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Dizygotic twinning in the Hawaiian monk seal

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Twinning is extremely rare in pinnipeds. Instances in which a female is observed simultaneously nursing 2 pups may represent alloparenting, or nonfilial nursing, a relatively common trait in pinnipeds. We explored the incidence of twinning in the Hawaiian monk seal (*Monachus schauinslandi*). We observed 7 sets of putative twins (i.e., an isolated female nursing 2 newborn pups) of 4,965 total births recorded between 1983 and 2008 (minimum twinning rate = 0.1%). Genetic specimens from the mother and both pups were available only for 5 of the 7 sets. Microsatellite genotyping revealed all of these to be dizygotic twins, with low probability of false assignment ($P < 0.001$). On average, Hawaiian monk seal twins were less likely to survive to weaning than singletons born in the same year and on the same island ($P = 0.008$). Those that weaned were smaller in size (i.e., axillary girths 1–4 *SD*s lower than singletons). In the critically endangered Hawaiian monk seal twinning appears to be a rare trait that results in overall lower survival rates but also offers a small opportunity for stock enhancement.

Key words: fecundity, maternal investment, microsatellite, multiple maternity, *Monachus schauinslandi*

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Twinning is a rare event in pinnipeds (true seals, sea lions, fur seals, and walruses). Antarctic fur seals (*Arctocephalus gazella*) exhibited a twinning rate of 0.06% over 9 breeding seasons at Bird Island, South Georgia (Hoffman and Forcada 2009). The incidence of live twin births in Weddell seals (*Leptonychotes weddellii*) was 0.1% during 3 seasons at Erebus Bay, McMurdo Sound (Gelatt et al. 2001). In a population of southern elephant seals (*Mirounga leonina*) twin births accounted for 0.38% of annual births, and only 20% of twin dyads survived to 18 months (compared to 47% of singletons—McMahon and Hindell 2003). At least 1 incidence of twinning has been observed in the following species: walrus (*Odobenus rosmarus*—Fay et al. 1991), Australian sea lion (*Neophoca cinerea*—Ling 1986), New Zealand fur seal (*Arctocephalus forsteri*—Dowell et al. 2008), Steller sea lion (*Eumetopias jubatus*—Maniscalco and Parker 2009), and captive gray seals (*Halichoerus grypus*—Spotte and Stake 1982).

The low incidence of twinning could reflect selection against a maladaptive trait (Trillmich 1990). Multiple maternity generally leads to smaller pup size at birth or weaning and can result in the death of 1 or both offspring. For phocids (true seals) and otariids (sea lions and fur seals) parturition and nursing are separated spatially from foraging (Boness and Bowen 1996). Therefore, females are either required to fast while nursing (most phocids) or to leave pups unattended while they forage at sea (otariids and harbor seals [*Phoca vitulina*—Boness and Bowen 1996] and Mediterranean monk seals [*Monachus monachus*—Pastor and Aguilar 2003]). Foraging species can have difficulty in relocating both pups upon return from the sea (Dowell et al. 2008). Fasting species have shorter lactation periods (4–53 days) and greater milk fat content (47–61%), producing faster growth rates (2.0–7.1 kg/day) in their nursing pups (Boness and Bowen 1996). These females lose considerable mass (e.g., ~40% in gray seals and northern elephant seals [*Mirounga angustirostis*]) during lactation (Boness and Bowen 1996) and might not be able to nurse 2 pups to an adequate size before weaning. Generally, at least 1 and often both progeny die at birth or prior to weaning. Unless 1 pup dies early on, twins are of small size and decreased body condition at weaning, reducing their chances of survival to reproduction (Dowell et al. 2008;

