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Enrichment reduces stereotypical behaviors and improves foraging development in rehabilitating Eastern Pacific Harbor Seals (*Phoca vitulina richardii*)

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ABSTRACT

There is empirical support for the efficacy of enrichment in decreasing stereotypical behaviors and increasing naturalistic behaviors in laboratory, agricultural, and zoological settings. However, little research has been done on the possible value of enrichment in facilitating appropriate behavioral development of rescued wildlife in rehabilitative captivity. Eastern Pacific harbor seal pups (*Phoca vitulina richardii*) often strand on the west coast of California due to maternal separation or malnutrition and need to develop skills essential for reintroduction success while in rehabilitation. In the current study, we designated four enclosures at The Marine Mammal Center in Sausalito, CA, as enrichment or control. Behavioral data were collected on 32 pups in these enclosures throughout the 2016 stranding season (April – July). In three enrichment sessions per day, pups were exposed to stimuli that elicited behaviors related to foraging and exploration (e.g., diving, tactile investigation, locomotor coordination). Stereotypical behaviors (e.g., flipper-chewing, suckling) were recorded daily when no enrichment was present. Extent of interaction with enrichment, number of stereotypical behaviors, and number of days to independently forage (free-feed) were used to determine the efficacy of enrichment for stereotypy reduction and development of foraging skills. We found a positive relationship between number of stereotypical behaviors and days to free-feed such that the more stereotypical behaviors were expressed, the more days it took the pups to free-feed ($p = 0.06$). When exposed to enrichment, pups displayed a trend toward fewer stereotypical behaviors than pups in a standard (unenriched) environment ($p = 0.09$). There were no differences in number of days it took to free-feed between enrichment and control pups but there was a negative relationship between the extent of engagement with enrichment and number of days to free-feed that approached significance ($p = 0.07$). This pattern of strong statistical trends suggests that enrichment can be used to reduce stereotypical behaviors and encourage naturalistic behaviors in wildlife rehabilitation settings, promoting the likelihood that rehabilitated animals will succeed when reintroduced to the wild.

1. Introduction

1.1. Enrichment in captive settings

In the wild, animals are exposed to a continuously varying array of stimuli that are relevant to their needs to forage, find shelter, avoid predators, and attract mates (Wells, 2009). In captivity, opportunities to react to multi-sensory stimulation and use physical and cognitive skills are often limited and can lead to stereotypical behaviors and a reduction in natural behaviors (Markowitz, 1982; Mason et al., 2006; Wells, 2009). Enrichment is a term used to describe husbandry activities that aim to improve physical and psychological health and well-being, decrease undesirable behaviors, and encourage animals to display a wider range of natural behaviors (Markowitz, 1982; National Academy of Sciences, 2011; Swaisgood and Shepherdson, 2005). Enrichment in zoological parks has been shown to reduce cortisol levels and stereotypic pacing in polar bears (*Ursus maritimus*; Shepherdson et al., 2013), elicit foraging and exploration in western lowland gorillas (*Gorilla gorilla gorilla*; Ryan et al., 2012), and improve hunting efficiency in black-footed ferrets (*Mustela nigripes*; Vargas and Anderson, 1999). Similar benefits are seen in laboratory and agricultural settings (Francis et al., 2002; Latham and Mason, 2007; Ninomiya, 2014).

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However, enrichment has not been applied to animals who will be returned to the wild after temporary captivity, as is the case for wildlife in rehabilitation.

1.2. The need for enrichment in wildlife rehabilitation settings

Wildlife rehabilitation programs rescue wild animals suffering from injury, illness, or displacement by placing them under temporary human care to receive veterinary treatment prior to release back into the wild (Moore et al., 2007; Seddon et al., 2007). If rehabilitation is successful, reintroduced animals will be able to find and consume food, avoid predators, and reproduce (Harrington et al., 2012; Reading et al., 2013). Considering that the primary emphasis in rehabilitation settings is on treating injury and disease, it is perhaps not surprising that little attention is given to other factors, such as enrichment, that may facilitate successful reintroduction. However, the few published investigations of captive-born animals that were followed post-reintroduction point to the value of enrichment. For instance, Stoinski et al. (2003) examined behaviors in captive-born golden lion tamarins (*Leontopithecus rosalia rosalia*) that were introduced into the Poço das Antas Biological Reserve in Brazil. Locomotor and foraging abilities at release were found to be the key variables that lead to survival beyond 6 months, both of which can easily be targeted with enrichment protocols.

