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The effect of visitor density and interaction on the behavior of four ray species (Hypanus sabina, Hypanus say, Pseudobatos lentiginosus, and Rhinoptera bonasus) housed in an aquatic touch pool

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The effect of visitor density and interaction on the behavior of four ray species (*Hypanus sabina, Hypanus say, Pseudobatos lentiginosus,* and *Rhinoptera bonasus*) housed in an aquatic touch pool

by

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CERTIFICATE OF APPROVAL

The thesis "The effect of visitor density and interaction intensity on the behavior of four ray species (*Hypanus sabina, Hypanus say, Pseudobatos lentiginosus,* and *Rhinoptera bonasus*) housed in an aquatic touch pool" submitted by Aimee Little.

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ABSTRACT

Human-Animal Interactions (HAI) in zoological institutions are thought to be important in helping visitors to establish a connection with animals and thus making them more likely to contribute to conservation efforts. However, animals can respond to visitor interaction in both negative and positive ways. The growing focus on animal welfare in zoological institutions emphasizes the need for assessing different environmental inputs, including visitor interaction, and how these inputs influence behavioral outputs associated with welfare. A touch pool exhibit presents a novel interactive experience that allows visitors to directly interact with various aquatic species, including elasmobranchs, whose conservation has important implications for the marine ecosystem. Yet there is little information on how interactive experiences impact the welfare of elasmobranchs in touch pools. This study assessed the role of visitor density and activity on the behavior of four different elasmobranch species in a touch pool exhibit.

Experimental conditions were implemented to assess how regulating visitor interactions with the animals impacted elasmobranch behavior. Higher visitor numbers, frequencies of interaction, and frequencies of intense interactions from visitors were correlated with higher instances of aggression and negative reactions to visitors from the animals. Findings showed that the presence of food also had a significant effect on the occurrence of aggression and negative reactions, and it is likely that increased food provisioning during the experimental conditions lead to higher rates of aggression and negative reactions than expected. This study proposes the regulation of visitor density, interactions, and feedings to reduce aggression and negative reactions among elasmobranchs in a touch pool exhibit.

Results indicated that the pro-social *Rhinoptera bonasus* was much more likely to engage in interactions with visitors and was more likely to show aggressive behaviors compared to the other three less-social species. Studies of elasmobranchs in captivity and in the wild have shown

that species can aggressively compete for valued resources, and there is the potential for this competition to negatively affect health and well-being of elasmobranchs if dominant species or individuals repeatedly target the same animals. Findings demonstrate that species compatibility is an important consideration for touch pool exhibits, and cross-institutional comparison should be carried out to determine if pro-social elasmobranch species are better suited to interactive experiences compared to less-social species.

I. INTRODUCTION

The Role of Welfare Science within the Zoological Institution

It is critical for AZA-accredited zoos and aquariums to promote high visitor attendance to generate the revenue necessary to support their mission statement values of conservation, education, research, and welfare (Ballantyne et al., 2007; Fernandez et al., 2009; Godinez and Fernandez, 2019). Achieving these goals are especially important in the modern era, as many critics of zoological facilities argue that AZA institutions contribute too little to the conservation of endangered ecosystems, and that they compromise the welfare of wild species by placing them in captive environments for “entertainment” (Hancocks, 2001; Rose et al., 2009; Godinez and Fernandez, 2019).

At one point in time, entertainment and recreation were primary motivations for most zoo visitors (Reade and Waran 1996; Altman, 1998; Morgan and Hodgkinson 1999; Tofield et al. 2003). However, the motivations of modern visitors are beginning to shift as there is increasing public interest and concern for the welfare of animals living within zoos and aquariums (Miller, 2012; Draper et al., 2013; Draper, 2016; Sherwen and Hemsworth, 2019). Recent research shows that the most important contributing factor toward visitor satisfaction at zoos is “seeing animals that are well cared for,” as opposed to being able to get close to the animals or watch them get fed (Ballantyne & Packer, 2016). If animal welfare is compromised (or perceived to be compromised), then the mission of zoological institutions and their value to society could also be compromised. Therefore, modern zoos and aquariums must achieve and maintain high standards of animal welfare to find success in the face of growing public interest and scrutiny.

In more recent years, welfare science has gained greater importance within zoos and aquariums, especially when it comes to emphasizing the use of data to make informed decisions

intended to promote better animal care and management (Kagan et al., 2015; Sherwen and Hemsworth, 2019; Miller, 2020). As animal welfare science continues to advance, the focus on developing negative indicators of welfare to minimize suffering has shifted towards also developing positive indicators to measure "agency," control, and opportunities to "thrive" (Kagan et al., 2015; Mellor, 2015; Mellor and Beausoleil, 2015). Thus, to understand an animal's welfare state, both negative and positive indicators should be assessed when trying to evaluate how different environmental inputs can impact the mental and physical welfare of animals living within zoos and aquariums.