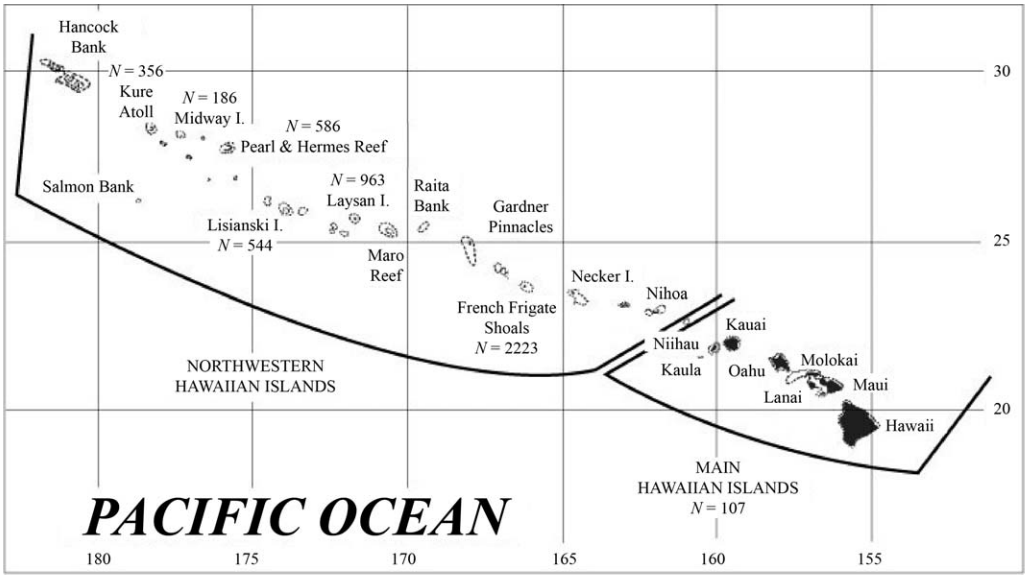

Fig. 1.—Range of the Hawaiian monk seal and number of births recorded at each island from 1983 to 2008.

Haase 2007; McMahon and Hindell 2003). Therefore, twinning usually leads to a reduced reproductive rate in pinnipeds.

Field observations of putative twins can represent alloparenting or fostering, in which a female nurses a nonfilial pup. Despite the high energetic costs of nursing, many pinniped species exhibit fostering. The relative frequency of fostering in otariids ranges greatly (0.17–11%—Dowell et al. 2008). Of 11 putative pairs of Antarctic fur seal twins, only 3 (27.3%) were genuine as indicated by parentage analyses using microsatellites (Hoffman and Forcada 2009). In general, fostering appears to be more common in phocids than in other pinnipeds (Stirling 1975). In a study of Weddell seals 4 of 7 putative twin dyads were instances of fostering (Gelatt et al. 2001). On average, 10% of Sable Island harbor seals engaged in fostering (Schaeff et al. 1999). Fostering also has been observed in northern elephant seals (27%—Riedman and Le Boeuf 1982) and gray seals (3–28%—Perry et al. 1998). The Hawaiian monk seal (*Monachus schauinslandi*) has exhibited fostering rates of up to 90% at French Frigate Shoals, where the density of mother–pup pairs is relatively high for the species (Boness et al. 1998).

The Hawaiian monk seal is a large-bodied monachine seal endemic to the Hawaiian Archipelago (Fig. 1). The majority of the species inhabits the Northwestern Hawaiian Islands, which include Kure Atoll, Midway Atoll, Pearl and Hermes Reef, Lisianski Island, Laysan Island, French Frigate Shoals, Necker Island, and Nihoa Island. A small and increasing number of seals occupy the main Hawaiian Islands of Ni'ihau, Kaua'i, O'ahu, Moloka'i, Maui, Lanai, Kaho'olawe, and Hawai'i (Baker and Johanos 2004). Although births occur throughout the year, parturition is most common between February and August (Johanos et al. 1994; Johnson and Johnson 1980). On average, pups weigh 14–17 kg at birth (Kenyon and Rice 1959; Wirtz 1968) and 60–75 kg at weaning (Craig and Ragen 1999; Kenyon and Rice 1959). Similar to other phocids, the Hawaiian monk seal experiences a high energetic cost of lactation; parturient females fast or rarely feed while nursing pups (~5–6 weeks) and lose considerable weight (Antonelis et al. 2006; Johanos et al. 1994; Kenyon and Rice 1959). Although fostering and pup switching is common in the species, it appears to have minimal impact on net pup survival, although pups lost before weaning were not included in the analysis (Boness 1990).