Enrichment in rehabilitative settings might promote successful reintroduction in at least two ways. First, as noted above, stereotypies are known to interfere with overall health and the development and expression of species-typical behavior in captive animals (Fraser, 2008; Ninomiya, 2014). To the extent that enriched animals are more engaged in activities that are inconsistent with stereotypies (e.g., playing instead of pacing), enrichment may promote overall health and behavioral development in animals prior to release.

Second, properly designed enrichment can target the development of the particular skills an animal needs to succeed in the wild. In the case of many marine mammals, this includes diving and foraging underwater. A typically developing wild harbor seal pup (*Phoca vitulina)* begins diving within days of its birth, accompanying its mother in her search for food (Bekkby and Bjørge, 2000; Bowen et al., 1999). Diving requires the development of physiological adaptations that allow extended periods of breath-holding. Thomas and Ono (2015) found that rehabilitating harbor seal pups' blood oxygen storage capabilities were deficient compared to wild seal pups, a result that may be accounted for in part by the fact that pups in rehabilitation spend a limited amount of time under water. Likewise, wild seal pups become successful foragers early in their lives, learning to catch and eat whole fish by the time they are four weeks old (Frost et al., 2006; Reidman, 1990). Captive pups have little opportunity to engage in naturalistic activities that promote interest in and the capture of fish, both of which could be targeted in enrichment activities. However, we were unable to find published accounts of the effects of enrichment used in a rehabilitation setting. Indeed, Harrington and colleagues (2012) reviewed 199 animal rehabilitation case studies and found that animal welfare while in captivity was mentioned in only 6% of those studies.

1.3. The current study

Eastern Pacific harbor seal pups (*Phoca vitulina richardii*) often strand on the west coast of California due to maternal separation or malnutrition. In 2016, 123 harbor seal pups were rescued at brought to The Marine Mammal Center (TMMC; marinemammalcenter.org) in Sausalito, California. Unlike other pinniped species, these pups are often rescued as neonates, and need to develop skills essential for reintroduction success while in rehabilitation centers. The goal of our study was to examine the effects of enrichment on harbor seal pups in rehabilitation, specifically on stereotypical behaviors and the ability to free-feed, which is defined as the ability for the animal to eat fish on their own with no human assistance. First, we evaluated the assumption that stereotypies are associated with inferior development. We hypothesized that there would be a relationship between stereotypical behaviors and development of free-feeding, such that higher rates of stereotypical behaviors would be associated with delays in the development of free-feeding (H1). We also predicted that pups exposed to enrichment would display significantly fewer stereotypical behaviors (H2) and progress to free-feeding in fewer days (H3) than pups maintained in a standard (unenriched) environment. Finally, we predicted that more interaction with enrichment stimuli would be associated with fewer days to free-feeding among the enriched pups (H4).

2. Methods

2.1. Subjects: husbandry and housing

In the spring and summer of 2016, neonatal Eastern Pacific harbor seals (*Phoca vitulina richardii*) pups stranded on the beaches between San Luis Obispo County and Mendocino County were rescued and brought to TMMC. Pups were assessed by veterinary staff upon admission and given a diagnosis for stranding. Pups were stabilized in indoor "condos" using subcutaneous fluids and tube feedings of a high-protein milk powder containing low levels of lactose and fortified with vitamins, minerals, and fish oil. When health had stabilized and teeth buds developed, pups were placed with conspecifics of similar developmental age (as determined by health status and a pup's ability to process and consume frozen fish offered by staff) in one of four outdoor enclosures (5.2 m x 3.7 m) containing a 5488-liter pool (3.0 m x 2.4 m) filled with recirculating treated salt water. Each enclosure was designated as either an enrichment pool or control pool for the duration of the stranding season. When living in these pools, the pups were fed a daily diet that mixed formula tube-feedings with frozen herring feedings in amounts based on body weight. Pups progressed from being hand fed whole fish by staff on the deck of the pool, to assisted hand feeds in the water, to following fish that were pulled on a string through the water, and finally to free-feeding, in which pups captured and ate thawed dead fish that were thrown into the pool. This four-stage process, termed "fish school" by TMMC, took an average of 3–4 weeks, with each session varying in duration based on the pups' behavior and development. Once pups were free-feeding proficiently and in stable health, they were transferred to larger, "pre-release" pools where pups continued to gain weight in order to qualify for release. To test the hypotheses in the current study, data collection took place from the beginning of fish school until transfer to the pre-release pools (i.e., at the end of fish school).

