Incorporating Captive Animal Behavior into the Conservation of Threatened Species, *Hippocampus ingens*

Dominique T. Richardson

Paul J. Narguizian

Department of Biological Sciences
California State University, Los Angeles
5151 State University Drive, Los Angeles, California 90032, USA.

Abstract

Reintroduction, bolstering wild populations of threatened species with captive-raised animals, is a potential way to aid vulnerable seahorses. Previous reintroductions, including those of other organisms, have not always been successful, as captive animals do not have the same behaviors as their wild counterparts. Although they are naturally ambush predators, captive seahorses are promptly weaned onto a dead food diet due to the high cost of live food. To determine if the weaning process affects the behavior and food preference of a potential reintroduction species, we recorded and analyzed the behavior of Hippocampus ingens, raised at the Cabrillo Marine Aquarium (CMA), when feeding on live and dead foods. These post-weaned seahorses prefer dead food ($p<0.001$) and their feeding behavior is significantly altered by the weaning process: they act as scavengers instead of ambush predators. This discrepancy in behavior can directly affect the survival of the seahorses upon reintroduction and possibly change their ecological niche. This work will help prepare reintroduction animals both for survival and to fulfill their ecological role, ensuring the long-term success of conservation efforts.

Keywords: Captive animal behavior, Reintroduction, Feeding behavior, Pacific seahorse

1. Introduction

Reintroduction, in which captive raised animals of threatened species are released to re-establish or to reinforce wild populations of their species (Kleiman, 1989), is one commonly used method to aid threatened, endangered and critical organisms. This process uses captive-raised animals that, usually, must meet a set of criteria before being re-released into the wild to form a new population or contribute to an already existing population (Kleiman, 1989). While this method has largely been used for terrestrial vertebrates, a similar method of restocking is common in commercial fisheries (Bell et al., 2006) and is beginning to be used to strengthen populations of endangered fish as well (Rakes et al., 1999).

It is increasingly common for reintroduction groups to ensure that captive-raised animals have behaviors appropriate for survival after reintroduction (Caro, 1999; Curio, 1999; Kleiman, 1989; Lindburg and Fitch-Snyder, 1994). Many studies have been done showing the importance of having proper anti-predator behaviors, mating behaviors and other natural behaviors—such as good locomotor skills or natural activity patterns—in captive-raised animals to ensure their survival (Alvarez and Nicieza, 2003; Hossain et al., 2002; Kellison et al., 2000; Stoinski and Beck, 2004; Stoinski et al., 2003; Stunz and Minello, 2001). This is an understandable concern for costly reintroduction efforts and in the restocking of fisheries where survival is also commercially beneficial.

However, very few studies have been done on proper predatory behaviors of reintroduced animals, including fishes. If captive-raised animals are to be reintroduced to strengthen natural but threatened populations, it is important not only that the reintroduced animals survive but also that they perform their natural ecological function. Some previous reintroductions suggest that the type of food a captive animal is raised on impacts the type of food they seek after release (Keith-Lucas et al., 1999; Stoinski and Beck, 2004; Stoinski et al., 2003; Vargas and Anderson, 1996). A discrepancy between natural diet and the food sought by an animal could have a significant impact, particularly if the animal is a predator.

Predators may play an important role in their ecosystems, maintaining prey populations (Terborgh et al., 1999) and trophic systems (Finke and Denno, 2005) or increasing biodiversity (Paine, 1969). If captive predators are raised on frozen or dead food, this may impact both the types of food they seek and how they seek this food after reintroduction, leading to a difference in the realized niche of a captive-raised animal and its wild counterpart.

Many seahorse species, including the Pacific Seahorse (*Hippocampus ingens*) are listed on the IUCN Redlist (IUCN Redlist, 2011), threatened by overharvesting for medicines and aquaria and by habitat degradation (Koldewey and Martin-Smith, 2010; Vincent et al., 2011). When seahorses are raised in captivity they are generally for commercial purposes, however reintroduction to bolster natural populations is also an option (Vincent and Koldewey, 2006; Vincent et al., 2011). Unfortunately seahorses are both costly and difficult to raise in captivity (Fenner, 1998; Lunn and Hall, 1998). Due to this expense and difficulty, there has been a push to raise captive seahorses on frozen enriched food species (Wilson and Vincent, 2000; Woods, 2003; Woods and Valentino, 2003). Because the Pacific Seahorse and other seahorses are important predators in their benthic ecosystems—ambushing live, moving food—(Felicio et al., 2006; Tipton and Bell, 1988) their loss would have a significant impact on these ecosystems. However, this also means that if seahorses are reintroduced it is important that they retain natural feeding behavior of ambushing live prey in order to maintain their specific predatory role in their ecosystem.

