

EFFECTS OF RESEARCH HANDLING ON THE ENDANGERED HAWAIIAN MONK SEAL

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ABSTRACT

We examined the effects of research handling on free-ranging endangered Hawaiian monk seals, *Monachus schauinslandi*, by analyzing differences in subsequent year survival, migration, and condition between handled seals and controls during 1983–1998. Each of 549 handled seals was matched to a control seal of the same age, sex, location, and year. Handling included instrumentation with telemetry devices ($n = 93$), blood sampling ($n = 19$), and tagging ($n = 437$). No significant differences were found between handled seals and their controls in one-year resighting rates, observed migration rates, or condition. Resighting rates of handled and control seals were high (80%–100%). Available sample sizes were sufficient to detect reasonably small (9%–20%) differences in resighting rates had they existed among instrumented or tagged seals and controls ($\alpha = 0.05$, power = 0.90). Too few seals were captured for blood sampling to detect even large differences in their resighting rates. However, blood samples were drawn from most instrumented seals, and there was no indication that this larger group suffered harmful effects. Duration of restraint during flipper tagging had no effect on subsequent probability of resighting. Our analysis suggests that conservative selection procedures and careful handling techniques have no deleterious effects on Hawaiian monk seals.

Key words: Hawaiian monk seal, *Monachus schauinslandi*, handling effects, endangered species.

A familiar dilemma in conducting wildlife research is that invasive methods are often required to obtain important data, yet handling may negatively affect individual research animals and potentially alter the very parameters of interest. With endangered species, this dilemma is greatly intensified in that research insights are often critical for devising sound conservation strategies, whereas the loss or harm of a small number of individuals can have important population repercussions. It is of interest, therefore, to carefully assess whether

past and present research practices have had negative effects on individuals and populations (*e.g.*, Ginsberg *et al.* 1995).

In general, handling effects are rarely explicitly addressed by the experimental design of research projects on free-ranging animals. While there are exceptions (Henderson and Johanos 1988; Boyd *et al.* 1991; Koopman *et al.* 1995; Walker and Boveng 1995; Castellini *et al.* 1996; Atkinson *et al.* 1998; Donohue 1998, 2000), this is especially true of marine mammal research. The reasons for this are at least threefold. First, in most cases, handled animals do not appear to be harmed in any obvious way when they are released and, consequently, handling effects are assumed to be negligible. Second, logistics and cost often preclude studies which control for handling effects. A study with controls for handled treatment subjects may require resources to track and monitor a control group in addition to the treatment group, the latter being of central interest. Perhaps most importantly, appropriate controls to test for handling effects are often unavailable. Control animals should be comparable to treatment animals in every way except for handling. This requires thorough knowledge (*e.g.*, age, sex, condition) of the animals in the population of interest. Additionally, it is not always possible to measure a handling effect. For example, time-depth recorders (TDRs) are used to electronically log information about the diving behavior of marine mammals. It is conceivable that carrying the instrument itself might influence the animal's diving behavior through increased hydrodynamic drag or altered buoyancy (Bannasch *et al.* 1994, Watson and Granger 1998, Webb *et al.* 1998). These potential effects are difficult to quantify because there would be no diving record for control animals. Finally, to test for effects of handling, one must follow treatment and control subjects for a meaningful period to properly assess effects. This is sometimes not feasible, especially in abundant, wide-ranging populations that have high natural mortality or low site fidelity.

Assessment of research handling effects is absolutely critical to the conservation of the Hawaiian monk seal, *Monachus schauinslandi*. The only endangered marine mammal residing entirely within U.S. waters, its present abundance is approximately 1,400 animals (Forney *et al.* 1999). Beach counts (an historical index of abundance) have declined by 60% since the late 1950s, and 4%–5% annually from 1985 to 1993. Counts stabilized from 1993 to 2000, but will likely decline in the near future due to a highly inverted age structure resulting from 10 yr of low juvenile survival at French Frigate Shoals, the largest of the six main reproductive subpopulations (Craig and Ragen 1999, Forney *et al.* 1999). The species is known to be sensitive to human disturbance. Historically, seals have reduced their use of, or completely abandoned, pupping and resting islands as a result of regular human presence and harassment (Kenyon 1972, Ragen 1999, Ragen and Lavigne 1999).