2.1.1. Subject criteria for inclusion in the sample

Upon release from the condos, pups were assigned to one of the four pools depending primarily on vacancies in those pools. Pups were sometimes moved from one pool to another for husbandry reasons. Careful records were kept of how long each pup spent in the control and enrichment pools, and after data collection, only those subjects who fulfilled the following criteria were included in the sample: a) spent at least 80% of its days in either an enrichment or control pool; b) had a malnutrition and/or maternal abandonment diagnosis (i.e. no trauma or clinical disease diagnoses); c) had no health issues that required additional veterinary intervention after transfer from the condos; and d) required no medication aside from a short course of antibiotics given upon admit to prevent umbilical infection. The final sample was 32 pups, 17 in the enrichment group and 15 in the control group. Pups were identified by wooden tags with ID numbers attached to their heads using temporary epoxy, as well as numbered flipper tags.

2.2. Enrichment stimuli

Five enrichment stimuli were designed with an emphasis on

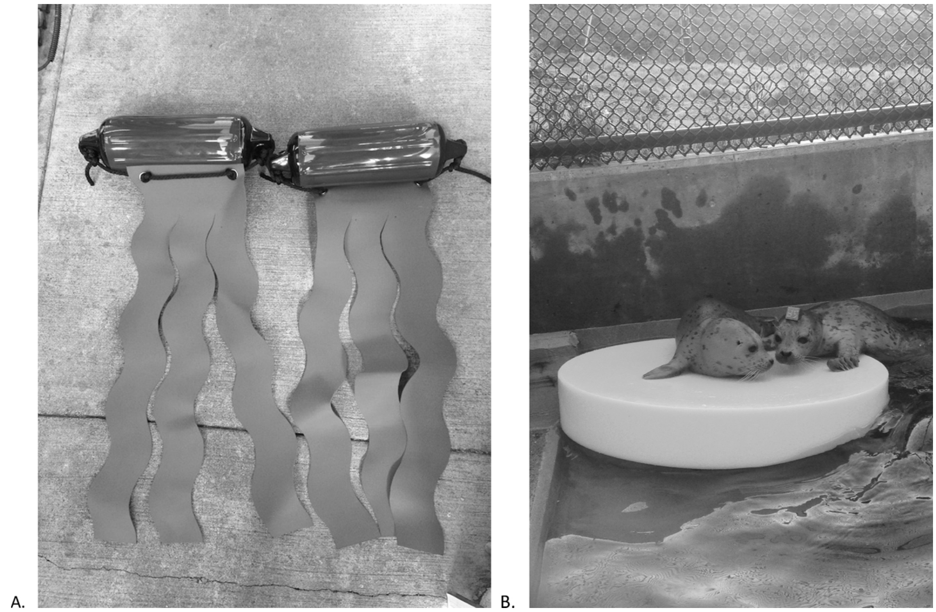

Fig. 1. Structural enrichment. Floating kelp was made of mitter curtains and marine buoys to simulate artificial kelp (A); subjects interacting with pontoon (B).

functionality, durability, animal safety, and easy cleaning. They were similar to stimuli that have been used successfully with permanently captive pinnipeds (Hocking et al., 2015; Hunter et al., 2002). Three of the enrichment stimuli (floating kelp, underwater hose currents, and floating pontoon) added structural complexity and novelty to the subjects' environment (see Fig. 1). The other two enrichment stimuli (feeding box and suspended kelp strand) were designed to encourage foraging skills in naturalistic situations and thus these stimuli included fish (see Fig. 2). At the beginning of each week, a randomized order of stimulus presentation was created for each enrichment session.