As with other pinnipeds, Hawaiian monk seals generally give birth to a single pup; however, instances have occurred in which an isolated female was observed with 2 young pups, often with attached umbilical cords or placentas nearby. The objectives of this study were to confirm behavioral observations indicative of multiple maternity with genetic analyses and to estimate the historical occurrence of twinning within the species.

Materials and Methods

Annual population assessments were conducted at 6 subpopulations in the Northwestern Hawaiian Islands during most years from 1983 to 2008. Seasonal field camps, typically 2–5 months during the pupping and breeding season, were established at Kure Atoll, Midway Atoll, Pearl and Hermes Reef, Lisianski Island, Laysan Island, and French Frigate Shoals. Mother–pup pairs also have been monitored opportunistically in the main Hawaiian Islands (including the island of Oahu) during the same period. Pupping beaches were surveyed at regular intervals throughout the season. Twinning was inferred if an isolated female attended 2 young pups of similar size, often with attached umbilical cords and placentas nearby. To facilitate individual identification some nursing and weaned pups were marked with unique numbers using hair bleach. Pup attendance and general condition of mothers and pups were monitored regularly. On multiple occasions researchers intervened to reunite preweaned pups that had become separated from their mothers. Upon weaning, pups were double-tagged using unique plastic tags (Temple Tag, Inc., Temple, Texas) and, since 1991, implanted with Biomark Passive Integrated Transponder tags (Biomark, Boise, Idaho—Baker and Thompson 2007). Tissue plugs for genetic analyses were collected during tagging and were preserved in ethanol or a 20% dimethylsulfoxide saturated salt solution or by freezing. During tagging, measurements were taken, including dorsal straight length, axillary girth (which is a good predictor of survivorship to 1 year of age—Baker 2008), and in some years body mass. Full necropsies were conducted on dead pups, including the collection of tissue specimens and measurements. Tagged pups were monitored (i.e., resighted, with location and condition data collected) throughout the remaining season and during subsequent years. Handling of live seals conformed to guidelines of the American Society of Mammalogists (Gannon et al. 2007), and the project was approved by the University of Hawai'i Institutional Animal Care and Use Committee.

We extracted genomic DNA from all tissue plugs using the DNeasy Tissue Kit (Qiagen, Valencia, California). We amplified the DNA of all specimens (mothers and putative twin dyads) at 17 Hawaiian monk seal microsatellite loci (Schultz et al. 2009, 2010) and 1 locus isolated from the gray seal (Allen et al. 1995). Two multiplexed polymerase chain reactions were performed as follows: 1× polymerase chain reaction mix (Qiagen), 2 μM of primer mix, and 30–50 ng of genomic DNA. The polymerase chain reaction protocol consisted of 15 min initial denaturation at 95°C, followed by 35 cycles of denaturation (94°C, 30 s), annealing (60°C, 30 s), and extension (72°C, 30 s), with a final extension (72°C, 30 min) to ensure the addition of a terminal adenine. Amplified products were run on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California) by the Core Sequencing Facility at the Hawai'i Institute of Marine Biology and scored using the software GENEMAPPER 4.0 (Applied Biosystems). Population-level analyses revealed no evidence of deviation from Hardy–Weinberg equilibrium, null alleles, or large allele dropout (Schultz et al. 2009). To reduce microsatellite scoring bias putative mother–twin groups were analyzed independently and scored without reference to family groups.

To verify maternity of each set of twins we compared genotypes of pups and putative mothers. Presence of at least 1 shared allele at every locus was considered confirmation of maternity. The genotypes of twin pups were compared to determine whether they were of monozygotic (all alleles identical) or dizygotic (some differing alleles) origin. The Hawaiian monk seal has the lowest genetic diversity of all mammals tested to date (Schultz et al. 2009). Because resolution was limited, we calculated the probability that a randomly chosen individual could be mistaken for the parent of the pup. Following Gelatt et al. (2001) and Hoffman and Forcada (2009), we calculated the probability as the product (at all loci) of $(1 - p)^2$ for each locus at which the pup was homozygous and $(1 - q - r)^2$ for each heterozygous locus, where p, q, and r are allelic frequencies of specific loci in the general population, as described in Schultz et al. (2009) and Schultz et al. (2010). Survival rates of twins and nontwins were compared using chi-square (χ^2) analysis in IBM SPSS Statistics (Chicago, Illinois).

