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Open the Cage: Handling, not captivity, affects escape responses of the red sea urchin, *Strongylocentrotus franciscanus* to the sunflower seastar, *Pycnopodia helianthoides*

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Abstract

The effects of handling and captivity of red sea urchins, *Strongylocentrotus franciscanus*, on its escape response to the predatory sunflower seastar, *Pycnopodia helianthoides*, were measured. One hundred and twenty four individuals of *S. franciscanus* were collected and subjected to 1 d, 2 d, 3 d and 4 d captivity treatments. Handling effects due to the tagging process were controlled for with 30 individuals. 1 d, 2 d and handling control treatments exhibited significantly lower escape responses compared to wild control organisms (all p values < 0.05). No effect of captivity time on escape responses was seen as handling controls did not differ from the captivity treatments. These results suggest that *S. franciscanus* would succeed after being reintroduced to the wild environment following captivity.

Keywords — Predator escape, captivity, *Strongylocentrotus franciscanus*

1. Introduction

Human populations have drastic effects on the natural world particularly through habitat change [1, 2]. These environmental changes, a portion of human-induced rapid environmental change (HIREC), can result in behavioural responses from various organisms [3]. Scientific studies often place their subjects in new environmental conditions to study their behavioural responses [4]. While captive environments offer opportunities for experimental manipulation, they may have effects on study organisms that magnify or dampen their intended responses. The environmental changes that scientists subject their study organisms to must be carefully acknowledged as well as the resulting effects that their reintroduction into native habitats may have [5].

Animals kept in captivity will quickly undergo behavioural responses similar to HIREC [6]. These studies often look at the evolution of captive animals over generations and therefore genotypes [6, 7], while relatively few studies look at the effects of short term responses (within one generation) of captive populations [4]. Changes in behaviours due to captivity can be caused by many stressors including proximity to humans and restricted movement [8]. These short term behavioural responses may result in lowered survival in the wild following reintroduction or

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negative consequences to the native environment such as the introduction of disease [9]. Handling, another common stressor during captivity, sometimes changes organism behaviour [10]. Understanding how quickly behavioural responses occur due to handling and captivity, along with the effects they have on survival in the wild, should impact reintroduction policy decision making.

Bamfield Marine Science Center (BMSC) recently proposed euthanizing collected organisms that are kept in captivity for more than 72 hours. BMSC has a strong history of animal care and takes in a wide variety of marine taxa but recognizes that the acquisition of study organisms disrupts the natural environment [4]. Among other reasons cited, the low probability of organism survival following reintroduction was an important factor in the decision making process [4]. The release of sport fish shows relatively low survival due to the aggressive nature in which the fish are caught and hatchery reared salmon often have difficulty recognizing predators [11, 12]. The Seattle Aquarium released a Giant Pacific Octopus successfully but there are few other studies that look into the reintroduction of invertebrates [4, 13]. It stands to reason that lower invertebrates, collected with less invasive procedures, would have a higher likelihood of survival following the reintroduction to their native environment [14]. Marine research stations need more evidence of reintroduction success to make well informed policy decisions.

Echinoderms are popular subjects for scientific research [14]. Forming a large portion of the specimens collected by BMSC, they are an appropriate study organism to educate changes to animal care policies [15]. Ordinary behavioural responses may change following captivity and could impact the ability of an echinoid to survive. Urchins have been known to exhibit escape responses in the presence of predatory sea stars [15, 16]. If responses are minimized due to increased stress, a symptom of captive and handled animals, lower survival could be expected [3]. *Strongylocentrotus franciscanus* is a common urchin to Barkley Sound that exhibits an escape response to *Pycnopodia helianthoides* [17]. The escape response of *S. franciscanus* in wild and reintroduced populations will be measured.

The primary predictions are:

1. Initial escape responses, the distance covered in 10 seconds following contact with *P. helianthoides*, decreases as time in captivity increases.

2. Escape response strength, the total distance covered in 30 seconds following contact with *P. helianthoides*, decreases as time in captivity increases.

3. Initial escape responses will be negatively affected by handling.

4. Escape response strength will be negatively affected by handling.

2. Materials and Methods

Five study sites were marked with anchored buoys, separated by 10 meters, along a transect running parallel to the coast at a depth of 8 meters at Aguilar Point near Bamfield, British Columbia (48.839444 125.141111; Figure 1). The bottom substrate was mostly a mixture of boulder and sand with little evidence of a dominant macro algal species at the time of survey. Five teams of two divers conducted the experiment between May 11 and May 17, 2016.