2.3. Procedure

Enrichment sessions took place three times a day, each for 30 min (0700 h, 1300 h, 1700 h), in both enrichment enclosures. Data were collected at the morning and afternoon sessions. A sham exposure in the control group was performed simultaneously whereby the research assistant remained in the control group's enclosure for the same amount of time as in the enrichment enclosure, mimicking the process of installing the enrichment stimulus. At the end of the enrichment session, the enrichment stimulus was removed from the enclosure, and a sham removal was performed in the control group by mimicking the removal of the enrichment stimulus. During enrichment sessions, interactions with the stimulus were live coded using instantaneous scan sampling at 1-minute intervals. At each scan, a subject's activity was recorded on a scale from 0 to 2, where 0 = not physically or visually engaged with the stimulus; 1 = visual inspection of stimulus; 2 = visually and physically engaged with the stimulus. Physical engagement was operationally defined as contact with stimulus using muzzle, vibrissae, or flippers in an unpatterned motion; this includes but not limited to pushing, mouthing, or scratching at enrichment stimulus. Visual engagement was operationally defined as head oriented and/or directed visual contact towards enrichment stimulus during the time of the instantaneous scan. At the end of data collection, the total engagement score was divided by total number of days in fish school to calculate an enrichment score for each subject in the enrichment group.

Stereotypical behaviors (SB) were recorded twice a day for 30 min at each session (1000 h, 1400 h). Data were live-scored in two of the four pools for each session (e.g., pool 1 and 2 in the AM and pool 3 and 4 in the PM) and pools were counterbalanced across data collection. Stereotypical behaviors were live-scored using one-zero sampling at each minute (0 = no SB observed in that minute; 1 = one or more SBs observed in that minute) and included flipper-chewing, self-scratching, suckling (on self, objects, or others), and swimming in repetitive circular patterns (Mason et al., 2006; Newberry and Swanson, 2008; Smith and Litchfield, 2010; Weary et al., 2008). At the end of data collection, the average number of scans per session in which stereotypical behaviors occurred was divided by the number of days in fish school to get a single stereotypical behavior score for each subject in the enrichment and control groups.

The number of days to free-feed was collected from patient charts and defined as the number of days from the first day of fish school until the pup was free-feeding proficiently at all meals and eligible for movement to the pre-release pools.

Six research assistants were responsible for live-score data collection. Inter-rater reliability (IRR) was calculated using absolute mixed model Intraclass Correlation Coefficients (ICC_3) before data collection began by scoring videos from three, 30-minute enrichment sessions and three, 30-minute stereotypical behaviors sessions that were recorded during the pilot study (summer 2015). IRR among raters was high ($r > 0.85$). IRR was calculated again half way through data collection, and it remained at $r > 0.85$.

2.4. Statistical analyses

We used IBM SPSS Statistics for Macintosh, Version 23.0 software for statistical analysis. Due to non-normality of the data as indicated by violated assumptions of linearity and homoscedasticity, non-parametric correlations (Spearman's) were used to test the relationships between stereotypical behavior scores and days to free-feed in the enrichment and control groups (H1), and between extent of engagement with the stimuli and days to free-feed (H4). For H2 and H3, where data from the enrichment and control groups were compared, there were violations of normality or homogeneity of variance in the data. Therefore, we used bootstrapped independent t-tests (10,000 samples) to generate the comparisons, as bootstrapping does not rely on theoretical distributions to generate p values (Howell, 2012). Alpha level was set at $p < 0.05$; where appropriate means (M) and 95% confidence intervals (CI) are given.