While the Hawaiian monk seal is faced with a precarious future, some degree of handling remains critical for research and conservation of the species. For example, most of the individuals are known and identified annually, often through the application of tags. Fitting seals with telemetry instruments provides invaluable information about the species' at-sea habitat requirements and

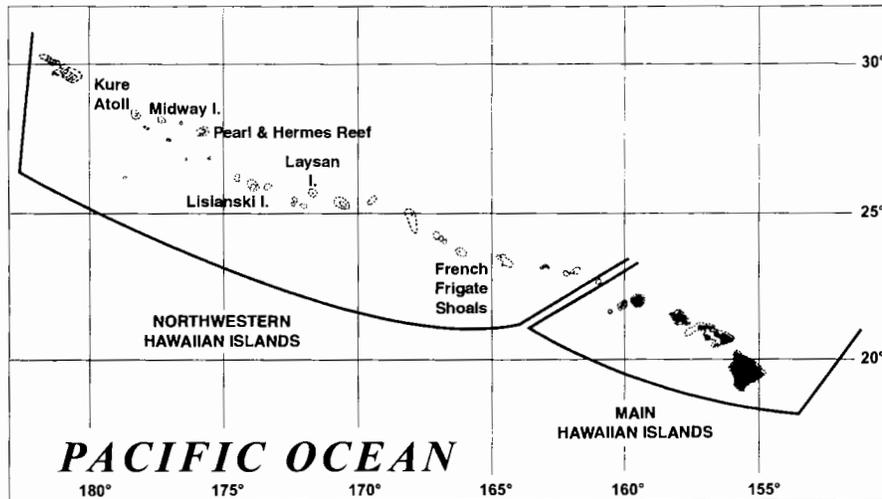


Figure 1. Hawaiian Archipelago. Six main subpopulations of Hawaiian monk seal are at French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll, and Kure Atoll.

potential for interaction with commercial fisheries (Abernathy 1999; Parrish *et al.* 2000, 2002). Collection of blood and other samples allows detection of disease processes that may influence the species (Aguirre,¹ Banish and Gilmartin 1988). It is imperative that potential adverse effects of these research activities on Hawaiian monk seals be addressed so that they can be weighed against the benefits to be gained from future studies.

In this study we examine data accumulated over the past two decades to test the null hypothesis that Hawaiian monk seals released after handling, and carefully matched control seals, did not differ in their rates of subsequent survival, site fidelity, or condition. Additionally, rare cases of wild seals dying under restraint are discussed.

METHODS

Hawaiian monk seals are distributed throughout the Hawaiian Islands but are primarily concentrated at six main reproductive locations (Fig. 1), where annual research camps have been deployed during most years since 1983. The majority of monk seals are identifiable from year to year using flipper tags, temporary bleach marks, or natural markings and scars. Additionally, most of the seals' ages are known, estimated within 1–2 yr, or a minimum age is known. Jolly-Seber estimates of survival indicate that the probability of re-

¹ Aguirre, A. A. 2000. Health assessment and disease status studies of the Hawaiian monk seal (*Monachus schauinslandi*). National Marine Fisheries Service Center Administrative Report H-00-01 (unpublished). 44 pp. Available from SWFSC, Honolulu Laboratory, 2570 Dole St., Honolulu, HI 96822.

sighting seals, given they are alive, is typically >0.90 for all ages and subpopulations (Craig and Ragen 1999; NMFS, unpublished data).

All cases of wild Hawaiian monk seals captured and handled for any purpose have been thoroughly documented since 1983. Strict guidelines are employed when selecting seals to handle to minimize adverse impacts on the animals. Thus, mother-pup pairs are never captured and restrained, molting seals and females believed to be pregnant are not handled, wounded and emaciated seals are avoided, captures are avoided during hot, windless weather, and seals are typically cooled with sea water during restraint.

Responses recorded one year after the handling event include whether a seal (1) is resighted, (2) returns to the same subpopulation or migrates, and (3) demonstrates a notable decline in health or condition (*i.e.*, emaciation, shark inflicted wounds, *etc.*). In this study we analyzed research handling occurring between 1983 and 1997, and the above responses measured through 1998. We specifically analyzed three types of handling: instrumentation with electronic devices, blood and disease sampling, and tagging.

Instrumentation involved capturing seals with a hoop net and physically restraining them until they were sedated with valium administered *via* the extra dural vein. Animals were not weighed, but their mass was visually assessed prior to capture and dosages were estimated to range from 0.10 to 0.30 mg/kg. Various instruments were then attached to the seals' dorsal pelage using epoxy glue. Instruments included time-depth recorders (TDRs),² satellite-linked time-depth recorders (SLTDRs),³ video-recorders (CRITTERCAM),⁴ and Geographic Positioning System (GPS) data loggers.⁵ After release, seals were either recaptured 2–119 d later and instruments were removed, or seals were not recaptured and the instruments were shed no later than the subsequent molt. Mass of instruments ranged from 0.2–2.4 kg, and the largest, CRITTERCAMs and GPS tags, were recovered within 31 d.

