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Safeguarding Diversity: Challenges in Developing a Genome Resource Bank for the California Sea Otter

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The U.S. Fish and Wildlife Service (FWS) estimates that there are currently 2,400 California sea otters (Enhydra lutris nereis) with a range of only 240 miles along the central California coast. The relatively low number and small range of the California sea otter is cause for great concern about the fate of this unique marine species because of the potential for oil spills within the region (see Bonnell et al., this issue). In fact, a major oil spill (>1,000 barrels) within the range of the California sea otter is considered to be the most serious threat to the survival of this species (U.S. Fish and Wildlife Service 1996).

The threat of lost genetic diversity

In the event of a major oil spill, the California sea otter population may lose not only a large number of animals, but also a significant proportion of existing genetic diversity. For any species, the potential for inbreeding increases when the total number of animals in a population is reduced. In naturally outbreeding species, such as the sea otter, inbreeding depresses reproductive fitness by decreasing genetic variation. In fact, inbreeding depression is a major influence on the cycle of extinction where lowered reproductive fitness results in fewer offspring that, in turn, further increases the chances of inbreeding (see Figure 1).

Figure 1. A potential scenario for extinction. Smal population size, resulting from either one or a combination of factors, increases the opportunity for incestuous matings. Inbreeding depress is expressed by reduced reproductive fitness and increased susceptibility to disease. Each subsequent reduction in population size further limits genetic variation, eventually leading to extinction (adapted from Wildt 1994).

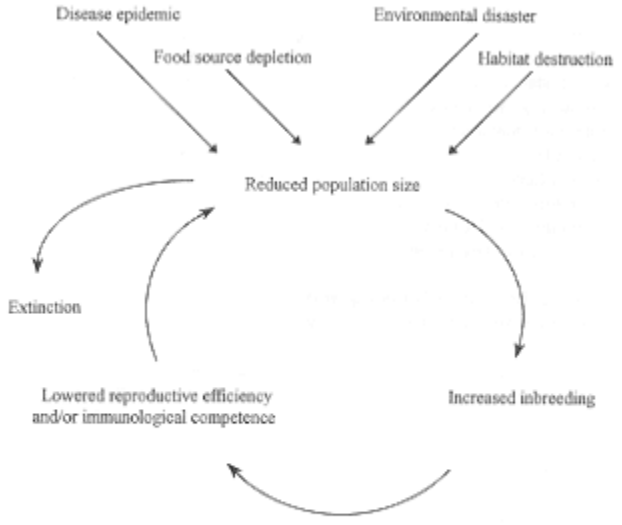

Figure 1. A potential scenario for extinction. Small population size, resulting from either one or a combination of factors, increases the opportunity for incestuous matings. Inbreeding depression is expressed by reduced reproductive fitness and increased susceptibility to disease. Each subsequent reduction in population size further limits genetic variation, eventually leading to extinction (adapted from Wildt 1994).

Recent evidence indicates that endangered species actually may give little warning of impending crises due to inbreeding because of a threshold relationship between inbreeding and extinction rates (Frankham 1995). Additionally, inbreeding is more likely to contribute to population decline for species with low reproductive rates. The reproductive rate of the sea otter is characteristically low, with an estimated annual birth rate of 0.9 pups per female in California (Riedman et al. 1994). The reproductive efficiency of the California sea otter is compromised further by a relatively high level of pup mortality. For example, in the Monterey area, a 40% pre-weaning pup mortality rate was estimated for 1985-1991 (Riedman et al. 1994).

The viability of wildlife depends, in part, on the level of genetic variation within species, populations, and individuals (Wildt et al. 1993). Because the possibility of an oil spill within the California sea otter's habitat is a constant threat to the continued viability of this population, conservation efforts should include the development of a Genome Resource Bank.