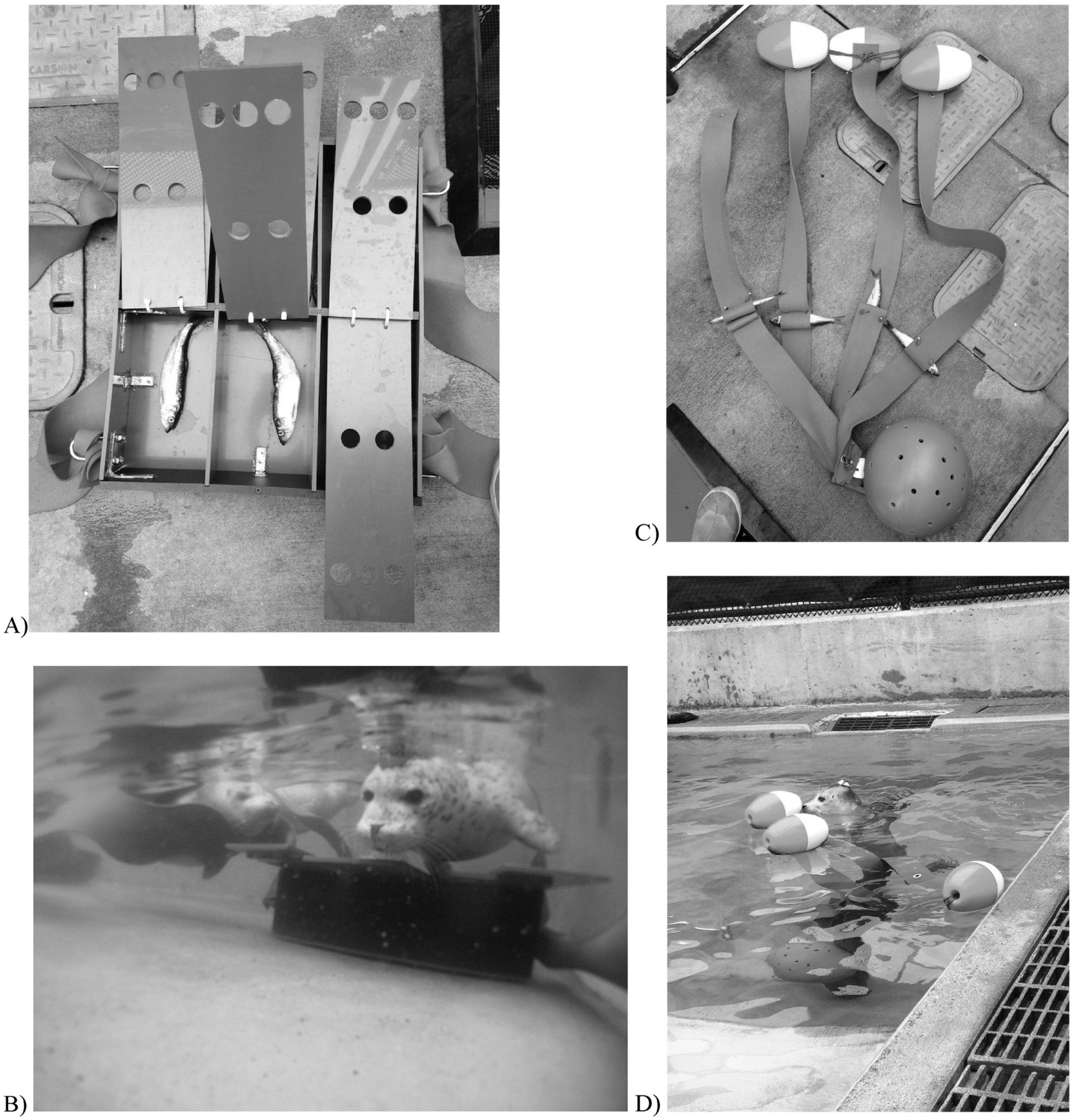

Fig. 2. Foraging enrichment. Feeding box (53.34 cm x 47.62 cm x 12.7 cm) (A). A subject interacting with the feeding box underwater (B). Suspended kelp strand was made of mitter curtains as artificial kelp with sewn pockets for fish, pool buoys, and a weighted jollyball. (C). Subject interacting with suspended kelp strand (D).

2.5. Ethical approval

The research reported here was approved by the TMMC IACUC (2015-2).

3. Results

3.1. Stereotypical behaviors and free-feeding (H1)

A Spearman's correlation was used to investigate the relationship between the average stereotypical behavior scores and days to free-feeding using all of the pups in the sample. As shown in Fig. 3, there was a positive correlation at the level of a statistically significant trend, such that the more stereotypical behaviors that were expressed, the longer it took subjects to free-feed, $r_s = 0.34$, $p = 0.06$, $R^2 = 0.12$.

3.2. Stereotypical behaviors in enriched versus control pups (H2)

A boot-strapped independent *t*-test (10,000 samples) was used to analyze the difference between the average number of scans in which stereotypical behaviors occurred in control and enriched pups. In accordance with our prediction, subjects in the enrichment group ($M = 0.04$, 95% CI [0.03, 0.06]) exhibited a statistical trend toward fewer stereotypical behaviors than subjects in the control group ($M = 0.06$, 95% CI [0.05, 0.08]), $p = 0.09$ (Fig. 4). The effect size ($d = 0.59$) using the control group's standard deviation is considered to be medium range.

