 Amazon river dolphins maintained at the Valencia Aquarium (including a captive-conceived female born in 2009) died in a 4 mo period of causes generally attributed to poor conditions, e.g. poor water quality and ingestion of foreign objects including debris from the deterioration of railings that surrounded enclosures (Table 6; AN Venezuela 2011a,b).

There has been success breeding captive narrowridged finless porpoises *Neophocaena asiaeorien*-

Table 5. Reported live captive births for Amazon river dolphins *Inia geoffrensis*, Yangtze finless porpoise *Neophocoena asiaeorientalis* asiaeorientalis, and Irrawaddy dolphins *Orcaella brevirostris* (Mahakam River subpopulation) held in captivity between 1956 and 2011. -: no information available

| Species                  | Institution   | Identification         | Sex    | Date of<br>birth | Date of death | Survival<br>time          | Source   |
|--------------------------|---|------------------------|--------|------------------|---------------|---------------------------|--|
| I. geoffrensis           | Fort Worth<br>Zoological Park<br>Marineland of<br>Florida |                        | -      | 1970<br>-        | 1970          | Died at<br>birth<br>15 d  | Huffman (1970),<br>Caldwell et al. (1989)<br>Caldwell & Caldwell (1972),<br>Caldwell et al. (1989) |
|                          | Valencia<br>Aquarium                                      | Telemachus             | Female | Nov 2000         | 2005          | ~5 yr                     | Pelaez (2010), Rojas (2010)  |
|                          | Valencia<br>Aquarium                                      | Zeus                   | Male   | Nov 2005         | Alive in 2011 | -                         | Boede (2005)   |
|                          | Valencia<br>Aquarium                                      | Helena II              | Female | 23 Oct<br>2009   | 14 Apr 2011   | 1 yr 173 d                | Pelaez (2010), Rojas (2011)  |
| N. p.<br>asiaeorientalis | Institute of<br>Hydrobiology                              | -                      | Male   | 2005             | Alive in 2011 | -                         | Wang et al. (2005),<br>Wang (2009)   |
|                          | Institute of<br>Hydrobiology                              | -                      | Male   | 2007             | 2007          | 39 d                      | Wang (2009)  |
|                          | Institute of<br>Hydrobiology                              | -                      | Male   | 2008             | 2008          | 5 d                       | Wang (2009)  |
| O. brevirostris          | Jaya Ancol<br>Oceanarium (                                | Isui<br>79GSA105mOb17) | Female | 4 Jul<br>1979    | Deceased      | Unknown;<br>alive in 1984 | Tas'an et al. (1980),<br>Tas'an & Leatherwood<br>(1984), Beasley (2007)                            |
|                          | Jaya Ancol<br>Oceanarium                                  | -                      | -      | -                | Deceased      | Unknown;<br>alive in 1984 | Tas'an et al. (1980),<br>Tas'an & Leatherwood<br>(1984), Beasley (2007)                            |

Table 6. Inia geoffrensis. Amazon river dolphins captured from 1975 to 1994 in the Orinoco River (Apure River tributary, Guariquito River), Venezuela, and taken into captivity at the Valencia Aquarium (Venezuela) as reported by Boede et al. (1998). Survival times are reported if known. –: no information available