The Visitors-Animal Relationship in Zoos and its Effect on Welfare

When zoological institutions facilitate learning about animals, and the animals are shown to be well-cared for, visitors develop more positive perceptions of animals in zoos and become more supportive of zoo-based conservation efforts (Povey and Rios, 2002; Anderson et al., 2003; Hosey, 2005). However, while large and excited crowds provide financial support towards a zoo's conservation goals, these same crowds have been shown to influence the behavior and welfare of animals (Hosey, 2000; Hosey, 2008). This dependency on visitors leads to difficult management decisions to balance visitor satisfaction with animal welfare. When visitors are unable to view or interact with zoo animals, it is more likely to negatively affect visitors' experience and their willingness to contribute towards zoo-based conservation (Reade and Waran, 1996; Woods, 2002; Fernandez et al., 2009; Godinez and Fernandez, 2019). Likewise, when animals display negative behavioral indicators like avoidance or aggression toward visitors, it is also more likely to negatively affect visitors' perception of animal welfare and their willingness to financially support the zoo (Miller, 2013; Godinez and Fernandez, 2019; Sherwen and Hemsworth, 2019). This apparent conflict between the goals of conservation, visitor

education and engagement, and animal welfare makes it critical for zoological institutions to understand the relationships between animals and visitors so that visitors can enjoy an engaging educational experience without compromising the welfare of zoo animals.

The most studied aspects of human impacts on animal welfare focus on interactions that lead to poor welfare outcomes (Hemsworth, 2018). Previous research has demonstrated that human behaviors such as shouting, sudden movements and loud noises can lead to stress and fear responses which can have an impact on welfare (Broom, 1986; Broom, 2001). However, it has also been acknowledged that animals can also experience positive emotions associated with humans, usually as the result of gentle handling and petting (Hemsworth, 2003). To better understand the role of human behaviors in animal welfare outcomes, Human-Animal Relationship (HAR) research developed as its own specialty within the field of welfare science to study how interactions between humans and animals lead to the development of different learned responses from animals (Cole and Fraser, 2018). While there has been less HAR research involving wild species, zoo animals are exposed to both familiar and unfamiliar humans daily. This includes frequent, as well as close and intense, interactions with visitors. By far the most common approach to studying visitor effects has been to assess behavioral changes of zoo animals in response to different visitor conditions (Sherwen and Hemsworth, 2019). The proposed model of HAR research for the zoological setting classifies relationships, their behavioral indicators, and their subsequent effects on welfare in three different ways (Hosey, 2000; Hosey, 2008; Hosey; 2013):

1) A negative relationship, in which an animal responds to interactions with aggression, avoidance, or other stereotypic behaviors associated with fear and stress, and thus has negative impacts on welfare.

2) A neutral relationship, in which an animal has no perceived reaction to interactions, and thus has no impact on welfare.
3) A positive relationship, in which an animal responds to interactions with affiliation, exploration, play, and other non-stereotypic behaviors associated with positive emotional states, and thus has positive impacts on welfare.

There are numerous studies that demonstrate zoo visitors can be a source of stress for many primate species (Hosey, 2000), especially when visitors are loud, large in number, and make attempts to "interact" with the animals (Fernandez et al., 2009). Large visitor numbers have been associated with increased aggression towards conspecifics for gorillas (*Gorilla gorilla gorilla*) (Wells, 2005), as well as for other primates including cotton-top tamarins (*Saguinus oedipus*), Diana monkeys (*Cercopithecus diana*), and ring-tailed lemurs (*Lemur catta*) (Chamove et al., 1998). While fewer studies have been conducted with non-primate species, an increase in stereotypic behavior with large visitor numbers has been observed in jaguars (*Panthera onca*) (Sellinger and Ha, 2005), fennec foxes (*Fennecus zerda*) (Carlstead, 1991), and brown bears (*Ursus arctos*) (Soriano et al., 2013). Increased avoidance has also been observed in little penguins (*Eudyptula minor*) (Sherwen et al., 2015) and quokkas (*Setonix brachyurus*) (Learmonth et al., 2018) when visitor numbers increase.