Results

In 26 years of extensive field observation only 7 putative twin births (of 4,965 total births) were observed that met our criterion for possible multiple maternity (i.e., an isolated female was observed with 2 newborn pups; Table 1). Because the collection of tissue specimens was not implemented until 1988, the 1st incidence of twinning (1983) could not be confirmed. No tissue specimen was collected from the mother (seal R003; Table 2) of putative twins born in 1998, although the pups shared at least 1 allele at all but 2 loci.

Genetic analyses of the remaining 5 sets of putative twins confirmed multiple maternity in all instances. All pups shared at least 1 allele per locus with their mother (Table 2). The probability of randomly generating genotypes of pups was low (Table 1), and thus we were confident in assigning maternity. We were unable to achieve a complete genotype for seal TS40 due to the degraded nature of the sample; however, her pups possessed at least 1 of her alleles at each locus tested ($n = 9$). None of the pup dyads shared all alleles at all loci, a result which is indicative of dizygotic twins.

Because our criteria for multiple maternity appears to be sufficiently stringent (i.e., 5 of 5 testable pup dyads were confirmed as twins), we assumed that all putative twins were genuine and included all 7 sets in summary analyses. The prevalence of twinning in the Hawaiian monk seal is 0.1% regardless of whether we include the 2 sets of putative twins for which genetic data are unavailable. Of 14 total pups, 5 were found dead shortly after birth, 8 survived to weaning, and 1 died shortly before weaning. In general, both twins of a dyad either died or survived to weaning. The 2 exceptions are the 2002 French Frigate Shoals pup that died from a shark bite 2–7 days before weaning and the 2008 Kure pup that was nursed as a singleton or nontwin (Table 1). The pooled survival rate of twins from birth to weaning (57.1%) was significantly lower ($\chi^2_1 = 6.93$; $P = 0.0084$) than that of nontwins (85.6%; Table 3). Although the pooled survival rate of twins from weaning to year 1 (40.0%) was lower than that of nontwins (58.0%), the result was not significant ($\chi^2_1 = 0.61$; $P = 0.43$), as a result of our small sample size (low statistical power). The 2006 Midway twins (which were supplementally fed after weaning) and the 2008 Kure pup (which was raised as a singleton) were not included in this final analysis.

Although axillary girth of the 1983 twins was not measured, these twins were reported to be smaller than normal upon weaning. Of the remaining 6 pups that survived to weaning, 5 had smaller axillary girth (82.0–96.5 cm; 1–4 *SD*s lower) than the average nontwins born on that island and during that year (Table 1). The 2008 Kure pup weaned at above average (axillary girth = 115 cm); however, this pup was raised as a singleton because its twin died at or soon after birth.

Table 1.—Hawaiian monk seal twinning events from 1983 to 2008. Putative twin dyads listed by mother and pup identification number (ID) and sex (M = male; F = female), year, island (FFS = French Frigate Shoals; Mid = Midway Atoll; Kure = Kure Atoll), days nursed, outcome, dorsal straight length (DSL) and axillary girth (AG) at death or weaning compared with the mean (and *SD*) days nursed, DSL, and AG of singleton births for that island and year. Mass of pups at death or weaning, age (years) of pup when last sighted, and probability (*P*) of a random seal being mistaken as the parent are given where known.