Blood and disease sampling procedures began with capture and sedation as described above. Subsequently, blood was drawn from the extra dural vein, rectal body temperature was measured, and a variety of samples were taken, including ocular, nasal, vaginal, and rectal swabs, and blubber biopsies (as described in Iverson *et al.* 1997). The combination of samples taken varied, depending upon study objectives.

Weaned pups were tagged on each rear flipper with a unique plastic TempleTM tag, measuring 4.9×1.7 cm. Because nearly all pups were tagged in this manner, there was no control group available against which handling effects could be measured. Therefore, to investigate the potential effects of tagging, we analyzed only seals older than pups that were either being tagged for the first time or had lost or broken tags replaced. Tagging was a brief

² Wildlife Computers, Inc., Redmond, WA, U.S.A.

³ Telonics, Inc., Mesa, AZ, U.S.A.

⁴ National Geographic Television, Washington DC, U.S.A.

⁵ Lotek Marine Technologies, Inc., St. John's, Newfoundland, Canada.

procedure which involved simply capturing, usually with a hoop net, and restraining the seals without chemical sedation while tags were applied.

In many cases a single capture involved two or all three types of handling described above. That is, while seals were sedated for instrumentation, the opportunity was often taken to obtain blood samples and replace missing tags. For this reason, we coded each handling event with multiple procedures as the most intensive type. Handling to apply tags was brief and did not involve chemical sedation, so it was judged the least intensive. Blood and disease sampling was considered an intermediate procedure. Instrumentation, because it involved carrying an instrument with its associated hydrodynamic drag and usually a subsequent recapture, was deemed the most intensive type of handling.

Control seals were selected to be as similar as possible to the handled seals in every respect. That is, each handled seal was matched with a control individual of the same sex and the same age that was present during the same year at the same location. In most cases it was possible to find controls meeting all these criteria. However, in cases where the handled seal's age was not known exactly, a seal with the same estimated or minimum age was used. For each instrumented seal we determined a control candidate list of all seals which met the above criteria, and one was selected using a random number generator. No seal served as a control for more than one handled seal in any year. However, an individual sometimes served as a control in more than one year, such that measured responses were treated as statistically independent events between years. A further restriction was that seals were not eligible as controls for instrumented or bled seals if they were noted as having been wounded, moribund, or compromised in any way during the year. This measure was taken because such seals would not have been chosen for capture. It was not possible to apply this criterion to the controls for tagged seals, as the required information on individual condition was not readily available for all years when tagging occurred. However, this affected only a small number of seals ($n = 4$) for all instrumentation and bleeding events. Seals were also excluded from the potential control list if they had died, or were believed to have died, during the period when researchers were in the field in the current year. We were interested in comparing survival of handled *versus* control seals during the post-handling period. By excluding from the control group the animals known or likely to be dead, we avoided matching a handled seal to a control which may have already been dead when the handling occurred. This measure also made tests for handling effects on survival conservative. To ensure that both handled and control seals would have equal probabilities of being resighted in the subsequent year, given they were alive, all had good identifying marks (either tags or distinctive scars). All controls for tagged seals had at least one (usually two or more) tags which had been applied in previous years.

Relative proportions of handled and control seals resighted, migrating, or in poor condition in the following year were analyzed using log-likelihood ratio tests and logistic regression (Hosmer and Lemeshow 1989). In multiple logistic regression models, independent variables (handled versus control, age,

Table 1. Number and type of electronic instrument deployments on Hawaiian monk seals 1992–1997. SLTDR = satellite-linked time-depth recorder, TDR = time-depth recorder, GPS = geographic positioning system data logger. SLTDRs were deployed at French Frigate Shoals, with exception of 24 at Pearl and Hermes Reef in 1997. TDRs were deployed at Laysan Island. All CRITTERCAM and GPS units were deployed at French Frigate Shoals.