Yet research has also shown evidence for visitor interactions to elicit positive behavioral responses from zoo animals. For example, both prairie dogs (*Cynomys ludovicianus*) (Eltorai and Sussman, 2010) and orangutans (*Pongo abelii*) (Bloomfield et al., 2015) appeared to be attracted toward visitor areas in their respective studies, while Asian small-clawed otters (*Aonyx* cinereus) showed an increase in playing and "begging" behaviors directed at visitors as a method of seeking interaction (Anderson et al., 2003). Similarly, Nimon and Dalziel (1992) found that a

long-billed corella (*Cacatua tenuirostris*) also engaged in "attention-seeking" behaviors to initiate interactions with visitors, but the animal would retreat more when visitor numbers increased. The authors concluded this change in behavior suggested a tolerance threshold for interaction. It is possible that different species may exhibit different tolerance thresholds towards visitor interaction, based on traits such as size or sociality, which may explain why different species vary in their response to visitors (Hosey, 2000; Hosey, 2005).

Interactive Experiences and their Effect on Animal Welfare

While most visitor effect studies have focused on species housed in traditional display exhibits, there have been far fewer studies conducted on animals that participate in "close encounter" experiences. It has become increasingly popular for zoos and aquariums to provide interactive programs that allow visitors to come into direct contact with animals. There is some research that suggests these experiences can help visitors to develop an emotional or empathetic connection with zoo animals, which in turn encourages them to act towards adopting attitudes and behaviors that support conservation (Gendron, 2004; Smith et al., 2008; Ballantyne et al., 2011; Ward and Sherwen, 2018). But not much research has been done to investigate how these experiences affect HARs in zoos from the animal perspective (Ward and Sherwen, 2018; Jones et al., 2016).

The limited number of studies that focus on the behavior and welfare of animals involved in these interactive experiences have shown mixed results. One study on dolphins in a visitor swimming program found that common dolphins (*Delphinus delphis*) retreated both during and within the 15 minutes following interactions with visitors (Kyngdon et al., 2003), but another study found that bottlenose dolphins (*Tursiops truncatus*) increased their playing behaviors during interactions with visitors (Trone et al., 2005). But Normando et al. (2018) observed that

the time giraffes spent engaged with visitors in a feeding program did not influence the occurrence of stereotypic behavior. The authors concluded that this may have occurred because giraffe feeding programs are designed to allow giraffes the "choice" of interacting with visitors, and so giraffes may be less likely to react negatively towards visitors because they can successfully choose to avoid interactions.

Anderson et al. (2002) also demonstrated how providing animals with choice influenced the occurrence of negative behaviors in a study with African pygmy goats (*Capra hircus*) and Romanov sheep (*Ovis aries*) in a petting zoo enclosure. When the authors observed that higher rates of aggression and avoidance behaviors occurred when visitor numbers increased, they introduced a retreat area for the animals that visitors could not access, which greatly reduced the occurrence of those behaviors. Many welfare studies indicate that giving animals the ability to make choices and exert control over their environments can help to reduce behavior associated with stress, as well as promote positive behaviors (Davis, 2005; Kagan et al, 2015). Therefore, if close encounter experiences are important for the emotional and educational experience of visitors, then it is important for zoological institutions to investigate if these experiences can 1) be beneficial to animal welfare and 2) be altered in ways to promote positive indicators of welfare while reducing any behaviors associated with stress and negative welfare, such as providing animals with the choice to engage in or avoid interactions.

Interactive Touch Pools and Elasmobranch Welfare

In addition to the previously mentioned types of close encounter experiences involving zoo animals, aquatic touch pools are also a common and popular feature of many zoological institutions. These interactive experiences allow visitors to directly engage with a variety of different aquatic species by touching and even sometimes feeding them (Sahrmann et al., 2015;

Ogle, 2016; Firchau, 2017; Biasetti, 2020). Marine invertebrates such as sea stars and anemones are the most used animals in touch pools, but due to advances in aquatic husbandry combined with the incentive for zoological facilities to provide “bigger” and more personal visitor experiences, many zoological institutions have incorporated several different elasmobranch species into these programs (Firchau, 2017). Touch pools are thought to help visitors develop a more positive emotional connection with these animals and to help educate them on the importance of elasmobranch conservation (Gendron, 2004). Research has highlighted that touch pools provide opportunities for families to engage in social learning (Rowe and Kisiel, 2012), and there is evidence that interacting with animals at a touch pool can lead to reduced mental stress (Sahrmann et al. 2016). It should also be noted that while the average visitor spends between 12 seconds and two minutes at a traditional display exhibit (Serrell, 1980; Bitgood et al, 1988; Marcellini & Jensen 1988), the average visitor time spent at a touch pool is 10 minutes (Firchau, 2017). This suggests that touch pools are successful for increasing visitor engagement using animal interactions.