Using Pacific seahorses, we conducted an experiment to determine if the usual process of weaning captive-raised predators to dead food affects food preference and feeding behavior at reintroduction age. The seahorses were raised and weaned at the Cabrillo Marine Aquarium according to standard aquarium procedure. At maturity we performed a food preference and feeding behavior test on the seahorses, offering natural live and dead foods found in their habitat. We digitally recorded the feeding sessions and used the frequency of feeding strikes on each food type to determine food preference. From the recordings we also quantified the feeding behavior of these seahorses to determine if the behavior itself is influenced by diet. If the type of food captive-raised seahorses are fed affects their food preference or feeding behavior at reintroduction age, it is possible that this will impact the seahorse's ecosystem after reintroduction. Although unnecessary for animals bred for captivity, if diet affects behavior and preference of these seahorses it may be worth the expense and effort for reintroduction groups to raise their animals on natural prey to ensure survival and proper ecosystem function after reintroduction.

2. Materials and Methods

2.1 General Care and Maintenance

The sample population consisted of four separate cohorts, 49 total individuals, born between January 2009 and July 2009. All Pacific seahorses (*H. ingens*), born in captivity, were raised to maturity with water quality, temperature, light exposure, and tank transfers controlled and in accordance with the guidelines in Seahorse Husbandry in Public Aquaria (Bull, 2002). The juvenile seahorses were started on a live prey diet, and promptly weaned to an exclusively dead prey diet (Bull, 2002), which is the common course of action in aquaria (Bull 2002). This weaning process involves feeding the new-born Pacific seahorses live nauplii while they are in a conical tank. After they have settled out and are transferred to a semi-kreisel tank, finely chopped frozen mysids are fed 3 times daily, while live nauplii are constantly available. At approximately 2 to 3 months old, these animals are transferred to a rectangular tank of appropriate size for the number of individuals (Seahorse Husbandry in Public Aquaria 2002). They are removed from the constant food source and fed frozen mysids with vitamin powder, supplemented with live *Artemia*, three times daily. Although there is some flexibility in the timescale of the weaning process based on the response of the seahorses, by approximately 3 to 4 months of age, the seahorses are fed a diet of exclusively frozen mysids, with added vitamin powder, three times daily: a diet they remain on permanently. Strict aquarium procedures ensured the four separate cohorts had a very similar upbringing

2.2 Experiment

We conducted the experiments at maturity, approximately 5 months old and after the completion of the weaning process. We offered the respective cohorts two different types of food—live and dead—and recorded the feeding sessions to observe preference and behavior. The experiments were run during the first feeding of the day to ensure the seahorses were actively feeding.

Each group was offered ½ of a normal feeding amount of either live mysids and *Artemia* or frozen mysids with vitamin powder—randomly assigned in treatment order. Proportions ½ of normal feeding amounts were used to ensure the seahorses remained hungry and continued feeding throughout the entire feeding session. We recorded the feed for 10 minutes, starting at the moment the food entered the water, using a JVC-Everio High-Definition Digital Camcorder (GZHD300BU, Victor Company of Japan, Japan). After 10 minutes, the feeding was stopped by removing any food that remained in the water with a hydro-vacuum. After cleaning out the food and allowing enough time for the seahorses to settle (about three to five minutes), the second food treatment was offered, and the feeding was recorded for another 10 minutes. After 10 minutes, the feeding was stopped by removing any food that remained in the water with a hydro-vacuum.

We analyzed the recordings of the feeding sessions using JWatcher (version 1.0; Blumstein and Daniel, 2007) and an ethogram (Table 1) of feeding behaviors. The proportion of time each seahorse spent doing each behavior in response to live food and in response to dead food was calculated. Because the seahorses were filmed as a group during feeding sessions but analyzed individually, individuals were matched to themselves using unique visual features for identification. This ensured that behavioral responses to live food and dead food were compared within an individual seahorse, not between different seahorses. Behaviors were limited only to those associated with feeding; other behaviors, such as courting, were excluded as they were not observed during feedings and were also unnecessary for analyzing feeding behavior. The proportion of time each seahorse spent doing a behavior in response to a given treatment was normalized to account for time spent out of sight of the camera. Because the data was paired—each seahorse's behaviors were measured in response to live food and in response to dead food—we calculated the difference in responses between the two treatments: live food response minus dead food response.