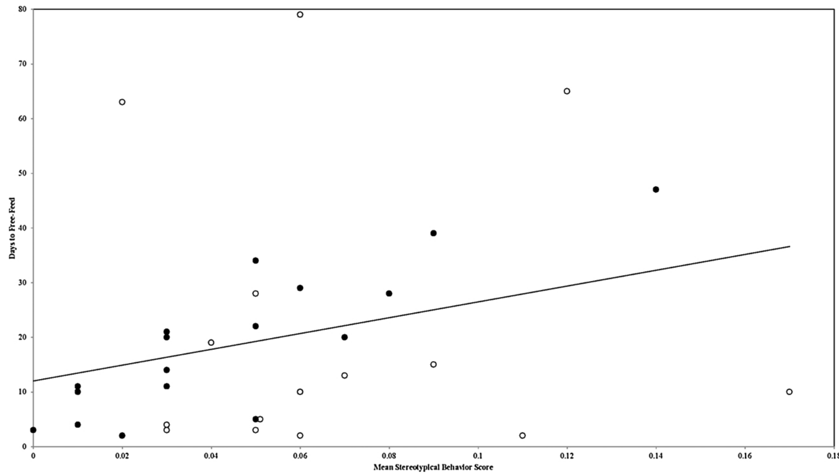

Fig. 3. The relationship between fequency of stereotypical behaviors and the number of days it took for subjects to feed on their own (r = 0.34, p = 0.06, r^2 = 0.12). The filled data points represent the enrichment group, the clear data points represent the control group.

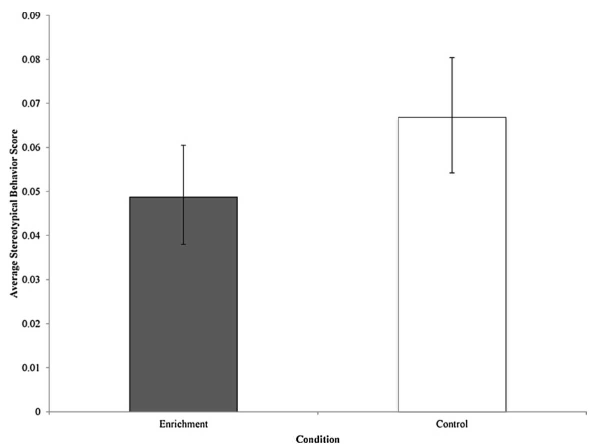

Fig. 4. Frequency of stereotypical behaviors in control (n = 15) and enriched (n = 17) pups (bootstrapped t-tests, p = 0.09, d = 0.59) Error bars represent 95% confidence intervals.

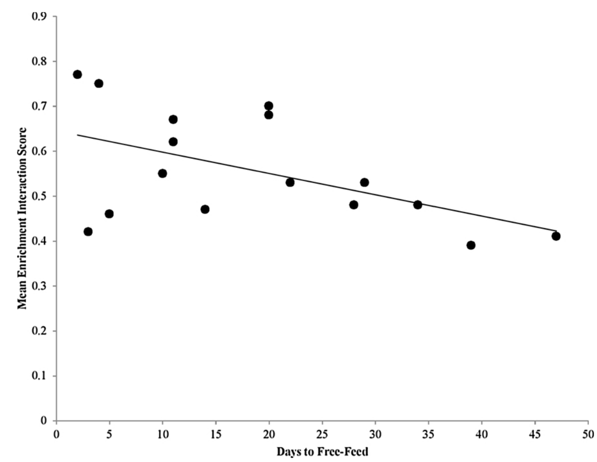

Fig. 5. Relationship between pups' frequency of interaction with enrichment stimuli and days to free-feeding (Spearman's r = -0.46, p = 0.07, R^2 = 0.21).

3.3. Time to free-feeding in enriched vs control pups (H3)

A bootstrapped independent t-test (10,000 samples) showed no significant differences (p = 0.72) in the number of days to free-feed between the enrichment (M = 18.82, 95% CI [12.79, 25.27]) and control (M = 21.4, 95% CI [9.33, 35.69]) groups. The comparison also yielded a small effect size (d = 0.15).