| Location  | Sex                   | Individual | Date<br>captured | Date of<br>death            | Survival<br>time          | Reported pathologies  |  |
|---|-----------------------|------------|------------------|-----------------------------|---------------------------|---|--|
| Apure River   | 5 male,<br>3 female   |            | 1975             | Deceased                    | Not known                 | -   |  |
|   | 1 male                | Arquimides | 1975             | Jun 1987                    | ~12 yr                    | Gastrointestinal obstruction, gastric ulcers  |  |
|   | 1 female              | Nelly      | 1975             | 6 Nov<br>1993               | ~18 yr                    | Warfarin poisoning (rodenticide)  |  |
| Guariquito<br>River   | 1 female              |            | 10 Jul<br>1987   | _                           | Released<br>after capture | -   |  |
|   | 1 male                | Ulyses     | 10 Jul<br>1987   | 13 Jan<br>2011 <sup>b</sup> | 23 yr 194 d               | Bronchopneumonia, hepatitis <sup>c</sup>  |  |
|   | 1 female              | Dalila     | 10 Jul<br>1987   |                             | Alive in 2011<br>(23 yr)  |   |  |
|   | 1 female              | Penelope   | 20 Apr<br>1994   | 25 Mar<br>2011 <sup>b</sup> | 16 yr 344 d               | Acute ulcerative gastritis, ingestion of<br>foreign material (note subsequent death of<br>offspring 'Helena II' from same cause;<br>see Table 5) <sup>c</sup> |  |
|   | 1 female              | Helena I   | 20 Apr<br>1994   | Deceased                    | Not known                 | -   |  |
|   | 1 female <sup>a</sup> | Artemis    | 19 Oct<br>1994   | 5 Feb<br>2011 <sup>b</sup>  | 16 yr 114d                | Hepatitis, pancreatitis, gastric ulcers <sup>c</sup>  |  |
| <sup>a</sup> Conceived in the wild (born 19 October 1994); <sup>b</sup> AN Venezuela (2011a); <sup>c</sup> Yucra (2011) |                       |            |                  |                             |                           |   |  |

talis in Japan since the mid-1970s (Furuta et al. 1976, Wang et al. 2000, Miyashita et al. 2005). Captive breeding of Yangtze finless porpoises N. a. asiaeorientalis in China has taken decades to organize and has included considerable trial and error (Perrin et al. 1989, Wang et al. 2000, 2006, Wang 2009). Currently, captive breeding is considered to be an integrated part of an overall conservation effort for this subspecies in China, including the establishment of reserve areas for wild populations (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 9 yr in captivity, 1 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).<sup>2</sup> The same female gave birth to a second calf in June 2007 and died 39 d later, followed by the death of the calf after 11 d. In July 2008, the second female gave birth to a calf, but did not lactate, and the calf died after 5 d (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 1 of the 2 captive males over an approximately 6 yr 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 *Orcaella brevirostris*, conceived in captivity, have occurred in 2 aquariums: 2 healthy dolphins were born at the Jaya Ancol Oceanarium (Tas'an et al. 1980, Tas'an &

<sup>&</sup>lt;sup>2</sup>This captive born male and a rehabilitated individual captured after sustaining injuries in 2008, were released into the Tian'e-Zhou Oxbow in April 2011 (http://wwfcn.panda. org/?3440/)

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Leatherwood 1984, Stacey 1996; Tables 2 & 6), and an unknown number of births has occurred at Oasis Sea World, Chantaburi Province (Perrin et al. 1996, Stacey 1996, Stacey & Arnold 1999); 3 captive-born Irrawaddy dolphins (ages approximately 8 to 10 yr) were reported to be housed at that facility in 2002 (Beasley 2002).

Among many other factors potentially affecting breeding success of small cetaceans, social grouping is often critical (e.g. avoiding possible suppression of spermatogenesis in subordinate male bottlenose dolphins; O'Brien & Robeck 2010a). Inappropriate social grouping can also have detrimental effects on health and longevity of small cetaceans in captivity. Unsuccessful breeding and short lifespan of Amazon river dolphins in captivity has been attributed, in part, to a lack of knowledge regarding aggressive behavior in social groupings (for example, larger males need to be separated from other animals) and to stressrelated diseases associated with transport and housing (Sylvestre 1985, Caldwell et al. 1989, Best & da Silva 1993). A comparison of pool size and number of individuals that had been housed among 13 institutions indicated a statistically significant correlation, likely to be related to water quality and space requirements among individuals, between dolphin survival and volume of water available (Bonar et al. 2007).

#### ASSISTED REPRODUCTIVE TECHNOLOGIES

Assisted reproductive technologies (ART) 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 1 sire to several females by extending semen; allowing more efficient breeding amongst institutions without animal transport) and a potentially shorter interval between generations (Andrabi & Maxwell 2007, Thomassen & Farstad 2009). O'Brien & Robeck (2010a) reviewed 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. However, 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 1-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 of 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 & Maxwell 2007). In addition, there are at least 2 initial criteria that must be met for AI and associated reproductive technologies to benefit endangered species. First, the captive 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 & Anderson 1991, Robeck et al. 1994). Second, to successfully use AI 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 et al. 1986, Wildt 1989, Robeck et al. 1994, 2004, 2005b).