However, the welfare impacts on elasmobranchs involved in these interactions are not well understood. Studies have shown that fish experience similar neuroendocrine and physiological stress responses to birds and mammals, and they will alter their behavior in response to noxious stimuli and prolonged periods of stress (Wedemeyer et al., 1990; Barton and Iwama, 1991; Wendelaar Bonga, 1997; Sneddon, 2003; Braithwaite and Huntingford, 2004; Brown, 2015; Fife-Cook and Franks, 2019). Touch pools expose elasmobranchs to frequent physical contact with visitors, and there is no evidence to suggest that they “enjoy” or purposefully seek out interactions with visitors (Biasetti et al., 2020). Based on other studies across taxa, visitor presence and interaction do have the potential to be a source of stress for

elasmobranchs. There is also the possibility that elasmobranchs in touch pools may experience injury as the result of encounters with visitors (Casamitjana, 2004), which can be detrimental to welfare. A small number of studies have identified and measured negative behavioral indicators for elasmobranchs in captivity, like higher instances of aggression and avoidance in response to husbandry practices such as feeding and training (Sabalones, 2004; Semeniuk and Rothley, 2008; Murphy et al., 2019), but there is currently a lack of studies that have attempted to measure similar behaviors for elasmobranchs living in touch pools.

Yet despite little empirical data available, zoos and aquariums have adopted a variety of methods in attempts to reduce the potential negative impacts of visitor interactions on the animals. This includes encouraging visitors to touch the animals in a certain way or providing spaces where the animals can avoid interaction with visitors (Biasetti et al., 2020). However, it is unknown if these methods are effective at reducing negative welfare impacts, or if they lead to more positive welfare states. Johnson et al. (2017) did provide evidence that the physiological health of elasmobranchs living within a touch pool was comparable to those living in a non-interactive exhibit. However, recent casualties at touch pools in both the United States and Canada (Controneo, 2014; D'Onofrio, 2015) make it apparent that there is a need for zoological institutions to identify and evaluate welfare indicators to better understand what management decisions will lead to optimal welfare for elasmobranchs in touch pools.

The purpose of this study was to investigate whether the presence and behavior of zoo visitors have an impact on the behavior of four different elasmobranch species living in a touch pool exhibit at the Jacksonville Zoo & Gardens (Jacksonville, FL). The species observed were: Atlantic stingray (*Hypanus sabina*), bluntnose stingray (*Hypanus say*), Atlantic guitarfish (*Pseudobatos lentiginosus*), and cownose ray (*Rhinoptera bonasus*). Both *R. bonasus* and *H.*

sabina are two of the most common elasmobranch species housed in aquatic touch pools (Firchau, 2017), but there is not much information regarding behavioral indicators of welfare for these species in captivity, especially in the context of an interactive exhibit. It is likely, however, that welfare will be dependent on how these species vary in their response to visitors based on species-specific traits such as activity, size, and sociality. *R. bonasus* is a social species that aggregates in large schools (Jacoby et al., 2012) and forages in groups (Ajemian and Powers, 2011). There are even anecdotal accounts that *R. bonasus* will seek out interaction and form "bonds" with their caretakers (McLaurin, 2016). In contrast, *H. sabina*, *H. say*, and *P. lentiginosus* are less gregarious in their natural habitats, typically only coming together in large groups for breeding, and while they can form small social groups, all three species are typically solitary (Snelson et al., 1988; Corcoran et al., 2013). It is possible that this difference in sociality between *R. bonasus* and the other three species will lead to different responses toward visitor presence and interaction.

Therefore, to address the larger goal of understanding how visitor interactions impact the welfare of elasmobranchs in a touch pool exhibit, the main objectives of this study were: 1) to determine if differences in visitor presence, density, and interaction type affect ray behavior, 2) to test if implementing conditions that control visitor density and interaction type will change ray behavior, and 3) to investigate if different species of elasmobranchs differ in their response to visitor presence, density, and activity. Based on previous studies with other zoo animals and the limited knowledge of elasmobranch behavior in captive settings, it was hypothesized that rays would exhibit more negative behaviors when there were more visitors, when there were more interactions, when the interactions were more intense, and when there were no limitations on where and how the interactions took place. In contrast, if rays do display affiliation toward

visitors, this would be more likely to occur when there were less visitors, when there were less interactions, when the interactions were less intense, and when there were limitations on where and how the interactions took place. Lastly, if species traits do influence how elasmobranch species respond to visitors, it was expected that *R. bonasus* would be more likely to interact with visitors and would display less aggressive behaviors than the other ray species in the JZG touch pool.

