Quantifying the Effect of Rehabilitation Pen Coverings on the Behavior of Captive Seabirds

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INTRODUCTION

Poor ventilation contributes to the development of aspergillosis, a frequent cause of morbidity and mortality in oiled seabirds. A study funded by the Oiled Wildlife Care Network found that ventilation was increased in rehabilitation pens covered with shade cloth[1], but because shade cloth is less opaque than more commonly used coverings (e.g., bed sheets and blankets), it may induce greater stress in captive birds due to increased visual contact with rehabilitators and the surrounding environment. To test this hypothesis we documented physiological and behavioral responses of common murres (*Uria aalge*) and large grebes (western and Clark's grebes, *Aechmorphorus occidentalis* and *Aechmorphorus clarkii*) after they were exposed to various stimuli while individually housed in pens covered with shade cloth and bed sheets. This study was approved by the UC Davis Institutional Animal Care and Use Committee (protocol #08-13426) and International Bird Rescue's Research Committee.

MATERIALS AND METHODS

Research Subjects: We used two cohorts of 5 birds selected from the rehabilitation populations at the San Francisco Bay Oiled Wildlife Care and Education Center and the Los Angeles Oiled Bird Care and Education Center. Each bird was housed on fresh water full time as part of the pre-release conditioning phase of the rehabilitation process and had a minimum packed cell volume of 35% measured one day before the study began. The rehabilitation flocks did not have sufficient numbers of birds to select a sufficient simple random sample for each treatment group so a crossover study design was used.

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Caging: In an empty room we placed vinyl soft side net bottom pens measuring 4'x2'x2' (LxWxH) in a single row. These pens are commonly used as standard OWCN rehabilitation equipment during oil spill responses affecting pelagic bird species. Each pen was covered with either green shade cloth (Easy Gardener Sun Screen Shade Cloth rated to block 87% of UV rays), or a white 130-thread count twin size flat bed sheet. Clips secured each covering to the sides of the pen.

Experimental Manipulation: Using hand nets we captured 2-3 birds from one cohort (murres or grebes) in rapid succession. After wrapping each bird in a bath towel, we placed Tegaderm® (3M, St. Paul, MN) bandage material on the plantar aspect of the feet and caudal aspect of the hocks (grebes only) and covered each foot with surgical stockinette (grebes and murres). Then we carried individual birds to one end of a randomly assigned soft side pen. Covering types alternated along the row of pens. At the far end of the pen, video cameras on tripods began recording each pen's interior. Next, handlers simultaneously laid each bird in the group on its left side on the net bottom inside the pen, then removed the towel, covered the pen, and exited the room.

Fifteen minutes later we sounded a brief (1-2 second), loud noise to apply an auditory stimulus. Thirty minutes later a single person stood by the side of each pen and bent over the covering in an attempt to visualize the bird inside. This visual stimulus lasted for 30 seconds. After 45 minutes, we removed the birds and collected blood samples in lithium heparin to determine plasma corticosterone levels.

We repeated the process at the same time on the following day. Birds were placed in the same pens, but material opposite from the previous day was used as the covering.

Behavioral Data Collection: One co-author (LAG) viewed the digital video recordings and captured individual behaviors using an event recorder (JWatcher™1.0, http://www.jwatcher.ucla.edu). She conducted focal animal sampling for three 5-minute intervals beginning: 1) immediately after the bird was placed on the net bottom and the towel removed (IM); 2) immediately after the auditory stimulus ended (AN); and 3) immediately after the observer became visible above the pen covering (AO). The data captured are shown in Tables 1 and 2.

Table 1
Behavior states coded during observation of video recordings.

Behavior	Description
Tonic immobility	Lack of movement due to environmental stimulus
Stand no scan	Weight rests on feet, or hocks and tail
Stand and scan	Looking around continuously
Sit no scan	Hocks bent and weight resting on keel
Sit and scan	Hocks bent and weight resting on keel and looking around continuously
Walk	Move from one area of the pen to another
Sleep	Lay head on dorsum

Table 2
Behavior events coded during observation of video recordings.