One hundred and twenty four individuals of *S. franciscanus* were collected from depths ranging from 6 to 9 meters at Aguilar Point by SCUBA over five collection dives. These urchins ranged in test diameter size from 23 to 92 millimetres. They were brought back to BMSC and placed in flowing salt water tanks for their captivity treatments. Urchins were not fed while in captivity. Urchin test diameters were measured with calipers and individuals were given a random treatment. 1 day captives ($n = 31$) were kept in the laboratory for a minimum of 24 hours, 2 day captives ($n = 29$) for a minimum of 48 hours, 3 day captives ($n = 30$) for a minimum of 72 hours and 4 day captives ($n = 34$) for a minimum of 96 hours. Individuals were identified by treatment group with the placement of two small (1 cm x 1 cm) rubber hosing tags on their spines. This was done by inserting a plastic pipette through a small hole in each tag and letting it off at the base of the spine after it was covered by the pipette. Once marked individuals satisfied a treatment requirement they were deployed by SCUBA equally at the anchor of the five study sites. Additionally, 30 *S. franciscanus* were subjected to a handling control treatment. These individuals were brought to the surface where they were tagged and redeployed equally among the study sites. Data were also collected on wild controls ($n = 57$) when time permitted following the underwater testing of treatment groups. Wild controls were haphazardly chosen by testing the third specimen encountered that satisfied the test diameter requirement of 3 to 9 cm during a standard roving survey.

Behavioural response data were collected the day following deployment. Predator escape response was induced by placing an individual of *S. franciscanus* on a flat surface and gently touching the tube feet on one side with *P. helianthoides*. The distance travelled along a standard transect tape was measured at 10 seconds, 20 seconds and 30 seconds following contact with the seastar.

Data were analyzed using a non-linear mixed effects model to describe the initial response (distance traveled within 10 seconds following contact) and response strength (total distance travelled within 30 seconds following contact) of *S. franciscanus* to contact with *P. helianthoides* using the R package "nlme" [18]. The fixed effect was treatment group while dive teams were included as random effects to account for variation between dive teams and the sites that they collected their data. All analyses were conducted in R [19].

3. Results

Initial predator escape responses did not vary significantly between captivity treatments and handling controls (Figure 1, Table 1). Response strengths, measured as total distance travelled in 30 sec, did not differ significantly between captivity treatments and handling controls (Figure 2, Table 2).

Wild urchins moved significantly faster in the first 10 seconds after contact with a seastar than all other urchins, except in four day captive treatments (Figure 1, Table 3).

Wild urchins moved greater distances over 30 seconds than urchins that had undergone handling processes (Figure 2, Table 4).

4. Discussion

Initial predator escape response and predator escape response strength appeared to be unaffected by length of captivity, which contradicts our first two predictions. This suggests that the amount of time *S. franciscanus* is subjected to captivity should not be a factor for determining whether an individual should be released back into the wild or euthanized. Instead, it appears that urchins that had been handled had weaker predator escape responses compared to the wild controls, which supports our third and fourth predictions. This suggests that the handling of wild urchins should be considered for reintroduction policies.

4.1. Responses to captivity

No significant differences in initial predator response nor predator response strength due to captivity time was found. Often behavioural responses change with captivity due to increased stress as an organism adjusts to its new environment [6]. Contrary to many other studies on organisms such as bony fishes and other vertebrates, these results suggest that captivity has little to no effect on the stress of *S. franciscanus* over the first 96 hours of captivity [20]. This might be due to the a high quality captive environment of the flow-through salt water system of BMSC, which can simulate the natural underwater environment of *S. franciscanus*. It could be expected that other, less advanced, captive environments might produce a negative effect on predator escape responses. Policies regarding the reintroduction of collected organisms should not be concerned about the captivity time of *S. franciscanus*, and possibly other echinoids, at BMSC.

4.2. Responses to handling

Urchins that experienced any handling prior to behavioural testing had weaker responses to predation than wild caught individuals. The process of collecting urchins, bringing them to the surface, placing tags and redeploying them appears to have put stress upon individuals. It is not clear from the study what portion of methodology changed the response of an individual to the presence of a predator. Three mechanisms are proposed by which this could occur: rapid changes in environmental pressure, tagging and increased detachment occurrences.