II. METHODS

Study Site and Groups

Observations were conducted on a population of rays housed in a mixed species aquatic touch pool exhibit at JZG between October 26th, 2020 and March 1st, 2021. Study animals consisted of four different species groups that varied in age, sex ratio, and size (Table 1). These groups were two female *H. say*, five *H. sabina,* one male *P. lentiginosus,* and 33 *R. bonasus*. The *H. sabina* and *R. bonasus* groups contained approximately even numbers of both sexes, for a total of 42 individuals across four species groups when the study began (Table 1). However, during the study, three male *R. bonasus* and three female *R. bonasus* were dispositioned to another zoological facility on January 13th, 2021. This change in *R. bonasus* group size was accounted for in the group data scan collected from January 13th, 2021, to March 1st, 2021. In addition, there was also an octagon-shaped net enclosure at the pool's center, where three young-of-the-year *R. bonasus* individuals were kept separate from the adult individuals throughout the course of this study. These individuals were also excluded from analysis due to physical inability to interact with visitors.

The study animals were housed in a large aquatic tank (9.14 m x 12.19 m; 66,245 L) with a shallow depth (0.76 m) intended to facilitate animal-visitor interactions. Water was delivered to the pool through a saltwater filtration system composed of five sand filters, 4 canister filters with carbon, 1 canister with Phosguard, 1 UV sterilizer, 1 foam fractionator, 1 fluidized bed, and ozone. Due to the physiological needs of the animals, the water conditions were monitored daily. Dissolved oxygen (DO) was generally between 85-95% saturation, or 6.0-7.0 mg/L, pH was maintained between 8.0-8.2, and salinity was kept around 24-25 ppt. Light-colored sand served as

the predominant substrate for the enclosure, with a one rock present to act as a hide location for the rays.

During all times of the year, visitors are permitted to interact with the rays at any location around the pool, except in the farthest corner from the visitor entrance (Figure 1). This area is inaccessible to visitors due to a waterfall filtration system. However, visitors can walk behind the waterfall to get access to the other side of the touch pool. The daily feeding schedule for the rays consists of one keeper feeding in the morning and one keeper feeding in the evening. This keeper-fed diet consists of shrimp, squid, and silversides. On-site education guides are also provided with two servings of shrimp and silversides for public feeding sessions. These feedings occur daily, with one serving of food allotted for morning feedings and one serving for afternoon feedings. Only visitors who purchase a "Total Experience" ticket at Admissions are permitted to feed the rays. If visitors do not pay for this package, they can still choose to engage with the touch pool by either observing the rays as they swim around, or by interacting with rays through physical touch. As a safety measure for both the animals and the visitors, exhibit guides are present to monitor visitor activity around the touch pool. There is also a sign posted to encourage visitors to rinse their hands before interacting with the animals, as well as a sanitizing station for them to wash their hands after touching and/or feeding the animals.

Manipulation of Visitor Density and Activity

JZG's pool was observed under three experimental conditions that modified 1) where visitor interactions occurred and then 2) how visitors could interact with the rays. During condition 1 (hereafter referred to as the "control condition"), no alterations were made to where visitors interacted with the rays or how they interacted with the rays. In condition 2 (hereafter referred to as the "retreat condition"), the back portion of the touch pool (the experimental area)

was blocked off to visitors by a physical barrier, providing a "retreat" area for rays where no visitor interactions occurred (Figure 1). In condition 3 (hereafter referred to as the "limited access condition"), the experimental area was reopened with limited access, controlled by a volunteer, who was instructed to escort small groups visitors at random to participate in interactions with more detailed instructions and closer supervision to reduce unpredictability and intensity of visitor interactions. This included both touch-only interactions, as well as feeding interactions if visitors chose to feed the rays while in the experimental area.

Data Collection

A total of 49.5 hours of touch pool observations were collected between October 26th, 2020, and March 1st, 2021. 16.5 hours were collected during all three conditions of the study. Animals and visitors were observed three days a week (Monday, Wednesday, and Friday) between the hours of 8:00-2:00 for three daily 30-minute observations that occurred within the timeframes of 8:15-9:15, 10:15-11:15, and 12:15-1:15, with an hour gap between observations. The first observation occurred during the morning keeper feeding before zoo operation hours, while the other two observations occurred during zoo operation hours when visitors were present. Visitor density and sound level (dBA) data were collected in real-time using the group scan format on ZooMonitor (Ross et al., 2016). Ray and visitor behaviors were coded afterwards using video footage. Inter-observer Reliability (IOR) for video coding was obtained for each condition until 80% reliability was achieved. To determine IOR, observation sessions were randomly selected for individuals to compare 1) ray proximity scan values recorded on an Excel template, and 2) ray and visitor all-occurrence values based on an export report provided by BORIS (Friard and Gamba, 2016), a free access event-logging software for coding live and recorded behavioral observations. When observations were taking place, an observer stood at the

end of the touch pool closest to the visitor entrance (Figure 1). Because this study occurred during the COVID-19 pandemic, the observer stood six feet from the edge of the pool to maintain social distancing when collecting visitor density and sound level data. Video cameras were also set up prior to every observation. For the control condition, one above-water camera and one underwater camera were utilized to collect video footage. Then for the retreat condition and the limited access condition, a second above-water camera was added when the experimental area started being used. All data collection protocols were approved by the institutions' IACUC and Research and Review Committee.