Behavior	Description
Pick	Use bill to pick foot coverings or at something in the pen
Vocalize	Vocalization event
Jump	Jump up and flap wings as if to escape
Flap	Open wings fully and flap wings without jumping
Preen	Run bill through feathers or rub back of head on body
Elimination	Droppings excreted

Plasma Corticosterone: We harvested plasma from whole blood samples immediately after collection. These were frozen at -80°C and shipped on dry ice to the University of Miami to measure plasma corticosterone levels using a radioimmunoassay.

Statistical Analysis: We analyzed data using SAS 9.2 software (SAS Institute, Cary, NC). Corticosterone values for each group were compared between treatments with a paired T-test. We used the Shapiro-Wilk test to confirm differences were normally distributed prior to performing paired T-tests. For behavioral data, we used the Wilcoxon signed-rank test to compare differences between the proportion of time birds under each treatment exhibited the behavior states described in Table 1 and the behavior events described in Table 2. For the behavioral analyses there were five birds in the murre cohort, but because of a camera malfunction, the grebe cohort was limited to four birds. Results of all tests were considered significant at a $p\text{-value} < 0.05$.

RESULTS

Plasma Corticosterone: There were no significant differences in the plasma corticosterone levels for either group of birds (Murres: T=2.55, P=0.0630; Grebes: T=1.92, P=0.866). Table 3 provides descriptive statistics for each cohort.

Table 3
Statistics for plasma corticosterone levels by cohort and treatment.

Cohort	Treatment	Mean	Std Dev	Minimum	Maximum
Murre	Sheet	103.9	36.3	62.8	159.4
Murre	Shade Cloth	54.3	16.6	40.7	82.8
Grebe	Sheet	14.7	6.3	4.3	21.3
Grebe	Shade Cloth	15.8	15.8	9.9	22.3

Behavioral Observations: Differences in the proportion and number of behaviors exhibited by birds under both type of coverings following each type of stimulus were not significant in either cohort. P-values for the observed behaviors grouped by type of stimulus may be found in Table 4. P-values could not be calculated for coded observations that did not quantitatively differ between the two experimental treatments. An asterisk represents these behaviors. Descriptive statistics are provided in Tables 5-10.

Table 4
P-values from Wilcoxon signed-rank tests of behavioral differences between treatment groups following each stimulus. An asterisk indicates the quantitative difference between treatment groups equaled zero.

	IM		AN		AO	
Stimulus	Grebes	Murres	Grebes	Murres	Grebes	Murres
Tonic immobility	*	*	*	*	1.0000	*
Stand no scan	*	*	*	1.0000	*	1.0000
Stand and scan	0.5000	0.0625	*	0.0625	*	0.0625
Sit no scan	*	*	0.5000	*	0.5000	*
Sit and scan	0.5000	*	0.2500	*	0.5000	1.0000
Walk	1.0000	0.0625	1.0000	0.0625	*	0.0625
Sleep	*	*	1.0000	*	1.0000	*
Pick	0.2500	1.0000	0.2500	1.0000	0.2500	*
Vocalize	0.2500	*	*	*	1.0000	*
Jump	*	1.0000	*	0.5000	*	1.0000
Flap	*	0.1250	*	0.5000	*	0.2500
Preen	0.1250	0.2500	0.5000	0.1250	0.5000	0.2500
Elimination	0.5000	0.1250	1.0000	0.5000	*	0.5000

Table 5
Observation statistics for grebes grouped by treatment (observation period=IM).

Covering	Variable	Mean	Std Dev	Minimum	Maximum
Shade Cloth	SitNoScan	0	0	0	0
	Immobile	0	0	0	0
	StandNoScan	0	0	0	0
	StandScan	0.0253000	0.0506000	0	0.1012000
	SitScan	0.9681750	0.0636500	0.8727000	1.0000000
	TotalScan	0.9934750	0.0130500	0.9739000	1.0000000
	Walk	0.0065250	0.0130500	0	0.0261000
	Sleep	0	0	0	0
	Preen	35.7500000	54.4571697	0	115.0000000
	Vocalize	1.2500000	1.8929694	0	4.0000000
	Flap	0	0	0	0
	Jump	0	0	0	0
	Eliminate	0	0	0	0
	Pick	6.5000000	9.9498744	0	21.0000000
Sheet	SitNoScan	0	0	0	0
	Immobile	0	0	0	0
	StandNoScan	0	0	0	0
	StandScan	0.0063750	0.0127500	0	0.0255000
	SitScan	0.9776500	0.0301959	0.9361000	1.0000000
	TotalScan	0.9840250	0.0319500	0.9361000	1.0000000
	Walk	0.0159500	0.0319000	0	0.0638000
	Sleep	0	0	0	0
	Preen	54.7500000	38.8705115	3.0000000	91.0000000
	Vocalize	0.2500000	0.5000000	0	1.0000000
	Flap	0	0	0	0
	Jump	0	0	0	0
	Eliminate	0.5000000	0.5773503	0	1.0000000
	Pick	10.0000000	10.0995049	0	24.0000000