The process of bringing *S. franciscanus* from depth to the surface could have a negative impact on escape response. Many fish species cannot survive the process of being brought to the surface so it is possible that a rapid change in pressure could affect other organisms [21]. However, the impact would likely not be as significant in echinoids as they have a water vascular system that does not allow for large expansions of air within the body cavity [22]. For this reason, changes in environmental pressure during handling is the least likely mechanism for which predator escape responses change.

The tags put onto *S. franciscanus* may have negatively affected the predator escape as tagging has been shown in other studies to affect behavioural responses [23]. The placement of the rubber hosing sometimes resulted in the breaking of urchin spines and may restrict spine and tube feet movement. Two small tags however cover very little of the urchins surface area and obstructed few, if any, of urchins spines and tube feet. Furthermore, there appeared to be little tag rejection during visual roving surveys of the study organisms. Since it is common for echinoids to autotomize appendages that are obtrusive or injured, it is likely that our tagging techniques were relatively unobtrusive [24].The tags may have had a slightly negative impact on predator escape responses but not enough to explain the significantly different results between handled individuals and wild controls.

All individuals that had previously been handled underwent more detachment processes than wild caught individuals. When *S. franciscanus* feels threatened by detachment its natural response is to grip tighter to the substrate with its many tube feet [25]. If divers were not capable of conducting a swift detachment procedure, it is likely that many tube feet and spines were injured. Since tube feet and spine condition are critical components of escape response speed, increasing detachment occurrences should have a strong negative impact on escape response [26, 27].

Furthermore, urchins have been shown to have a tremendous ability to regenerate tube feet [28]. This may explain the increased predator escape response by the four day captives as this treatment group did not show significantly weaker predator responses compared to the wild controls. If increased days in captivity means more opportunity for the regeneration of tube feet, it is possible that more days in captivity could be beneficial for predator responses when compared to handling controls. Further experiments would need to be conducted over a longer period of time to see what long term effects captivity would have on predator escape response. It appears that this mechanism has the largest impact on predation escape response and suggest its further study.

It appears that the handling process had a negative impact on predator escape response while captivity appeared to have no effect. The lack of ecologically relevant changes to escape response due to captivity should not affect reintroduction. Thus, BMSC review their reintroduction policy for animal care to account for the handling of organisms and continue to review the effects of captivity on the reintroduction of wild organisms to their native habitat.

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6. Appendix

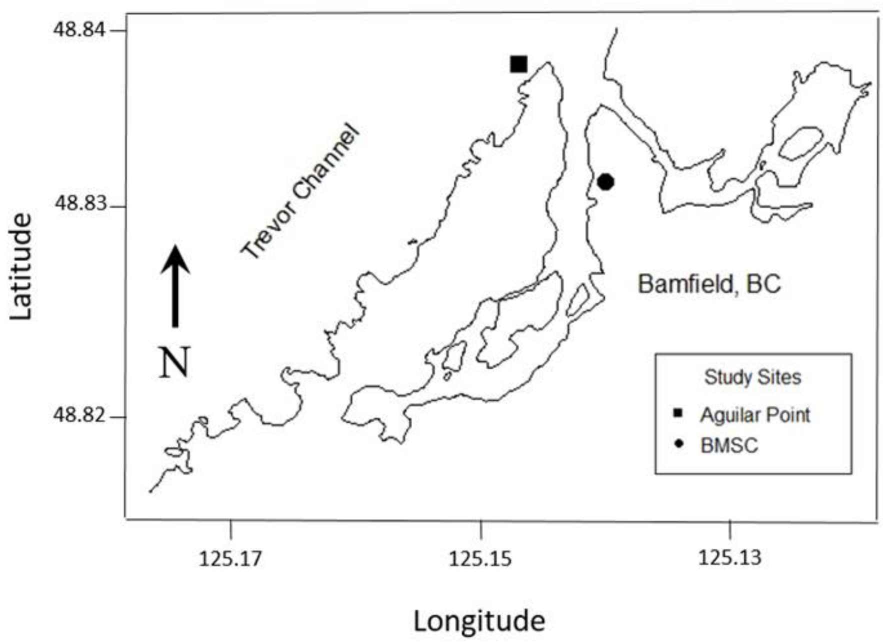

Figure 1: *Map of study areas in and around Bamfield, British Columbia.*

Table 1: *Model predictions and experimental observations of C. maenas and H. nudus distributions among good and poor patches (N = 3, averages ±0.33 absolute error).*