Instrument type	1992	1995	1996	1997	Total
SLTDR	3	4	18	31	56
CRITTERCAM		7	7	10	24
TDR	8				8
GPS			5		5
Total	11	11	30	41	93

sex, year, and location) were assessed using the model-building strategy suggested in Hosmer and Lemeshow (1989). When contingency table expected values were <1 , or when 20% of expected values were <5 (Zar 1984), Fisher's exact test was used. Tests were considered significant at $\alpha < 0.05$. All statistical tests and associated P -values were obtained using S-Plus software. Power of performed tests was assessed using Power And Sample Size (PASS 2000) software.

RESULTS

Instrumentation

A total of 93 instruments were deployed on monk seals during 1992–1997 (Table 1). SLTDRs were attached to 56 seals (38 males, 18 females) ranging from age 1 to at least age 18. All remaining instruments were deployed exclusively on adult males from a minimum of age 5 to an estimated age 18, including 24 CRITTERCAMs, 8 TDRs, and 5 GPS units. Total restraint time averaged 25.8 min. (SD = 7.3, range 9–47 min.)

There was no significant difference in the proportion of instrumented versus control seals resighted in the year subsequent to handling for all instrument types combined (log-likelihood ratio test, $P = 0.13$) nor for any particular type of instrument (Fisher's exact test, all $P > 0.48$, Table 2). Of the 87 instrumented and 81 controls resighted, none were observed as having migrated to a different subpopulation. Three instrumented and four control seals were observed with wounds (either from large shark bites or unknown causes), and these rates did not differ (Fisher's exact test, $P = 1.0$). Of 18 female seals with SLTDRs and their 18 controls, 17 (94%) from each group were seen the following year. Instruments were deployed during discrete studies at particular locations in various years (Table 1), and conditions and methods conceivably may have varied between studies. We therefore examined resighting rates during each discrete study and found no significant differences between instrumented and control seals in all cases (Fisher's exact test, all $P > 0.10$).

Table 2. Number and percentage of instrumented and control Hawaiian monk seals observed after one year, 1993–1998. Number of controls was equal to the number instrumented in all cases.

	Instrumented seals		Control seals	
	Seen	% Seen	Seen	% Seen
All instruments ($n = 93$)	87	94%	81	87%
SLTDR ($n = 56$)	53	95%	50	89%
CRITTERCAM ($n = 24$)	22	92%	19	79%
TDR ($n = 8$)	7	88%	8	100%
GPS ($n = 5$)	5	100%	4	80%

Blood and Disease Sampling

Eighty-two seals were sedated and blood samples were obtained during 1992–1997; however, 63 of the samples were taken simultaneously with instrumentation handling and have been addressed above. The remaining 19 seals were primarily captured for blood sampling and were matched with controls. Restraint time averaged 19.8 min. (SD = 3.4, range 13.0–26.3 min.). All 19 of the bled seals were resighted the year following their captures, and 17 of the 19 controls were resighted (Fisher's exact test, $P = 0.49$). No controls or blood-sampled seals were observed wounded or compromised in any way, and one blood-sampled seal and zero controls migrated the following year. All four females that were bled and all of their controls were resighted the next year. The small difference in migration of sampled versus control seals was not significant (Fisher's exact test, $P = 1.0$).

Tagging

A total of 750 monk seals (from age 1 to age 20) were tagged during 1983–1997 (Table 3). We matched 437 (252 males, 185 females) of those to controls with pre-existing tags (applied in previous years), and these individuals were used to statistically examine the effects of tagging. Of the 437 handled and control seals, 373 (85%) and 359 (82%), respectively, were resighted the following year (log-likelihood ratio test, $P = 0.20$, Fig. 2). Given the large sample sizes available from the tagging data set, it was possible to further explore the effects of various factors on resighting probabilities. In univariate logistic regression models, resight probabilities differed significantly among years ($P = 0.001$), and older seals were more likely to be seen ($P = 0.01$), while location ($P = 0.69$) and sex ($P = 0.18$) had no significant effect. In a multiple logistic regression, the best model included age, year, and their interaction (all $P < 0.05$). The latter factor is likely related to a temporal decline in juvenile survival which occurred after the late 1980s (Craig and Ragen 1999). This model was not significantly improved by including a variable indicating whether a seal had been tagged the previous year ($P = 0.19$).

We were particularly interested in detecting any negative effect of handling,

Table 3. Summary of Hawaiian monk seals older than pups tagged 1983–1997 by location and year. Numbers include individual seals tagged more than once in different years. Total number of taggings at all sites and all years was 750.