Table 6
Observation statistics for murres grouped by treatment (observation period=IM).

Covering	Variable	Mean	Std Dev	Minimum	Maximum
Shade Cloth	SitNoScan	0	0	0	0
	Immobile	0	0	0	0
	StandNoScan	0	0	0	0
	StandScan	0.8973800	0.0949594	0.7758000	0.9837000
	SitScan	0	0	0	0
	TotalScan	0.8973800	0.0949594	0.7758000	0.9837000
	Walk	0.1025800	0.0949348	0.0163000	0.2241000
	Sleep	0	0	0	0
	Preen	2.8000000	4.6583259	0	11.0000000
	Vocalize	0	0	0	0
	Flap	4.6000000	1.5165751	3.0000000	7.0000000
	Jump	0.6000000	1.3416408	0	3.0000000
	Eliminate	1.8000000	2.4899799	0	6.0000000
	Pick	0.2000000	0.4472136	0	1.0000000
Sheet	SitNoScan	0	0	0	0
	Immobile	0	0	0	0
	StandNoScan	0	0	0	0
	StandScan	0.9523800	0.0665366	0.8349000	0.9932000
	SitScan	0	0	0	0
	TotalScan	0.9523800	0.0665366	0.8349000	0.9932000
	Walk	0.0475800	0.0665542	0.0068000	0.1651000
	Sleep	0	0	0	0
	Preen	3.0000000	4.2426407	0	9.0000000
	Vocalize	0	0	0	0
	Flap	6.0000000	5.8309519	1.0000000	16.0000000
	Jump	0.2000000	0.4472136	0	1.0000000
	Eliminate	1.2000000	1.0954451	0	3.0000000
	Pick	0	0	0	0

Table 7
Observation statistics for grebes grouped by treatment (observation period=AN).

Covering	Variable	Mean	Std Dev	Minimum	Maximum
Shade Cloth	SitNoScan	0.0357250	0.0714500	0	0.1429000
	Immobile	0	0	0	0
	StandNoScan	0	0	0	0
	StandScan	0	0	0	0
	SitScan	0.9642750	0.0714500	0.8571000	1.0000000
	TotalScan	0.9642750	0.0714500	0.8571000	1.0000000
	Walk	0	0	0	0
	Sleep	0	0	0	0
	Preen	0	0	0	0
	Vocalize	0	0	0	0
	Flap	0	0	0	0
	Jump	0	0	0	0
	Eliminate	0	0	0	0
	Pick	1.7500000	3.5000000	0	7.0000000
Sheet	SitNoScan	0.1195500	0.2391000	0	0.4782000
	Immobile	0	0	0	0
	StandNoScan	0	0	0	0
	StandScan	0	0	0	0
	SitScan	0.8346750	0.3258677	0.3459000	1.0000000
	TotalScan	0.8346750	0.3258677	0.3459000	1.0000000
	Walk	0.0017750	0.0035500	0	0.0071000
	Sleep	0.0439750	0.0879500	0	0.1759000
	Preen	0.7500000	0.9574271	0	2.0000000
	Vocalize	0	0	0	0
	Flap	0	0	0	0
	Jump	0	0	0	0
	Eliminate	0.2500000	0.5000000	0	1.0000000
	Pick	1.2500000	1.2583057	0	3.0000000

Table 8
Observation statistics for murres grouped by treatment (observation period=AN).