There have been successes in the AI of 5 cetacean species, but these have occurred at only a few institutions after decades of species-specific research combined with highly refined clinical experience. Results of the first successful AI trials included the live births of 3 common bottlenose dolphins Tursiops truncatus (Robeck et al. 2005b), 2 killer whales Orcinus orca (Robeck et al. 2004), 1 beluga whale Delphinapterus leucas (O'Brien et al. 2008), and 5 Pacific white-sided dolphins Lagenorhynchus obliquidens (Robeck et al. 2009). O'Brien & Robeck (2010a) recounted collaboration amongst 36 researchers and institutions and that an additional 18 bottlenose dolphins, 2 killer whales, 3 belugas, and 1 Indo-Pacific bottlenose dolphin were produced from AI (6 of these were reported as still in utero from AI procedures conducted during 2010). This work marks a significant achievement for cetacean science and collections management and, if AI is proven replicable for these species (as it has been for common 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 captive

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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 and efforts ex situ (Wildt et al. 1992, O'Brien & 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 (e.g. Howard & Wildt 2009) and improved captive management of the species involved. 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 is often 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 & Wemmer 1999, Goodrowe et al. 2000, Andrabi & Maxwell 2007).

Some of the problems encountered during trials of AI in captive cetaceans have occurred at the methodological development stage (Robeck et al. 2004). Robeck et al. (2005b) noted that their success with common bottlenose dolphins highlights the value of strategic, systematic research into the basic reproductive physiology of a species for the development of ART. Because the common bottlenose dolphin has been most widely maintained in captivity, research on reproduction in this species has been particularly thorough (Schroeder 1990, Schroeder & Keller 1990, Robeck et al. 1994, 2005b). The earliest reports detailed reproductive behavior (e.g. Tavolga & Essapian 1957) and anatomy (Harrison 1969, Harrison et al. 1972, Harrison & McBreaty 1977, Bryden & 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 & Reilly 1984). In the late 1970s and 1980s, Cornell et al. (1977, 1987) reported results of research focused on 1 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, includ-

ing timing of ovulation and the estrous cycle, as well as aspects of the seasonality of reproduction in females (Sawyer-Steffan et al. 1983, Kirby & Ridgway 1984, Yoshioka et al. 1986, Kirby 1990, Schroeder 1990, Urian et al. 1996) and variation in testosterone levels among age classes and seasons in males (Harrison & Ridgway 1971, Yoshioka et al. 1986, Kirby 1990, Schroeder & Keller 1989). In addition, long-term, systematic evaluation of endocrine changes (in particular rapid detection of changes in urinary luteinizing hormone) elucidating ovarian activity and collection of these data in conjunction with ultrasound evaluation of ovarian changes and male reproductive characteristics (Brook 2001, Robeck et al. 2005b, Robeck and Monfort 2006) were necessary to achieve success with AI in bottlenose dolphins. Likewise, intricate details of reproductive endocrine physiology in female and male killer whales, beluga whales, Pacific white-sided dolphins, and Indo-Pacific bottlenose dolphins were needed to achieve the first successful AI in these species (Walker et al. 1988, Robeck et al. 1993, 2004, 2005a, 2009, O'Brien et al. 2008, O'Brien & Robeck 2010a).

Detailed knowledge of sperm characteristics and production (Fleming et al. 1981, Miller et al. 2002, Robeck & Monfort 2006, Yuen 2007, O'Brien et al. 2008), as well as semen preservation (Robeck & O'Brien 2004, O'Brien et al. 2008, Robeck et al. 2009, O'Brien & Robeck 2010a), have 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 & 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 & 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 & Robeck 2006, 2010a, O'Brien et al. 2009).

Species-specific research will be required to develop reproductive knowledge for the application of ART to captive propagation for any new small cetacean. Development of gamete preservation for biological resource banking and organized banking of spermatozoa may be of future value for conservation of small cetacean species (Wildt et al. 1997, O'Brien & Robeck 2010a).