3.4. Engagement with enrichment stimuli and time to free-feeding (H4)

One outlier, whose inclusion would have created heteroscedasticity in the distribution of scores, was removed for this analysis (n = 16). A negative correlation at the level of a statistically significant trend was found between the amount of time interacting with enrichment and the number of days it took the subjects to free-feed, Spearman's r_s = -0.46, p = 0.07, R^2 = 0.21 (see Fig. 5). Thus, the less that pups interacted with the stimuli, the longer it took them to free-feed. We then conducted a post-hoc examination of the differences between structural (kelp, currents, and pontoon stimuli) and foraging (feeding box and suspended kelp) stimuli by summing the interaction scores across stimuli within the two categories followed by a bootstrapped independent t-test (10,000 samples) that did not assume equal variances. We found that foraging enrichment elicited significantly more interaction than structural enrichment M = -0.39, SE = 0.07, p < 0.01, d = 1.34 (See Table 1).

Table 1
Means and standard deviations of interaction scores for each enrichment stimulus, as well as collapsed means and standard deviations for the two enrichment categories (in bold).

Enrichment Type	Enrichment Stimulus	*M ineraction score*	*SD*
Structural		**0.44**	**0.13**
	Floating Kelp	0.56	0.31
	Currents	0.31	0.09
	Pontoon	0.45	0.1
Foraging		**0.83**	**0.26**
	Feeding Box	0.88	0.29
	Suspended Kelp Strand	0.78	0.28

4. Discussion

The consistent significant trends in the results of this study support the hypothesis that enrichment can improve the welfare and development of rehabilitating harbor seal pups through reduction of stereotypies and improvement of foraging behaviors. Our data point to the merit of using enrichment as a husbandry tool, and reveal that enrichment is effective not only in zoological parks and agricultural settings, but in rehabilitation settings as well.

4.1. Stereotypical behaviors and the development of independent feeding

There is empirical evidence that stereotypies are associated with poor behavioral and physical health in captive animals (Mason and Latham, 2004), but there is less known about the possible effects of stereotypies on normal development, and nothing is known of this relationship in rescued immature seals. In pinniped rehabilitation, the development of independent feeding is the most salient (and arguably the most important) developmental milestone in terms of preparation to return to the wild. We found that harbor seal pups who engaged in higher rates of stereotypies took more time to develop free feeding skills than pups who expressed fewer stereotypies. Although this relationship failed to reach statistical significance ($p = 0.06$), it is consistent with the literature on the deleterious effects of stereotypies on captive animals. We are unable to assume a causal relationship from this correlation, but to the extent that stereotypies are a bellwether of overall health, interventions that reduce stereotypies are certainly advisable especially with developmentally-sensitive animals that are destined for wild reintroduction.

4.2. Enrichment and stereotypies

Our experimental manipulation allowed us to test the hypothesis that stereotypies are reduced when rehabilitating seal pups are provided with enrichment. Our data tentatively support this contention, with a statistical trend ($p = 0.09$) toward fewer stereotypies in the enriched group. Given that the result was seen despite the small sample size, this trend warrants consideration. Interestingly, the stereotypical behaviors of the rehabilitating seal pups in our sample were expressed at relatively low levels, regardless of group ($M_E = 4\%$ of observations, $M_C = 7\%$ of observations). Although low, stereotypical behaviors occurred in the control group at almost double the percentage as the enrichment group. One could argue that the decrease in SBs we observed in the enriched pups is not of clinical significance, given this low baseline rate. However, our evidence (above) that stereotypies were indicative of slower progress toward free feeding in our subjects suggests that even low rates of SBs may signal concerns about seal pup development.

The ethological needs model proposed by Hughes and Duncan (1988) may explain the relationship between SBs and free-feeding and why enrichment can be effective in mitigating stereotypies. According to this model, animals are motivated by internal and external factors to express species-typical behaviors and if there is not enough opportunity to do so, animals will engage in functionless, incomplete, or stereotypical behaviors instead. Enrichment breaks this cycle by satisfying the ethological needs (foraging and exploration in the current study) of animals. Notably, in our study SBs were measured between a half hour to three hours before or after enrichment sessions. The fact that there was a statistical trend that indicated a difference between conditions suggests the possibility of a carryover effect that acts even in the absence of an enrichment stimulus and lends support to our conclusion that the predicted effect was achieved (Krebs and Watters, 2017; Swaisgood et al., 2001).