Covering	Variable	Mean	Std Dev	Minimum	Maximum
Shade Cloth	SitNoScan	0	0	0	0
	Immobile	0	0	0	0
	StandNoScan	0.0470600	0.1052294	0	0.2353000
	StandScan	0.9262200	0.0938639	0.7605000	0.9836000
	SitScan	0	0	0	0
	TotalScan	0.9262200	0.0938639	0.7605000	0.9836000
	Walk	0.0267000	0.0196275	0.0042000	0.0556000
	Sleep	0	0	0	0
	Preen	4.0000000	6.2849025	0	15.0000000
	Vocalize	0	0	0	0
	Flap	1.6000000	0.8944272	1.0000000	3.0000000
	Jump	2.0000000	4.4721360	0	10.0000000
	Eliminate	0.8000000	1.3038405	0	3.0000000
	Pick	0	0	0	0
Sheet	SitNoScan	0	0	0	0
	Immobile	0	0	0	0
	StandNoScan	0	0	0	0
	StandScan	0.9507200	0.0638509	0.8472000	1.0000000
	SitScan	0	0	0	0
	TotalScan	0.9507200	0.0638509	0.8472000	1.0000000
	Walk	0.0492800	0.0638509	0	0.1528000
	Sleep	0	0	0	0
	Preen	5.4000000	10.4307238	0	24.0000000
	Vocalize	0	0	0	0
	Flap	1.6000000	0.8944272	1.0000000	3.0000000
	Jump	2.0000000	4.4721360	0	10.0000000
	Eliminate	0	0	0	0
	Pick	0.2000000	0.4472136	0	1.0000000

Table 9
Observation statistics for grebes grouped by treatment (observation period=AO).

Covering	Variable	Mean	Std Dev	Minimum	Maximum
Shade Cloth	SitNoScan	0.0061500	0.0123000	0	0.0246000
	Immobile	0.0154250	0.0308500	0	0.0617000
	StandNoScan	0	0	0	0
	StandScan	0	0	0	0
	SitScan	0.9530750	0.0938500	0.8123000	1.0000000
	TotalScan	0.9530750	0.0938500	0.8123000	1.0000000
	Walk	0	0	0	0
	Sleep	0.0253250	0.0506500	0	0.1013000
	Preen	1.7500000	2.3629078	0	5.0000000
	Vocalize	0.7500000	1.5000000	0	3.0000000
	Flap	0	0	0	0
	Jump	0	0	0	0
	Eliminate	0	0	0	0
	Pick	8.2500000	15.1959424	0	31.0000000
Sheet	SitNoScan	0.1546750	0.3093500	0	0.6187000
	Immobile	0	0	0	0
	StandNoScan	0	0	0	0
	StandScan	0	0	0	0
	SitScan	0.8453000	0.3094000	0.3812000	1.0000000
	TotalScan	0.8453000	0.3094000	0.3812000	1.0000000
	Walk	0	0	0	0
	Sleep	0	0	0	0
	Preen	0.2500000	0.5000000	0	1.0000000
	Vocalize	0	0	0	0
	Flap	0	0	0	0
	Jump	0	0	0	0
	Eliminate	0	0	0	0
	Pick	4.0000000	6.1644140	0	13.0000000

Table 10
Observation statistics for murres grouped by treatment (observation period=AO).

Covering	Variable	Mean	Std Dev	Minimum	Maximum
Shade Cloth	SitNoScan	0	0	0	0
	Immobile	0	0	0	0
	StandNoScan	0.0043000	0.0096151	0	0.0215000
	StandScan	0.9514400	0.0537084	0.8673000	0.9970000
	SitScan	0.000700000	0.0015652	0	0.0035000
	TotalScan	0.9521400	0.0534775	0.8673000	0.9970000
	Walk	0.0435200	0.0530806	0.0029000	0.1327000
	Sleep	0	0	0	0
	Preen	2.8000000	4.7644517	0	11.0000000
	Vocalize	0	0	0	0
	Flap	2.2000000	1.3038405	1.0000000	4.0000000
	Jump	1.4000000	3.1304952	0	7.0000000
	Eliminate	0.6000000	0.8944272	0	2.0000000
	Pick	0	0	0	0
Sheet	SitNoScan	0	0	0	0
	Immobile	0	0	0	0
	StandNoScan	0.0037400	0.0083629	0	0.0187000
	StandScan	0.9853800	0.0201428	0.9603000	1.0000000
	SitScan	0	0	0	0
	TotalScan	0.9853800	0.0201428	0.9603000	1.0000000
	Walk	0.0108600	0.0155135	0	0.0334000
	Sleep	0	0	0	0
	Preen	7.2000000	13.4052229	0	31.0000000
	Vocalize	0	0	0	0
	Flap	1.6000000	0.8944272	1.0000000	3.0000000
	Jump	0	0	0	0
	Eliminate	0	0	0	0
	Pick	0	0	0	0