#### LOGISTICAL REQUIREMENTS FOR CAPTIVE BREEDING

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. Captive breeding programs as a means of species conservation should not be instituted ad hoc, but should be systematically developed to integrate potential solutions to multiple risk factors. Recently revised IUCN guidelines on the management of ex situ populations for conservation include a broad outline for the assessment of feasibility and risk (both risk factors in the wild and risk imposed on the target taxon by the process of ex situ conservation) as a part of the overall decision-making process for implementing ex situ conservation efforts (IUCN 2012). Guidelines include stepwise risk assessment criteria that may prove useful for determining if and when individuals should be removed from the wild and for prioritization of captive breeding efforts. Decisions must be made regarding the number of individuals that can be removed from the wild population and the risk criteria that must be assessed on a per species basis prior to initiating a captive breeding program. Once fully established, these guidelines may prove useful to developing strategies for the conservation of small cetacean species.

Additional requirements must be met to supply the proper housing, nutrition, behavioral stimulation, and preventive medicine required for the care of small cetaceans. While governmental and institutional organizations sometimes develop guidelines for marine mammals in the captive environment, special considerations are necessary to the maintenance of these animals, and, realistically, zoological institutions must often exceed minimum requirements to achieve success (Joseph & 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.'3

A captive breeding program should be undertaken only when *in situ* conservation is proving uncertain

and when there is a long-term commitment of the substantial financial resources required, including buy-in from local and national government agencies. In addition, a captive breeding program should be developed as an integrated component of an overall conservation effort for a threatened taxon, including the goals of public awareness, population management reinforcement or reintroduction, and other support to wild populations (including habitat protection, restoration, and management), long-term banking of biological samples, scientific research, and fundraising (IUCN 2012). High cost is inherent to such recovery programs. For example, total United States Fish and Wildlife Service (USFWS) California Condor Recovery Program costs have exceeded 35 million US dollars, and cost estimates for 2010 to 2014 for the USFWS Black Footed Ferret Species Action Plan total in excess of 30 million US dollars.<sup>4</sup>

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 & Ballou 1992, Ralls & 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 in aquariums.

The overall infrastructure of a captive breeding program for small cetaceans must include experienced personnel (handlers, trainers, nutritionists, veterinarians, scientists) and facilities that meet or exceed international standards 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 & O'Brien 2004, Robeck et al. 2004, 2005a,b, 2009, Robeck & Monfort 2006, O'Brien et al. 2008, Yuen et al. 2009, O'Brien & Robeck 2010a).

<sup>&</sup>lt;sup>3</sup>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

<sup>&</sup>lt;sup>4</sup>Information available at http://ecos.fws.gov/speciesProfile/

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 & Antrim 2010), this is not always the case. The best husbandry methods for small cetaceans are those that require the least amount of restraint and attain maximum cooperation from the animal (Ridgway et al. 1989). This approach is strongly evident in the research that has resulted in successful AI 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 & Monfort 2006, Yuen et al. 2009, O'Brien & 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 gualified, experienced veterinary personnel, and the implementation of ART will require the presence of a veterinarian with expertise in that field and ongoing participation of other qualified, experienced veterinary personnel and scientists.

## CHALLENGES OF REINTRODUCING CAPTIVE-BRED ANIMALS

Even if a species can be bred routinely in captivity, use of captive-bred individuals to reinforce wild populations or reintroductions of captive-bred animals to form a new population in suitable ecological habitat can be difficult, complex, 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 many more failures (Jule et al. 2008, Bowkett 2009).

Numerous factors must be considered in planning the reintroduction of captive-bred populations, including disease risk, the potential effects of transport stress, changes in the genetic configuration of the captive population, and the propagation of a sustainable captive population (Mathews et al. 2006, Teixeira et al. 2007, Ballou et al. 2010, Earnhardt 2010). In addition, behavior and complexities of social structure may affect reintroduction success. Although specific characteristics such as group size, feeding niche and activity patterns have been suggested to affect reintroduction success, many of these have not been tested experimentally to facilitate a better understanding of the factors critical to outcome (Stanley Price 1989, Mathews et al. 2005). In general, captiveborn individuals are less likely than wild-born conspecifics to survive and reproduce subsequent to release (Earnhardt 2010). Captive-bred individuals may suffer high mortality rates resulting from lack of anti-predator behavior or foraging skills or failure to integrate into social groups (Ralls & Ballou 2013). Some species may be behaviorally flexible and, therefore, good candidates for pre-release training in behaviors well suited to survival in the wild (e.g. preparation for foraging, predator avoidance; Beck et al. 1991, Wells et al. 1998, Griffin et al. 2000).