Genome Resource Banks

A Genome Resource Bank (GRB) is the systematic collection, cryopreservation and use of biological materials (gametes, embryos, tissues, blood products, and DNA) from a given species. Because cells can endure storage at low temperatures (-196°C), cryopreservation of gametes and/or embryos extends the generation interval of an individual indefinitely. GRBs essentially are frozen repositories that provide insurance against loss of genetic diversity by retaining viable germ plasm from founder animals for future generations (Wildt 1992). In the case of the California sea otter, a GRB would safeguard existing genetic diversity against not only environmental disasters, but also other unpredictable incidents like disease epidemics (see Thomas and Cole, this issue).

For several reasons, a GRB can be a powerful conservation tool. Most importantly, a GRB offers a high degree of security against loss of genetic diversity by providing an avenue for long-term storage of genetic material. Once established, a GRB can be used as a strategic method for managing exchange of genetic diversity among wild populations. In fact, successful cryopreservation of semen from free-ranging animals suggests that sperm banking also may be a viable approach to promoting genetic diversity in captive populations (Wildt 1989), as well as between captive and wild populations. Additionally, biomaterials stored in a GRB can be used for basic and applied research in genetic, disease and reproductive studies. For example, cryopreserved embryos can provide precise standards for quantifying subtle processes like genetic drift (Mazur 1984).

The effective use of a GRB requires an organized, systematic approach. The Genome Resource Banking Advisory Group, a scientific advisory group under the auspices of the American Zoo and Aquarium Association, serves to guide and facilitate the development of GRBs. Recently, the GRB Advisory Group developed a Resource Guide designed to assist in the formulation and implementation of GRBs. Issues to be considered when developing a GRB include: (1) current knowledge of life history and reproduction; (2) status in the wild; (3) type and amount of germ plasm to preserve in the context of management and genetic goals; (4) technical aspects of germ plasm collection; (5) storage, use and ownership; and (6) available resources and funding (AZA Genome Resource Bank Advisory Group 1996).

Genome Resource Banking and assisted reproductive technology

The potential benefits of GRBs for maintaining genetic diversity and preserving species depend heavily upon adequate usage of stored genetic material through assisted reproductive technologies such as artificial insemination, in vitro fertilization, and embryo transfer. Successful assisted reproductive technology, however, relies on knowledge of species-specific reproductive characteristics, including annual reproductive cycles, seminal traits, and timing of ovulation. Therefore, practical application of assisted reproductive technology and, ultimately, the GRB, requires a solid reproductive database.

Female reproduction

Much of the documented information about reproductive biology in the female California sea otter has been derived from comprehensive field studies (Kenyon 1969; Wendell et al. 1984; Siniff and Ralls 1991; Jameson and Johnson 1993; Riedman et al. 1994), as well as direct examination of reproductive tracts (Sinha et al. 1966; Sinha and Conaway 1968; Kenyon 1969; Schneider 1973; Bodkin et al. 1993). Females reach sexual maturity at 4-5 years of age and 75% of 14-15 year old females have been found to still be reproductively active (Bodkin et al. 1993). Females less than 3 years old have immature ovaries (Schneider 1973) ranging in size from 7-11 mm (Sinha and Conaway 1968.) In mature females, ovaries are 11-23 mm in length and enclosed within a bursa. Typically, a single preovulatory 8 mm follicle is present on the ovary; polyovular follicles are rare. Fresh ovulation sites, or corpora lutea, measure 5-8 mm in diameter; whereas corpora lutea of the preimplantation period and pregnancy range in size from 5-11 mm and 9-17 mm, respectively (Sinha and Conaway 1968). The period of delayed implantation appears to be variable and may account for the range of differences in gestation length (Jameson and Johnson 1993). On average, gestation is 6 months and is considered to consist of a 2-3 month pre-implanted and 4 month implanted phase. The peak pupping period for the California sea otter in the southern part of the range near Morro Bay occurs from January to March, with a smaller peak in the late summer to early fall (Siniff and Ralls 1991); whereas, in the Monterey area, seasonal trends in pupping are less pronounced (Riedman et al. 1994). Females return to estrus 1 day to several weeks after pup weaning. Interestingly, females that lose their pups prematurely soon return to estrus; thus, spring pupping mortality and subsequent mating may account for the second pupping peak in the fall. Estrus length, based on pair-bond dissolution, is estimated to be 3-4 days (Riedman and Estes 1990). Even though there appears to be a seasonal trend in pupping, births can take place throughout the year (Jameson and Johnson 1993).