DISCUSSION

The original study plan called for cohorts of 10 birds each, but ultimately we were limited to groups of five. This was because we were never able to identify enough birds that met our case definition in the same stage of rehabilitation at the same time. Because these species are susceptible to developing numerous secondary medical problems associated with prolonged periods of captivity, holding them in care while we waited to enroll additional birds in the study was not a viable option. Eventually, contractual obligations forced us to move forward and work with the largest groups available.

Tables 5-10 show that birds spent the greatest proportion of their time scanning the environment (Figure 1 depicts one example). Although differences between treatments were not statistically significant, this level of alertness suggests birds experienced some degree of environmental stress no matter which covering was used. P-values from Wilcoxon signed-ranked tests approached significance ($p=0.0625$) in murres for the proportion of time birds spent standing and scanning (Figure 2 depicts one example). Although the differences were not significant, the mean proportion of time spent scanning was consistently lower for the shade cloth treatment. Similarly, the difference in plasma corticosterone values approached significance for murres ($p=0.063$) with the mean value for the shade cloth treatment measuring roughly half that of the sheet treatment group. Elevated corticosterone levels are considered to be a physiologic response to stress in birds.[2] These results, combined with the behavioral data, suggest the shade cloth covering is potentially less stressful than a sheet. These findings might be improved by repeating the study with a larger sample of murres.

The goal of this study was to learn whether the effects of various environmental stimuli differed between birds housed in rehabilitation pens covered by either a shade cloth or bed sheet. It was important to assess whether the benefits of increased ventilation provided by shade cloth might be outweighed by increased stress to captive birds. Our study showed that there was no significant difference between the treatments based on the metrics analyzed, and suggests that shade cloth is an acceptable pen covering material.

Figure 1
Mean proportion of time murres engaged in behavior states (observation period=AO).