	Experimental Group				
	Day 1	Day 2	Day 3	Day 4	WC
t-values	-0.243	-0.059	-0.581	0.916	2.179
p-values	0.808	0.953	0.562	0.361	0.031*
n	31	29	30	34	57

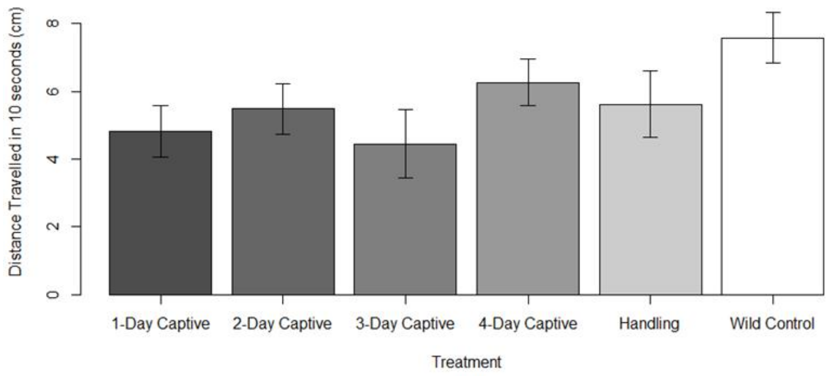

Figure 2: *Initial response values for captivity treatments, handling and wild control S. franciscanus. Wild control urchins travelled significantly further than handling control, 1 day captive, 2 day captive and 3 day captive treatments. Mean initial response distances are shown ±1 SE. Sample sizes are shown in Table 1 and Table 3.*

Table 2: *Summary of non-linear mixed effects model for mean predator escape strength of red sea urchins in different captivity treatments with HC as the baseline. Fixed effects are treatment groups and random effects are dive team. HC is handling controls, WC is wild controls and n is the sample size.*

	Experimental Group				
	Day 1	Day 2	Day 3	Day 4	WC
t-values	-0.794	-0.571	0.058	1.206	1.905
p-values	0.428	0.568	0.954	0.229	0.058
n	31	29	30	34	57

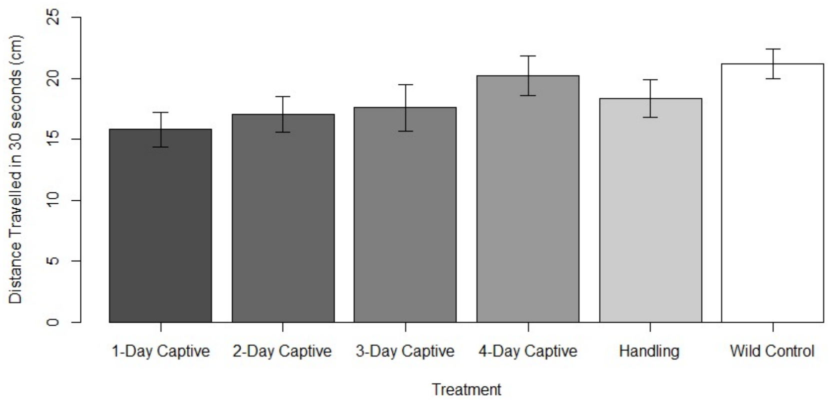

Figure 3: *Response strength values for captivity treatments, handling and wild control S. franciscanus. Handling control urchins travelled significantly further than 1 day and 2 day captivity treatments. Mean distance travelled are shown ±1 SE. Sample sizes are shown in Table 1 and Table 3.*

Table 3: *Summary of non-linear mixed effects model for mean initial escape response of red sea urchins in different captivity treatments with WC as the baseline. Fixed effects are treatment groups and random effects are dive team. HC is handling controls, WC is wild controls and n is the sample size.*

	Experimental Group				
	Day 1	Day 2	Day 3	Day 4	WC
t-values	-2.544	-2.288	-2.959	-1.275	-2.179
p-values	0.012*	0.023*	0.003**	0.204	0.031*
n	31	29	30	34	30

Table 4: *Summary of non-linear mixed effects model for mean space response strength of red sea urchins in different captivity treatments with WC as the baseline. Fixed effects are treatment groups and random effects are dive team. HC is handling controls, WC is wild controls and n is the sample size.*

	Experimental Group				
	Day 1	Day 2	Day 3	Day 4	WC
t-values	-2.895	-2.592	-1.924	-0.638	-1.905
p-values	0.004**	0.010*	0.056	0.524	0.058
n	31	29	30	34	30