Behavioral Observations

An ethogram was created by incorporating published work by Casamitjana (2004), Murphy et al. (2019), and *ad libitum* sampling of the JZG ray population (Table 2). Because individuals were difficult or impossible to distinguish on video footage, group behavioral data was taken for each species group in the exhibit. During the 30-minute observations, both ray and visitor behaviors were recorded as all-occurrence frequencies, except for one single event behavior for ray aggression. Visitor decibel level, visitor density, and ray proximity data were recorded at scan intervals every minute. Ray proximity was measured as the rays' spatial distance from visitors. Ray aggression and ray-visitor interaction behaviors were only counted if more than half of the rays' bodies were in frame for the underwater camera. Thus, aggression and interaction behaviors were only recorded when they occurred in the underwater camera view of the Visitor Area for the control condition and the retreat condition, and when they occurred in the underwater camera view of the Experimental Area for the limited access condition. The field of view was the same in both Visitor Area and the Experimental Area to ensure the same amount of area was being filmed in both locations. Aggression behaviors were denoted by what species

were involved in the interaction, and these were then classified as either the “aggressor” or the “aggressed.” Aggression was also categorized as either proximate or distant depending on whether the aggression occurred at the side of the pool within reach of visitors. Interaction behaviors were only counted when a visitor made direct physical contact with a ray using their hand(s). All-occurrence behaviors were captured on video footage and coded using the BORIS event-logging software (Friard and Gamba, 2016).

Prior to this study, renovations were completed around the touch pool exhibit to replace the tent canvas used for protection against weather conditions with a sturdier pavilion structure that also allowed for more natural light exposure. This resulted in a glare on the surface of the pool that reduced the visibility of the above-water camera. Because of this constraint, proximity data for the rays was only collected in the areas that were visible on the above-water camera (Figure 1). Rays were counted as proximate when they were close enough to be in reach of visitors at the side of the pool. Boundaries between the different areas of the pool were established and kept consistent throughout the study (Figure 1), and rays were counted in an area if more than half of the body passed over the established boundary. Visitor density for the entire pool was recorded using a category system due to the difficulty of counting large crowd sizes greater than 30 people. Exact visitor count was only collected for the Visitor Area in the control condition and the retreat condition, and then for the Experimental Area in the limited access condition. All scan observations were recorded using an interactive ethogram on the ZooMonitor behavioral data app (Ross et al., 2016).

Data Analysis

Data were collected for 11 days in all three conditions. Of these 11 days, data collection occurred for 10 observations during the morning keeper feeding time frame, 11 observations

during the morning visitor time frame, and 10 observations during the afternoon visitor time frame. This resulted in a total of 31 observations for each condition. One observation was missing from both the morning keeper feeding time frame and the afternoon visitor time frame due to one or more video camera(s) losing power and failing to record footage. However, the same amount of data ended up being collected for each time frame across all three conditions. Each observation video was initially 30-minutes in length. However, due to the dense number of behaviors that occurred during that amount of time (e.g., hundreds), only the middle 10-minutes of each observation was analyzed to improve manageability of the dataset. This resulted in roughly 5 hours of footage being analyzed for all three conditions. However, due to several unexpected husbandry events during the morning keeper feeding time frame that limited data collection for ray behavior, the morning keeper feeding observations were ultimately removed from analysis. Thus, the sample size was readjusted to 11 observations during the morning visitor timeframe and 10 afternoon observations during the afternoon visitor time frame for all three conditions.

All-occurrence behaviors were summed for each observation session to calculate the average rate per minute of aggression, interaction, reaction, and proximity percentage for each observation period. Proximity values for the rays were calculated in two ways: a percentage out of the total population for a species group, and a percentage out of the visible population for a species group. This meant that two proximate and distant percentages were calculated for each area of the pool per scan interval. However, only percentages out of the visible population for each species group were used for analysis. This was because of the high number of *R. bonasus* in the pool (n = 30 for first half of study; n = 24 for second half of study) compared to the other species (n = 8), and the fact that a relatively high number of *R. bonasus* individuals were out of

view during scan intervals. In addition, because the population sizes were small for *H. sabina, H. say*, and *R. lentiginosus,* these species were combined into the same group ("Flat-body") in the analysis when being compared to *R. bonasus* proximity percentages.