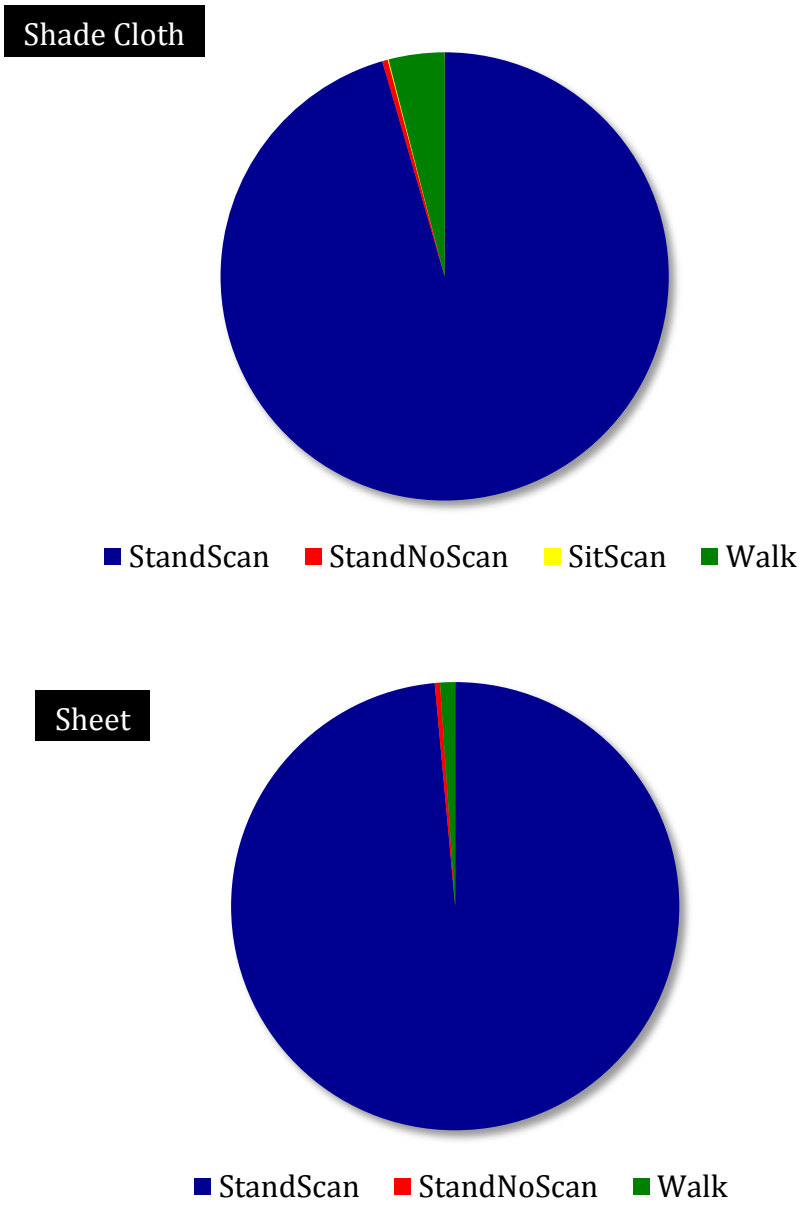
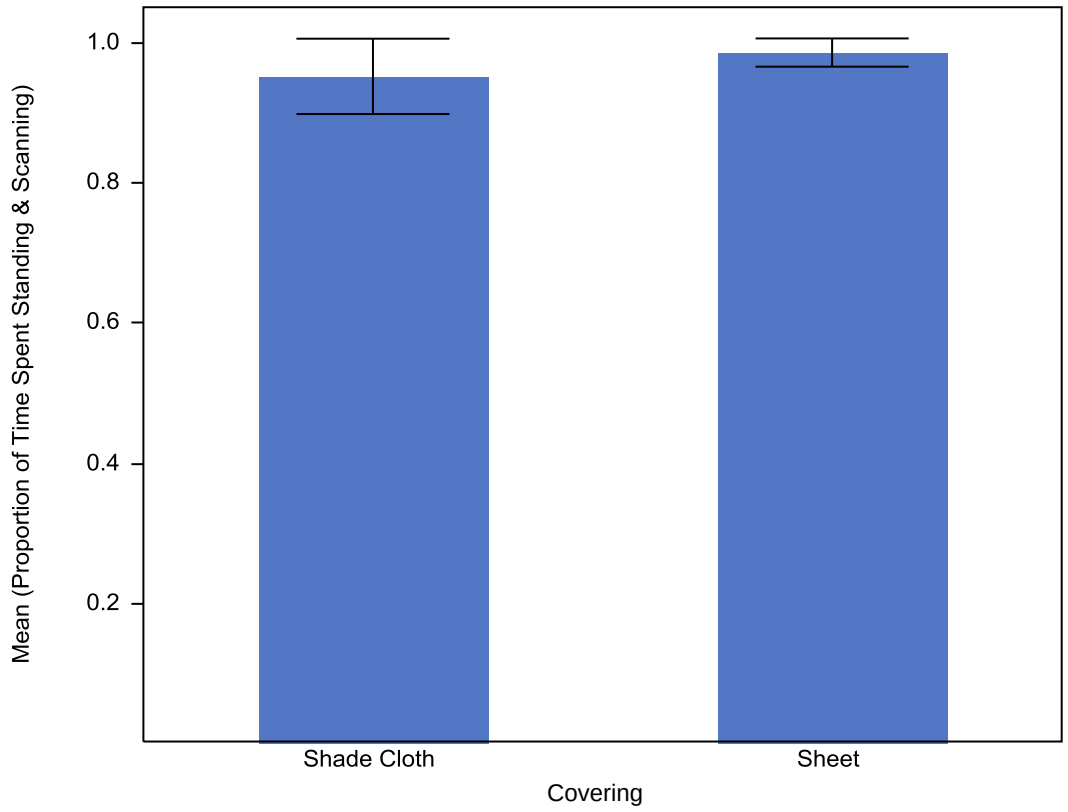


Figure 2
Mean proportion of time murres spent standing and scanning the environment by treatment group (observation period=AO). Error bars=1 standard deviation.



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