Year	Island	Mother ID	Mother age (years)	Pup ID	Pup sex	Days nursed	Mean days nursed	Outcome	DSL (cm)	Mean DSL (cm)	AG (cm)	Mean AG (cm)	Mass (kg)	Age last sighted (years)	*P*
1983	FFS	Y190	≥ 5	FF8300EN	M	39	41 (2)	Weaned						≥ 1	
				FF8300EP	M	39	41 (2)	Weaned						≤ 1	
1998	Oahu	R003	≥ 12	RYD1	F	0		Died day 0	102		57		16.8		1.2×10^{-13}
				RYD2	M	0		Died day 0	98		49		13.6		7.7×10^{-15}
2002	FFS	Y305	18	YEXE	M	1–4		Died day 1–4	89		45		10		1.0×10^{-15}
				YEXF	M	1–4		Died day 1–4	88		44		10		1.8×10^{-17}
2002	FFS	Y652	≥ 12	YE40	F	35–39	39 (8)	Weaned	122	125 (7)	97	105 (8)		7	1.0×10^{-20}
				YEXC	F	32–33	39 (8)	Died day 33[a]	120		84				1.0×10^{-20}
2006	Mid	KN88	19	PO22	F	42	37 (4)	Weaned	113	124 (11)	82	118 (11)	29.8	1	7.2×10^{-18}
				PO26	F	42	37 (4)	Weaned	114		89		36.1	1	2.0×10^{-13}
2007	Kure	TS40	15	KB22	F	>15	39 (6)	Weaned	114	134 (6)	92	114 (11)		≤ 1	1.8×10^{-13}
				KB24	M	>15	39 (6)	Weaned	107		88			≤ 1	4.2×10^{-13}
2008	Kure	B6AN	11	KWX1	F	0	46 (7)	Died day 0	99	126 (7)	49	111 (9)	11.8		1.0×10^{-20}
				KW28	F	44	46 (7)	Weaned	126		115			1[b]	4.0×10^{-20}

[a] Died due to lethal shark attack.
[b] Age when observed during 2009 field season.

Table 2.—Genotypes of Hawaiian monk seal mother and putative twin pups at 17 loci (Msc5 not shown because all seals shared the same allele). ID = identification number.

Seal ID	Hg6.3	Ms504	Ms23	Ms265	Ms9	Ms15	Ms647	Ms663	Msc17	Msc10	Msc19	Msc1	Msc9	Msc4	Msc3	Msc23	Msc13
R003																	
RYD1	227,237	308,308	355,355	158,162	305,305	203,203	117,117	294,294	117,117	141,141	118,118	161,167	208,212	167,167	126,126	164,164	200,200
RYD2	227,227	308,308	355,358	158,162	309,309	203,203	117,117	294,294	115,115	141,141	118,118	161,167	208,212	167,167	126,134	164,164	200,200
Y305	227,237	308,326	355,358	162,162	297,309	203,203	115,117	294,294	115,117	141,145	118,118	161,161	208,212	167,167	126,134	160,168	200,200
YEXE	227,237	308,326	344,358	162,162	297,309	203,203	115,117	294,294	115,117	145,145	118,118	161,163	208,216	159,167	126,134	160,164	194,200
YEXF	227,237	326,326	348,355	162,162	297,309	203,203	117,117	294,294	115,115	141,145	118,118	161,161	208,216	159,167	126,130	160,164	194,200
Y652	227,227	308,308	358,358	158,162	305,313	203,203	115,115	294,294	115,115	141,141	118,118	163,167	212,212	167,171	126,132	164,164	200,202
YE40	227,227	308,308	358,362	158,162	305,313	203,215	115,117	294,294	115,117	141,145	118,126	167,167	212,212	159,171	126,132	164,168	200,200
YEXC	227,227	308,308	358,362	162,162	313,313	203,203	115,117	294,294	115,117	141,145	118,126	167,167	212,212	171,171	126,134	164,168	200,200
KN88	227,227	308,308	348,355	158,158	309,313	203,215	115,117	290,294	117,117	141,145	118,118	167,167	212,212	167,167	130,134	164,164	194,202
PO22	227,227	308,308	344,355	158,162	313,313	212,215	115,115	290,294	117,117	141,145	118,118	161,167	212,212	167,167	130,136	164,164	194,202
PO26	227,227	308,308	348,355	158,162	297,309	203,215	115,117	294,294	117,117	145,145	118,118	167,167	212,212	159,167	130,130	164,164	194,202
TS40									115,117	145,145	118,134	167,167	212,212	159,159	132,132	164,168	200,200
KB22	227,227	308,326	348,348	158,158	309,309	203,203	115,117	290,294	117,117	145,145	118,118	161,167	212,212	159,159	132,134	160,164	200,202
KB24	227,227	308,308	348,348	158,162	309,309	203,212	117,117	294,294	115,115	141,141	118,118	161,163	212,212	159,167	134,134	160,164	200,200
B6AN	227,227	308,308	355,358	158,162	305,309	203,212	115,115	294,294	115,115	141,141	118,118	163,163	212,212	159,159	130,134	164,168	194,200
KW28	227,237	308,308	355,358	158,162	305,309	203,212	115,117	294,294	115,115	141,145	118,118	161,163	212,212	159,159	130,134	164,168	200,200
KWX1	227,227	308,326	355,358	158,158	305,313	212,212	115,117	294,294	115,115	141,145	118,118	161,163	212,212	159,167	130,134	164,168	200,200