Year	French Frigate Shoals	Laysan I.	Lisianski I.	Pearl and Hermes Reef	Midway Atoll	Kure Atoll
1983	—	1	—	—	—	—
1984	—	2	—	—	—	—
1985	—	9	—	—	—	—
1986	1	72	—	1	—	—
1987	12	6	—	—	—	—
1988	5	43	—	4	—	2
1989	47	11	—	2	—	—
1990	2	81	19	—	—	2
1991	49	1	—	15	1	—
1992	21	5	6	1	1	—
1993	29	1	2	3	—	3
1994	1	6	—	—	—	1
1995	107	—	10	38	—	2
1996	1	99	2	14	—	—
1997	4	—	2	3	—	—

which, if it occurred in just one year at one location, might conceivably have been masked by lumping the data. Thus, we further tested the individual year/location data sets independently. These analyses were limited to 13 year/location combinations with the number of seals in each group (tagged and controls) exceeding 10 (range 11–49). The critical α was adjusted using the Bonferroni procedure to account for these 13 repeated tests; thus, $\alpha = 0.004$

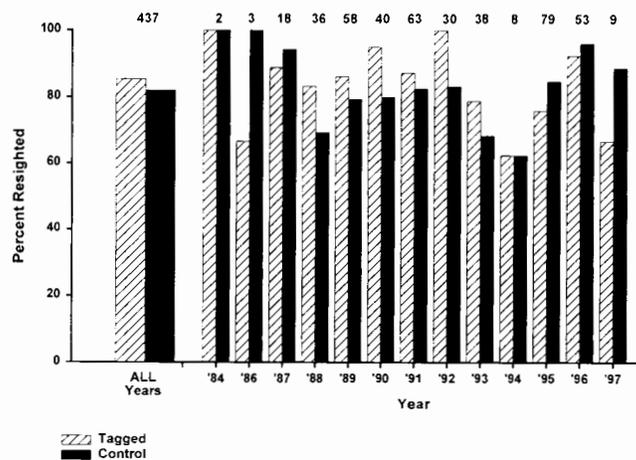


Figure 2. Percentage of Hawaiian monk seals handled for tagging and their controls which were seen in year subsequent to handling 1984–1997. Sample sizes (equal for tagged seals and controls) shown above bars.

(0.05/13). The only significant difference was at Laysan Island in 1990, when all 25 seals tagged were seen the following year, compared with 16 of 25 control seals (log-likelihood ratio test, $P = 0.003$). The only data set which suggested a possible deleterious effect of tagging was from French Frigate Shoals in 1995 where 33 of 47 (70%) tagged seals were subsequently resighted compared to 40 of 47 (85%) controls. This difference, however, was not statistically significant (log-likelihood ratio test, $P = 0.08$).

Migration was rarely observed. Only 4 of 373 seals seen one year after tagging and 2 of 359 controls were observed to have moved to another sub-population. This small difference was not significant (log-likelihood ratio test, $P = 0.43$).

Although there were no detectable effects of tagging when comparing handled seals to closely matched controls, we considered whether the length of time seals were physically restrained had any effect on their resight probability. For this analysis we examined resighting rates after 644 tagging events and recorded restraint time. The average restraint time was 3.3 min. (SD = 1.8, range 0.25–11.5 min). For all 644 taggings, restraint time did not affect the probability that seals were seen the subsequent year (logistic regression, $P = 0.36$). Similarly, when all year/location data sets were analyzed separately, restraint time had no significant effect (all $P > 0.20$).

Minimum Detectable Differences

We calculated the difference that could have been detected in resighting rates between handled and control monk seals, given our sample sizes (handled plus controls) and the observed rates among the control seals (with $\alpha = 0.05$, power = 0.90, and two-tailed hypotheses). For the instrumentation analysis ($n = 186$, control resighting rate = 0.87), a difference of 0.20 (*i.e.*, a resighting rate of 0.67 among instrumented seals) would have been detectable. Among the few seals that were blood sampled but not instrumented and their controls ($n = 38$, resighting rate = 0.89) only a larger difference could have been detected (0.47). For the tagging analysis ($n = 874$, control resighting rate = 0.82), a difference as small as 0.09 would have been detectable (*i.e.*, a drop in the resighting rate among tagged seals to 0.73).

Mortalities During Restraint

Results thus far have considered handling effects on released monk seals. Over the past 18 yr, five wild monk seals died during restraint or immediately thereafter during research and management activities that involved selecting and handling seals according to the protocols outlined in this paper. The number of monk seal handling events using these methods through 1999 exceeds 4,800 (including pup tagging), so the risk of death during handling is approximately 0.001. In most cases, the cause of death was not absolutely determined. The circumstances of the five deaths (all adult males) are described below.