The IUCN Reintroduction Specialist Group has provided guidelines for reintroductions and is a source of advice for those planning to reintroduce captive-bred taxa to the wild (www.iucnsscrsg.org). For example, the 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 species that have not been well-studied. 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 to the animals. Unless well-experienced personnel conduct the operation, drowning, trauma, capture wounds (from nets or other capture gear), and bacterial and other infections related to capture damage and transport conditions are likely to remain a major cause of death among free-ranging cetaceans during and soon after capture (Walker 1975, Ridgway et al. 1989, Wang et al. 2000, Fisher & Reeves 2005), especially in the riverine and tropical to sub-tropical habitat of many critically endangered small cetacean populations. Acclimation to captivity, which may be an especially acute challenge for riverdwelling species, including subpopulations of Irrawaddy dolphins Orcaella brevirostris, poses an additional risk of mortality. Some of the problems involving behavior, space requirements, and disease in these dolphins have been addressed over time. The techniques required to enable successful captive breeding of the majority of small cetaceans listed by the IUCN as Endangered or Critically Endangered have not been sufficiently developed for these species. In the future, development of these techniques should begin before a population is Critically Endangered.

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 the small subpopulations of cetaceans listed by the IUCN as Critically Endangered (Fisher & Reeves 2005), some of which have <100 individuals remaining. Removal of individuals from a cetacean population by live-capture is the population 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 a 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 (2012) 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. Major funding is required for a program to develop properly from the outset. In addition, 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. Many of the small cetacean species that are currently Endangered and Critically Endangered inhabit regions of underdeveloped nations, making achievement of logistical requirements incrementally more difficult. Also, we note that advancements in scientific knowledge and technology do not always translate into improved real-world practices. Even under the best of circumstances, developing a successful program takes many years of work. Assisted reproductive technologies, such as AI, 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 for a few extensively studied odontocete species can provide insight and experience for use in other cetacean species, and may even be expected to advance some aspects of the use of ART in other species, but species-specific research will still be required before ART can be used on any new species.

Captive-bred small cetaceans should not be reintroduced into an area until the major threats to their survival have been eliminated. The development of successful techniques for the release of captive-bred individuals into viable habitat poses additional challenges. We conclude that the realities and risks involved indicate that, under current and foreseeable conditions, 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 to prevent the extinction of Critically Endangered small cetaceans are habitat preservation and the elimination of bycatch (e.g. Beasley et al. 2009, Kreb et al. 2010, Ross et al. 2010, 2011, Gerrodette & Rojas-Bracho 2011).

#### NOTE ADDED IN PROOFS

Numerous studies have shown that the Yangtze finless porpoise Neophocaena asiaeorientalis asiaeorientalis is in decline. Mei et al. (2012) reported an accelerated decline of this subspecies based on life tables before and after 1993. Their individual-based Leslie matrix model predicted a high probability of extinction (86%) within the next 100 yr. As a result, Mei et al. (2012) stated that the current rate of porpoise decline exceeds the threshold for IUCN Critically Endangered status (loss of 80% of abundance or higher within 3 generations), and recommended reclassifying the status of the Yangtze finless porpoise to Critically Endangered. Porpoises in the 2 semi-natural reserves at Tian'e-Zhou and Tongling Reserve have produced calves (Wang et al. 2006), but they cannot be considered self-sustaining populations. Only 1 calf has survived at IHB. Since it is generally believed that conditions in the Yangtze River will not improve in the foreseeable future (Zhou et al. 1998), it may never be possible to reintroduce these animals back into the Yangtze River. Thus, the only freshwater porpoise in the world will be functionally extinct, and then may soon join the ranks of other extinct Yangtze megafauna such as the baiji and the 7-m Chinese paddlefish Psephurus gladius.

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