4.3. Foraging behavior development

Although there was not a group difference in the number of days it took for seal pups to achieve the developmental milestone of free-feeding, we found that pups who spent more time interacting with enrichment progressed to free-feeding more quickly. This relationship aligns with evidence in the literature that effective enrichment increases foraging behaviors in other species and is a promising result in terms of the possibility that enrichment improves the rate of development in rehabilitating pups (Charmoy et al., 2015; Hocking et al., 2015; Ryan et al., 2012). A new study by Greig et al. (2018) concluded that for harbor seal pups, "the best strategy to increase the probability of post release survival appears to be increasing pup mass prior to release" (p. 18). Carefully developed and properly implemented enrichment might enhance interest in feeding and perhaps improve rate of weight gain during rehabilitation.

The lack of significant difference in the enrichment and control group's days to free-feed is puzzling, especially considering the correlation between engagement with enrichment stimuli and time to free-feed reported above. The seals were randomly placed into experimental or control conditions but it is possible that seals from the control group were slightly healthier or older (age could only be estimated by the veterinary staff) than the pups in the experimental group, thereby blunting the effects of enrichment. Replication of this comparison is necessary in order to learn more about the effects of enrichment on progression to free-feeding.

4.4. Effects of enrichment type

Many types of enrichment reduce stereotypies, supporting the argument that stereotypical behaviors are due to the inability to perform behaviors that are normally elicited by internal states or external cues (Mason et al., 2006; Ninomiya, 2014; Wells, 2009). Operationalized as time spent interacting with the apparatus, there was a significant difference between the two categories (foraging vs structural) of enrichment in our study. As has been shown in other research, foraging enrichment is most likely to elicit responses from animals (Charmoy et al., 2015; Hocking et al., 2015; Hunter et al., 2002; Markowitz, 1982). However, this does not mean that foraging enrichment should be the only type of enrichment provided, in rehabilitation settings or otherwise. Individual preferences were evident, and some subjects showed the highest interaction scores with non-foraging enrichment items. Although foraging enrichment may encourage food-related behavior, structural enrichment promotes physical activity, investigatory behavior, and sensory stimulation that are likely to be important to development.

4.5. Limitations

There are several limitations to our study, mainly involving experimental control and sample size. For example, we could not eliminate all other influences from the daily lives of the seals (e.g., husbandry interactions with pool-mates), and the ages of the pups were only estimated given that exact birth dates could not be known, thus the average age of pups in the enrichment versus control groups may have been different. The control group exhibited a wider range of scores on most of our variables than the enrichment group. This may be due to greater variability in age and/or health status within the control group that occurred despite random assignment to condition. Such variability can easily mask group differences. In a clinical setting such as TMMC, sample size is not entirely under the control of researchers; in our case a common pox virus prevented a number of subjects from being included in the final sample. Furthermore, due to a record number of harbor seals at TMMC in the summer of 2016, pups were moved between pools more often than we had expected, which eliminated potential subjects due to the repeated crossing between enrichment and control pools. The associated reduction in statistical power may be responsible for the fact that many of our comparisons approached but did not reach the standard alpha level of $p < 0.05$. Nonetheless, the consistent pattern of our results points to the validity of our results and the potential for additional studies of psychological well-being in wildlife rehabilitation settings.

4.6. Conclusions

Our study is the first to empirically examine how enrichment can be utilized in rehabilitation settings with wild marine mammals under temporary human care. Pinniped rehabilitation facilities clinically assess and treat illnesses and injuries of wild, stranded seals with the goal of reintroduction. However, factors beyond clinical health such as psychological well-being and development of age-appropriate species-typical behavior need to be integrated into the daily husbandry activities for this unique population. Our data suggest that, as is true in zoos and aquariums, enrichment can be a useful welfare tool in rehabilitation by reducing stereotypical behaviors. Furthermore, interacting with enrichment may promote the development of free-feeding, thereby moving seals more quickly through the process of rehabilitation. Further investigation needs to be done to understand the effects of different types of enrichment on physiology and behavior as well as the developmental effects of enrichment at different points during the rehabilitation process. Finally, post-reintroduction tracking will be imperative to understand the long-term implications of enrichment in rehabilitation.

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