(1) In 1982 a seal died at Lisianski Island during restraint to attach a TDR. No sedation had been used. This project occurred before protocols and data collection were standardized; therefore, seals captured for this project were not analyzed above. The seal died while being forcibly mounted by another adult male seal soon after release from restraint. A combination of stress associated with the capture and the subsequent aggressive mounting is believed to have led to the death.

(2) In 1984 a seal died at Laysan Island. The seal had been captured for translocation from Laysan Island in an effort to reduce a male-biased sex ratio that was causing high adult female mortality. The animal was believed to have died due to capture stress.

(3) In 1992 a restrained seal died at Laysan Island during a blood sampling and instrumentation capture. Capture stress was noted as the cause of death.

(4) In 1994 a seal died at Laysan Island during capture for translocation. This seal died before being moved to a holding pen. The cause of death was undetermined.

(5) In 1999, a seal died at Midway Atoll while under sedation for blood sampling. The sedated seal stopped breathing and could not be revived. No clear cause of death was determined, but there was evidence that pulmonary congestion was present prior to capture.

DISCUSSION

Our analyses indicate there are no deleterious effects on survival, migration, or condition associated with the research handling most critical to conservation of Hawaiian monk seals (tagging, instrumentation, blood sampling). The results strongly suggest that if captured animals are released alive, they fare as well as non-handled seals. The extremely low rate of mortality during or immediately after capture (0.1%) will be difficult to improve upon. While the one-year resighting rates observed for handled and control seals were similar and suggested no negative impacts, it is further reassuring that our sample sizes would have been sufficient to detect relatively small differences had they existed. A previous study by Henderson and Johanos (1988) examined relatively short-term behavior and survival of tagged versus non-tagged weaned Hawaiian monk seal pups, an age group of the population which this study could not assess. Henderson and Johanos (1988) also found no indication that tagging pups resulted in measurable harmful effects.

Little is known about the at-sea foraging behavior or habitat use of Hawaiian monk seals, and this area of research is critical for identifying and protecting seal habitat and prey resources (DeLong *et al.* 1984; Abernathy 1999; Parrish *et al.* 2000, 2002). In this regard, it is encouraging that instrumentation conducted to date, including the large CRITTERCAM video units, appears not to have negatively impacted the survival or condition of the study subjects. That is not to say that attaching instruments does not affect the comfort, behavior, or even short-term foraging success of study animals: Walker and Boveng (1995) found that attachment of TDRs to foraging Ant-

arctic fur seals lengthened their foraging cycles. Such impacts are daunting when attempting to interpret measured at-sea behavior, and we can assume that seals handled by researchers do not benefit from the experience. Yet at the very least we are confident that the threat to monk seals' survival from handling is minimal.

Female monk seals, because of their obvious demographic importance, have typically been avoided when research activities involve relatively intensive procedures (instrumentation, blood sampling). However, the very fact that females are so important to the species' conservation makes learning more about their habitat requirements, prey selection, and health status all the more compelling. In the past, primarily adult male monk seals have been instrumented, and it was hoped that their behavior would not differ markedly from that of adult females. However, there is evidence that adult male and female monk seals differ in their foraging habitat (Abernathy 1999, Stewart⁶). These studies suggest that satellite tracking and diving studies should include both sexes, as adult males are not likely to be adequate proxies for inferring female behavior. Fortunately, this study indicates that females can be instrumented without compromising their survival.

One reason relatively few adult female monk seals have been captured for instrumentation and bleeding is the potential that sedation with valium might influence the development of a growing embryo or fetus. Whether sedation might affect subsequent pupping of pregnant monk seals could not be addressed given the data available, as this issue was confounded by the fact that obviously pregnant females have not been handled. It is not always possible to visually assess whether a female is pregnant prior to capture; however, by systematically avoiding the conspicuously pregnant, it is likely that a selective bias is introduced. This policy is unlikely to change, as there is no compelling reason to handle obviously pregnant females, when females less compromised can be chosen.

Because of the scarcity of the Hawaiian monk seal, effects of handling will be assessed periodically as more research is completed and more data become available. In the interim, this analysis allows us to move forward with future studies with the confidence that negative effects on handled seals will be negligible, if current field methodology and animal selection guidelines are consistently maintained.

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⁶ Personal communication from Dr. B. S. Stewart, Hubbs-SeaWorld Research Institute, 2595 Ingraham Street, San Diego, CA 92109, December 1999.

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