Table 3.—Hawaiian monk seal twin survival rates in comparison to singleton survival rates by island and year. FFS = French Frigate Shoals; Midway = Midway Atoll; Kure = Kure Atoll; NA = not applicable.

Year	Island	% twins surviving to weaning	% singletons surviving to weaning	% raised as twins surviving, weaning to age 1	% singletons surviving, weaning to age 1
1983	FFS	100	Unknown	50	Unknown
1998	Oahu	0	NA	NA	NA
2002	FFS	25	79	100	64
2006	Midway	100	85	NA	77
2007	Kure	100	100	0	37
2008	Kure	50	93	NA	36

Discussion

Extensive monitoring of the Hawaiian monk seal over 26 years has allowed us to estimate the rate of twinning in the Hawaiian monk seal (0.1%). Although parturition can occur throughout the year, seasonal monitoring lasted only 2–5 months. At some sites (i.e., Kure Atoll and Pearl and Hermes Reef) most births occurred prior to field monitoring, such that many mother–pup pairs were not observed during the nursing period. Therefore, the calculated incidence of multiple maternity in Hawaiian monk seals is a minimum estimate, but it is similar to previously published twinning rates in pinnipeds (Gelatt et al. 2001; Hoffman and Forcada 2009; McMahon and Hindell 2003).

Based on numerous field observations, Hawaiian monk seals rarely foster 2 pups simultaneously for an extended time period (Boness 1990). The parturient female frequently will become aggressive toward one pup, or the larger pup will outcompete the smaller one, which subsequently is abandoned. In 3 of the 4 cases in which both twins nursed (i.e., neither died at or shortly after birth), observers intervened a number of times to reunite pup(s) and mother. Thus, anthropogenic intervention could have increased the survival rate of Hawaiian monk seal twins.

Pup survival to weaning is linked to maternal condition and experience (Baker et al. 2007). In the Northwestern Hawaiian Islands the average age of 1st reproduction is 7–11 years (Harting et al. 2007). The known ages for 4 of the adult females at the time of twinning ranged from 11 to 19 years old. Five of the twin mothers had a minimum of 3–8 single births prior to twinning, and 5 twin mothers had 1–4 single births after producing twins. At least 3 of the females had a single birth the year before, and 3 gave birth to a single pup the year following the twinning event. Hawaiian monk seal females can bear pups annually, although most do not (Johanos et al. 1994). Therefore, many of the twin mothers in our study were experienced and in the prime of their reproductive years.

Five of the 7 twin dyads were born at 3 atolls over the last 7 years (2002–2008) of the 26-year study period when total births there were declining. Dizygotic twinning is a heritable trait in mammals, such that mothers of twins are more likely to twin again (Ely et al. 2006; Hoekstra et al. 2008). To our knowledge no adult female Hawaiian monk seal has produced multiple sets of twins, and further genetic analyses are required to determine if the mothers in this study are related. Twinning, however, provides a small opportunity for intervention in this critically endangered species ($n = 1{,}247$), which is declining at approximately 4% per year (Baker and Thompson 2007; Carretta et al. 2007). Captive feeding or translocation could improve the survival rate of twins and possibly increase the overall rate of twinning. The 2006 Midway twins, for example, had critically small weaning girths and were placed in captivity and fed for 9.5 months. These pups subsequently were satellite tagged and released at Midway Atoll. Initially, both seals apparently foraged successfully; however, after several months at liberty their body condition declined, and neither seal was observed during subsequent field seasons. Perhaps these seals would have fared better in the main Hawaiian Islands, where juveniles exhibit higher survival rates. Because of its rarity, even successful twinning is unlikely to have a major impact on the overall reproductive rate of the Hawaiian monk seal. Nevertheless, in a species threatened by extinction each birth is important.

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