Although a substantial amount of information about female reproductive biology has been accumulated, little is known about sea otter endocrinology. Biologists do not know for certain if females are (1) induced ovulators; (2) seasonally polyestrus or monoestrus; or (3) how to distinguish between pregnant and nonpregnant states. Such information is critical for the application of assisted reproductive technologies. The Seattle Aquarium and the Center for Wildlife Conservation, in cooperation with public aquariums and marine mammal biologists, are conducting a long-term project on sea otter endocrinology using a non-invasive hormone monitoring technique that measures steroid metabolites extracted from fecal samples. Over 350 samples have been collected from 2 female Alaskan sea otters spanning 1 year. Preliminary data reveal, for the first time, annual estrogen and progesterone profiles in a captive female (see Figure 2). Cyclic estrogen spikes occurred at 45-60 day intervals, increasing in magnitude until the largest spike, which was associated with full estrus behavior. Ovulation was confirmed by a 10-fold rise in progestins within 10 days following the estrogen spike and continued to rise until 30 days post estrogen spike. Both estrogens and progestins fell off slightly by day 50, and then showed a dramatic and sustained rise (3-fold increase for estrogens and 20-fold increase for progestins) 90 days post estrogen spike of a presumed pregnancy. These data suggest that the preimplantation period in this female lasted for 3 months. With the addition of more females to the dataset, it may be possible to fully characterize the period of delayed implantation in sea otters. Additionally, fecal cortisol metabolites also have been monitored successfully and show promise as an index of physiological stress in this species.

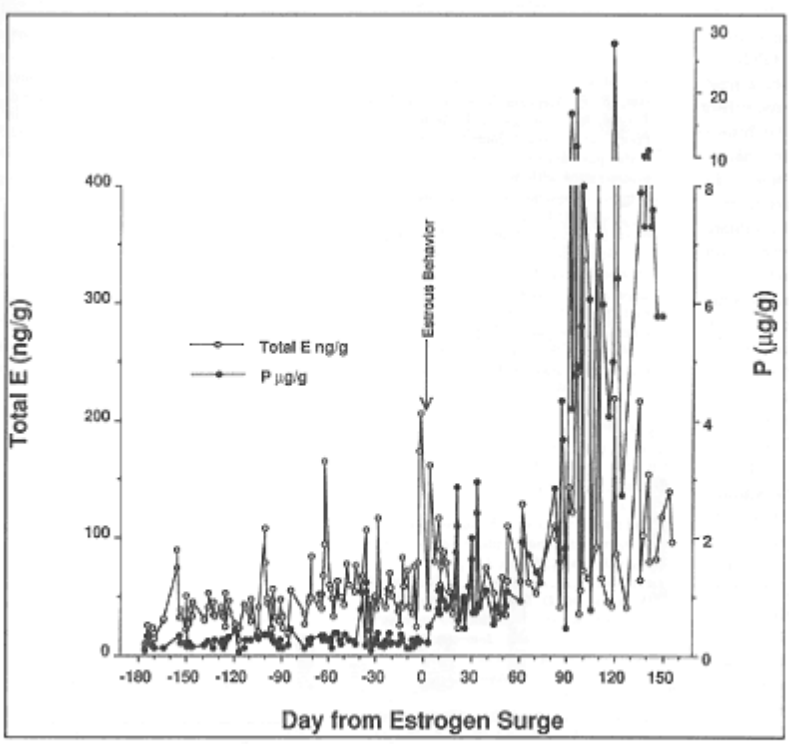

Figure 2. Total estrogen (E = estradiol and estrone) and progestins (P = progesterone metabolites) concentrations (per gram of dry fecal weight) across a full year of fecal samples from a single female sea otter at the Seattle Aquarium. Day 0 reflects the time of the estrogen spike which, for this female, occurred in January. Estrus behavior refers to overt and frequent female-initiated mating behavior with the male. This behavior was observed only during the single event indicated on the graph.