Overall Visitor Effect on Rays

To test the effect of several visitor variables on ray behavior, linear mixed models (LMM) were created to describe the relationship between a ray response variable and explanatory visitor variables using the LmerTest package (Kuznetsova, Brockhoff, & Christensen, 2017), version 3.1-3 on RStudio Desktop version 2021.9.1.372 (RStudio Team, 2021). Predicator variables included experimental condition (control, retreat condition, regulated interaction condition), visitor count, negative interactions, touch interactions, and feed occurrence. Behavioral variables of interest for the rays included aggression, negative reaction to visitor interaction, positive reaction to visitor interaction, and proximate percentage of rays in the observation area. For the purposes of this study, both startle and retreat behaviors were classified as negative reactions to visitor contact, while affiliative behaviors were classified as positive reactions to visitor contact. Date of observation and observation time frame were also incorporated into all models as random effects. The model specifications for each response variable was:

Visitor Effect Model <- lmer(BehaviorVariable ~ ExperimentalCondition + AvgVisitors + AverageTotalNegativeReactionCount + AverageTouchCount + FeedOccurrence + (1|Date) + (1|TimeFrame)

The optimal model was chosen using the drop1 function from the Stats R package to identify the variables with the most significant effect in the full model (Ekstrom, 2012), and then creating a reduced linear mixed model that contained only the statistically significant variables from the drop1 results. Based on the results of the linear mixed model analysis, a Spearman's rank correlation coefficient test was run to test the direction of significance for visitor count or interaction count on a ray response variable, and a Wilcoxon-signed rank test was run to determine the degree of significance for feed occurrence on a ray response variable. Additional analysis was conducted using RStudio Desktop version 2021.9.1.372 (RStudio Team, 2021).

Visitor Effect on Rays Between Experimental Conditions and Between Species

To test for how effective restricting an area to visitors was at reducing negative behaviors and increasing positive behaviors, a Wilcoxon-signed rank test was used to compare aggression, negative reactions, and positive reactions between the control condition and the retreat condition. Then, to test if more rays were present in the experimental area versus the visitor area when visitors were present around the pool, a Wilcoxon-signed rank test was used to compare the percentage of proximate rays and distant rays in each area out of the total amount of visible rays in the pool.

To determine how effective regulating visitor interaction intensity was at reducing negative behaviors and increasing positive behaviors, a Wilcoxon-signed test was used to compare the rate of aggression, negative reactions, and positive reactions per observation between the control condition and the limited access condition, and between the retreat condition and the limited access condition. A Wilcoxon signed-rank test was also used to compare the percentage of proximate rays in the respective observation area for every minute between the control condition and the limited access condition, and between the retreat condition and the

limited access condition. This was done to test if regulating interactions resulted in more rays being proximate than distant. Lastly, a Wilcoxon sum rank test was used to compare interaction, aggression, and proximate percentages per observation session between cownose rays and the "flat-body" group to test if *R. bonasus* displayed differences in behavior compared to *H. sabina*, *H. say*, and *P. lentiginosus*,

III. RESULTS

Visitor Effect on Ray Behavior

Visitor interactions with the rays were more frequent in the control condition (860) than the reatreat condition (543) and the limited access condition (534). Out of the interactions that occurred, more negative ray reactions were recorded in the control condition (366) compared to the retreat condition (194) and the limited access condition (251). However, there were less positive ray reactions in the control condition (43) compared to the retreat condition (54) and the limited access condition (66).

There was also less aggression in the control condition (527) compared to the retreat condition (683) and the limited access condition (874). Aggression increased from the control condition to the experimental conditions for *R. bonasus* (control condition = 367, retreat condition = 503, limited access condition = 640), *H. sabina* (control condition = 47, retreat condition = 59, limited access condition = 91), and *H. say* (control condition = 92, retreat condition = 98, limited access condition = 122). *P. lentiginosus* was the only species that a decrease in aggression was observed from control condition (15) to the retreat condition (11) and the limited access condition (6). Figure 2 shows the percentages of ray reactions to visitor interaction across all three conditions.

The results of the linear mixed model analysis revealed significant effects of different visitor variables on all four response variables. Once effects were determined, drop1 model was used to identify which visitor variables within the models showed the most significant relationship with the ray variables. This was determined by higher F-values and lower Pr values. Then, a simplified model was run that contained only the statistically significant variables among

experimental condition, visitors, negative interactions, touch interactions, and feed occurrences (Table 3).