2.3 Statistics

All statistical tests were run using SPSS v16 (SPSS Inc. 2008). To determine if the seahorses had a food type preference, we calculated the difference in the total number of total strikes made on each food type by the seahorses (# strikes made on live food minus # strikes made on dead food). Using total number of strikes, rather than successful strikes, provides a measure of how actively the seahorses were trying to catch prey instead of how successful they are at catching prey. We ran an ANOVA on the difference in total number of strikes to determine if cohort had an effect on the difference in number of strikes. This ANOVA also calculated an intercept, which shows if the calculated difference (# strikes made on live food minus # strikes made on dead food) was significantly different from 0, meaning there was a significant difference in the number of strikes made per food type. Post hoc tests were run to show which food type treatment was preferred.

To determine if food type significantly affected feeding behavior, we calculated the difference in the proportion of time spent on each behavior when feeding on live and dead food. For each behavior we ran an ANOVA to determine if cohort had an effect on the difference in proportion of time spent doing that particular behavior. This ANOVA also calculated an intercept, which shows if the calculated difference (live food response minus dead food response) was significantly different from 0, meaning there was a difference between the behavioral responses to food treatment. Post hoc tests were run to show which treatment had an effect. Bonferroni's Method was used to account for the seven tests (one for each behavior) that were run. The adjusted alpha for feeding behavior was 0.007 (alpha for food preference remains 0.05).

3. Results

3.1 Food Preference

There was no effect of cohort on the number of strikes made per food type ($p=0.094$, $\alpha=0.05$). Treatment significantly affected the number of strikes made per food type ($p<0.001$, $\alpha=0.05$). More strikes were made on dead food. Post-weaned seahorses make an average of 5.75 strikes per 10 minutes on dead food while making an average of 1.34 strikes per 10 minutes on live food (Figure 1).

3.2 Feeding Behavior

There was no effect of cohort on the difference in proportion of time spent performing any behaviors (Table 2). There was also no effect of treatment on the proportion of time the seahorses spent swim looking ($p=0.834$, $\alpha=0.007$), bottom looking ($p=0.069$, $\alpha=0.007$), swim tracking ($p=0.133$, $\alpha=0.007$), or attached tracking ($p=0.048$, $\alpha=0.007$).

There was a significant effect of treatment on the proportion of time the seahorses spent attached looking ($p=0.001$, $\alpha=0.007$), bottom tracking ($p=0.004$, $\alpha=0.007$) and striking ($p<0.001$, $\alpha=0.007$). Post-weaned seahorses spend more time attached looking when feeding on live food and more time bottom tracking and striking when feeding on dead food.

4. Discussion

4.1 Food Preference and Feeding Behavior

Because there was no significant effect of cohort on either food preference or feeding behavior the behaviors of all the seahorses can be analyzed together. Regardless of when they were born, the seahorses have similar food preferences and feeding behaviors. However, weaning to a dead food diet does have an effect on the food preference and feeding behavior of the seahorses.

Previous studies have shown that wild seahorses and juveniles prefer live food (Felicio et al., 2006; Woods, 2003), which is why the weaning process is necessary. However, these post-weaned seahorses prefer dead food. They make more than an average 1 strikes per 2 minutes on dead food (5.75/10min average) while making just over an average 1 strike per 10 minutes on live food (1.34/10min average). This, along with the increased time spent striking in response to dead food over live food, shows a strong preference for their weaned, dead food diet.

The captive diet is also having an effect on the feeding behavior of the seahorses. The seahorses spend more time attached looking, a normal resting behavior, when feeding on live food than when feeding on dead food. It is likely that this behavior is seen more commonly when the seahorses are feeding on live food because they are less interested in this food treatment. If the seahorses were interested in the live food, while it might take them longer to catch—lowering striking rates—one would still expect to see increased tracking time as the seahorses search for and prepare to catch their prey.

The post-weaned seahorses also spend more time bottom tracking when feeding on dead food. The dead food does not remain in suspension as long as live food, and eventually all falls out to the bottom of the tank. The increase in bottom tracking behavior shows that the seahorses are spending less time searching for food and instead spending more time focusing on the food and feeding. Although one might expect to see an increase in bottom looking time as well, it is possible that there was no significant difference in this behavior between treatments because the seahorses are still looking for food along the bottom, even when live food is in the tank.