Male reproduction

In contrast to female sea otters, limited information is available about the reproductive biology of male California sea otters. Male Alaskan sea otters reach sexual maturity at 5-6 years of age (Schneider 1978; Garshelis 1983); however, the onset of sexual maturity in California males has not been established (Riedman and Estes 1990). Reproductive tracts from "old" (exhibiting well-worn teeth) Alaskan males show no signs of diminished sperm production (Kenyon 1969). It also has been suggested that Alaskan males produce sperm throughout the year (Lensink 1962); however, testicular histology indicates that a mild periodicity is associated with spermatogenesis (Kenyon 1969). In California, mating activity of territorial males increases during September through November, and copulation occurs exclusively in the water (Riedman and Estes 1990). Only one report documents ejaculate traits in sea otters (Ballachey 1995); this study was conducted to assess damage in male Alaskan sea otters after the 1989 Exxon Valdez oil spill in Prince William Sound. Overall, ejaculates from adult male sea otters exhibited low sperm concentrations and contained an average of only 13.8% morphologically normal sperm cells per ejaculate. The most prevalent structural defects included mid-piece and tail abnormalities. More alarming, however, is the fact that high numbers of abnormal sperm were found in both oiled and unoiled (control) males, indicating that this may be characteristic of the species. It is clear that a comprehensive study of reproduction in the male sea otter needs to be conducted.

Conclusion and recommendations

Present realities require focusing on basic research to determine critical reproductive parameters for both female and male sea otters, including the timing of ovulation, number of normal sperm produced and optimal methods for cryopreserving sperm, before attempting artificial breeding or long-term storage of genetic material. Ideally, protocols for ovulation detection, semen collection and artificial insemination are developed using a model species before applying the technology to the endangered species. This

approach has been successful for a critically-endangered mustelid species, the black-footed ferret (Mustela nigripes). Semen cryopreservation and artificial insemination techniques were developed first for the domestic ferret (Mustela putorius furo; Howard et al. 1991). Determining optimal methodology in the model species enabled the production of black-footed ferret offspring by artificial insemination with frozen-thawed sperm (Howard et al. 1996). For the sea otter, the non-threatened Alaskan subspecies would be an excellent model for the threatened Californian subspecies. Additionally, the relative abundance of Alaskan sea otters in captivity provides a unique opportunity to develop these technologies for use in the field without disturbing wild populations.

GRBs, in combination with assisted reproductive technology, have enormous potential for conserving endangered species. There is a general consensus that GRBs will be contributing significantly to the conservation of rare wildlife species as we enter the 21st century (Wildt 1992). For the California sea otter, the availability of stored gamete and/or embryo samples is particularly relevant in the event that the current population experiences a drastic decline in number. Storing gamete samples, however, should not be viewed as the solution to sea otter conservation, but rather as one of many tools that will enable this species to thrive. All reasonable approaches should be explored and used to help conserve and understand the California sea otter while maximizing the efficient use of limited resources.

For wildlife populations that are susceptible to catastrophe, an organized effort to collect, evaluate, cryopreserve, store and use germ plasm should rank high on the list of conservation priorities. Because of the likelihood that an oil spill could dramatically affect the California sea otter population, there is an urgent need to establish a GRB before such a crisis occurs. Development of GRB will require a concerted effort from aquariums, reproductive biologists, field biologists and policy-makers, as well as funding commitments. In summary, the following reproductive studies are recommended to facilitate the development of a GRB: (1) conduct a comprehensive study on male reproduction that characterizes ejaculate traits and seasonality; (2) determine unknown aspects of female sea otter endocrinology, including estrous cycle length, timing of ovulation and seasonality; and (3) develop semen handling and cryopreservation protocols. Because of the precarious status of the California sea otter, these reproductive studies should be initiated as quickly as possible. A GRB, together with conservation activities such as improved pollution control, diligent habitat maintenance and protection, captive breeding/reintroduction programs and public awareness campaigns, promises to be an effective strategy for conserving the California sea otter.

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