There is no difference in swim looking or swim tracking in response to the two food treatments. These behaviors are not common ways for the seahorses to track down their live food. Because the dead food sinks to the bottom we do not expect to see an increase in these behaviors as a response to dead food.

Overall, the weaning process has changed their food preference and the way the Pacific seahorses feed. They prefer the food they have been weaned onto and their behavior has adjusted accordingly. Instead of waiting in cover to ambush their prey, the post-weaned seahorses are leaving their holdfasts to forage along the bottom. They have become scavengers instead of ambush predators.

4.2 Potential Consequences and Future Studies

It is necessary to take into account these changes in behavior for the seahorses' survival after reintroduction. If they are seeking dead food and ignoring live food, they may not obtain enough nourishment to survive. While live mysids are common in their environment, an abundance of dead mysids are not likely to be available for them to eat. Also, when seeking dead food along the bottom, they are not camouflaged and are subject to a higher risk of predation. By looking for dead food and scavenging, they are leaving cover and thus this change in diet lowers their overall chances for survival.

It is also necessary to take into account how these changes in behavior may affect the role the seahorses will play in their ecosystem. If the weaned seahorses continue to seek out dead food after reintroduction, this may prevent them from performing their role as an ambush predator, possibly impacting the ecosystem. For a reintroduction program to be successful and sustain itself for the long term, the released animals should not only survive, but also fulfill their ecological niche. Proper predatory training may be necessary to ensure both of these goals are met for reintroduced animals.

While this study shows the potential effects of weaning on food preference and feeding behavior of Pacific seahorses, future studies may provide a better understanding of the reasons for these developed food preferences, such as caloric difference between foods or difference in ease of capture. Understanding the causes in developed food preference may help reintroduction programs design cost effective care procedures—such as de-weaning processes or tank designs that keep dead food in suspension—that prevent the changes in feeding behavior that develop as side effects of weaning captive predators to dead food. In addition, reintroduction programs may also benefit from understanding the detailed impacts of these findings on seahorse survival and seahorse prey populations in the wild, as well as potential applications to other reintroduction species.

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Tables:

Table 1. Ethogram of Seahorse Feeding Behaviors

Behavior	**Description**
Swim Look	Free swimming with unfocussed, scanning eyes
Attach Look	Attached to a holdfast, not to another seahorse, with unfocussed, scanning eyes
Bottom Look	Not free swimming or attached, touching the bottom of the tank with unfocussed, scanning eyes
Swim Track	Eyes directed at prey, head or body following and approaching prey by swimming
Attach Track	Eyes directed at prey, head or body following and approaching prey by stretching while still attached
Bottom Track	Eyes directed at prey, head or body following and approaching prey dragging along the bottom
Strike	Quick movement of the head or snout to suck up prey
Out of sight	Not visible in camera frame

Table 2. Feeding Behavior Significance Values

Behavior	**Effect of Group (p-value, α=0.007)**	**Effect of treatment (p-value, α=0.007)**
Swim Look	0.054	0.834
Attach Look	0.472	0.001
Bottom Look	0.319	0.069
Swim Track	0.843	0.133
Attach Track	0.01	0.048
Bottom Track	0.221	0.004
Strike	0.921	<0.001

Figures:

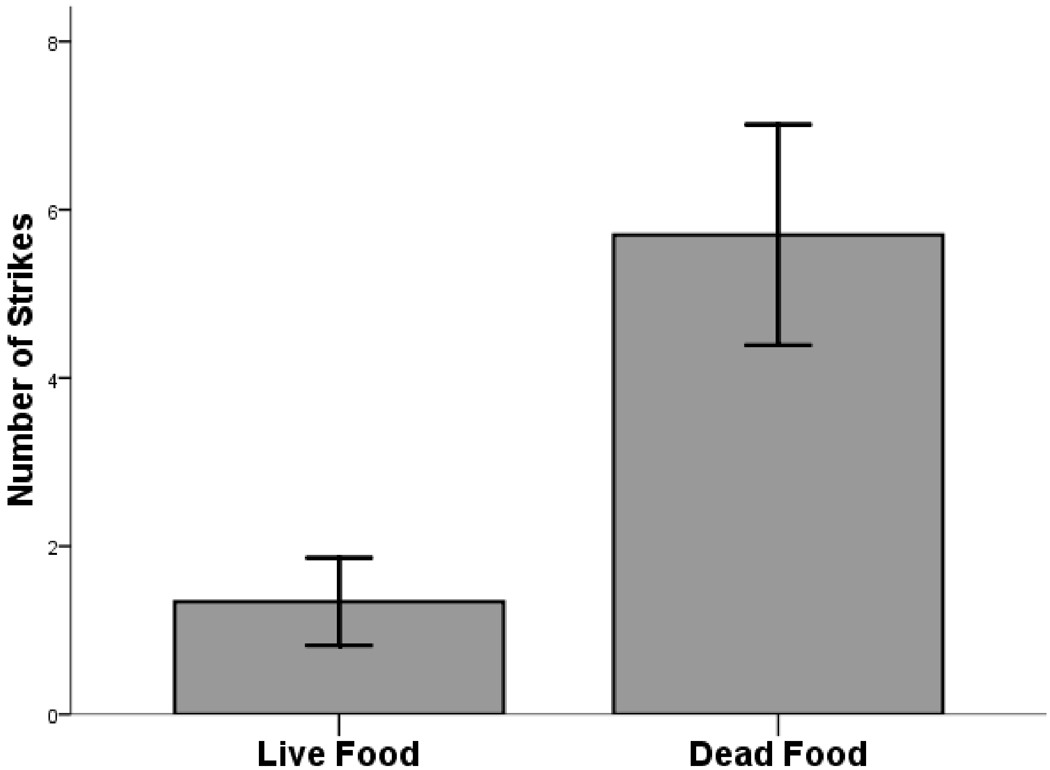

Figure 1. Average number of strikes made per food treatment during 10 minute feeding period. Error bars represent ± 2 standard errors.

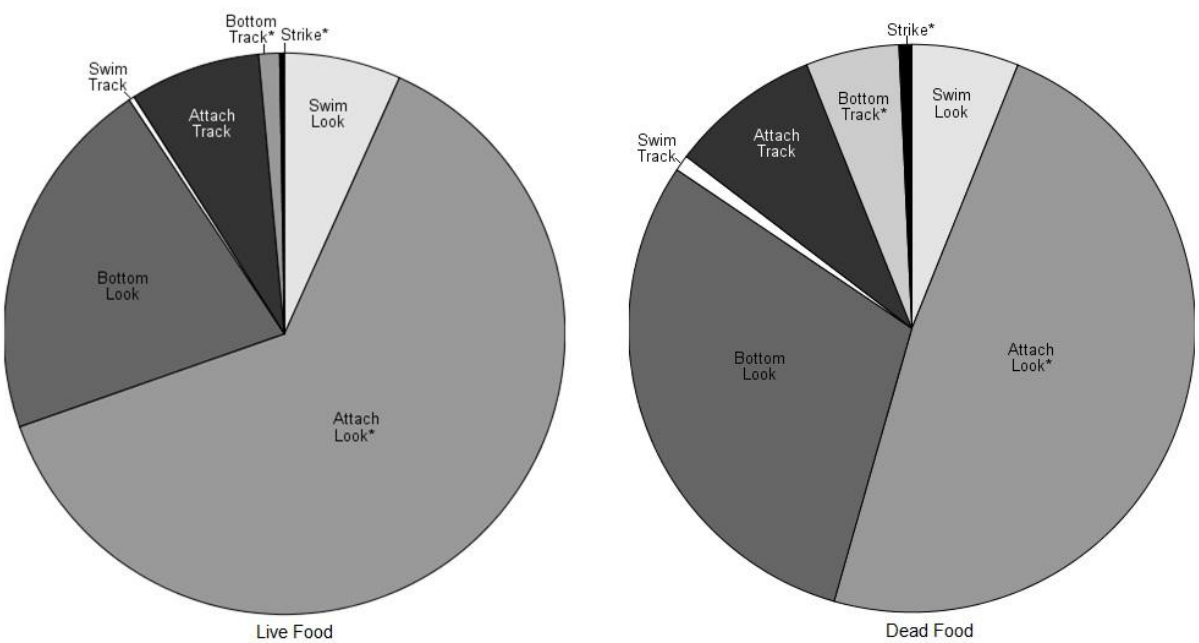

Figure 2. Comparison between the average proportion of time spent on behaviors when feeding on live versus dead food. Asterisks denote behaviors with a significant difference between treatments.