The best-fit model for aggression included three parameters with significant effect: experimental condition (drop1: F-value = 7.373, Pr = 0.010; LMM: estimate effect = 0.842, t = 2.823, p = 0.006), touch (drop1: F-value = 4.994, Pr = 0.030; LMM: estimate effect = 0.422, t = 3.511, p < 0.001), and feed occurrence (drop1: F-value = 23.932, Pr < 0.001; LMM: estimate effect = 2.770, t = 5.583, p < 0.001) (Table 3). Further analysis using a Spearman's correlation test showed there was a significant positive correlation between touch (M = 2.03, SD = 2.03) and average aggression per minute (M = 3.01, SD = 2.54; r(2) = 0.47, p < 0.001) (Figure 3). In addition, there was also a significant increase in the average occurrence of aggression per minute during sessions when food was present (Mdn = 5.1, SD = 2.90) than when food was not present (Mdn = 1.6, SD = 1.24; Z = 6.84, p < 0.001), as indicated by Wilcoxon signed rank test (Figure 4).

The best-fit model for negative reaction, carrying a cumulative model weight of 0.60, included four parameters with significant effect: visitors (drop1: F-value = 5.060, Pr value = 0.028; LMM: estimate effect = -0.079, t = -2.831, p = 0.007), negative interaction (drop1: F-value = 7.432, Pr value = 0.009; LMM: estimate effect = 0.256, t = 2.519, p = 0.015), touch (drop1: F-value = 366.024, Pr value < 0.001; LMM: estimate effect = 0.583, t = 19.327), and feed occurrence (drop1: F-value = 4.570, Pr value = 0.037; LMM: estimate effect = 0.245, t = 2.267, p = 0.027) (Table 3). Spearman's correlation test showed a significant positive correlation between average visitors per minute (M = 2.22, SD = 2.11) and average number of negative reactions per minute (M = 1.28, SD = 1.30; r(2) = 0.56, p < 0.001) (Figure 5). There was also a significant positive correlation between negative interactions (M = 0.41, SD = 0.56) and average

negative reactions per minute (M = 1.28, SD = 1.30; r(2) = 0.73, p < 0.001) (Figure 6). In addition, there was a significant positive correlation between touch (M = 2.03, SD = 2.03) and average negative reactions per minute (M = 1.28, SD = 1.30; r(2) = 0.96, p < 0.001) (Figure 7). Lastly, there was a significant difference in average occurrence of negative reactions when there was food present (Mdn = 1.95, SD = 1.33) versus when there was no food present (Mdn = 0.70, SD = 1.23; Z = -5.04, p < 0.001), as indicated by the Wilcoxon signed-rank test (Figure 8).

The best-fit model for positive reactions, carrying a cumulative model weight of 1.00, showed a significant effect of touch (drop1: F-value = 34.231, Pr value < 0.001; LMM: estimate effect = 0.104, t = 6.513, p < 0.001) (Table 3). There was a significant positive correlation between touch (M = 2.03, SD = 2.03) and the average positive reaction per minute (M = 0.26, SD = 0.33; Spearman r(2) = 0.67, p < 0.001) (Figure 9). The best-fit model for proximity, carrying a cumulative model weight of 1.00, included two parameters of significant effect: experimental condition (drop1: F-value = 32.748, Pr value < 0.001; LMM: estimate effect = -0.040, t = -7.150, p <0.001) and feed occurrence (drop1: F-value = 27.602, Pr value < 0.001; LMM: estimate effect = 0.054, t = 5.715, p < 0.001) (Table 3). However, there was no significant difference in the percentage of rays proximate to visitors per minute during observations when food was present (Mdn = 0.12, SD = 0.06) than when food was not present (Mdn = 0.09, SD = 0.04; Wilcoxon signed rank test, Z = -1.00, p = 0.316).

Comparison Between Retreat Area and No Retreat Area

The best-fit linear mixed model for aggression showed there was a significant effect of experimental condition. There was a significant increase in average aggression per minute from the control condition (Mdn = 1.80, SD = 1.38) to the retreat condition (Mdn = 2.80, SD = 1.75; Z = -2.09, p = 0.037) (Figure 10), as indicated by Wilcoxon signed rank test. There was also a

significant decrease in average negative reactions to visitor interaction from the control condition (Mdn = 1.60, SD = 1.08) and the retreat condition (Mdn = 0.60, SD = 1.05; Z = -2.19, p = 0.030) (Figure 11). In addition, there was also an increase in positive reactions to interaction from the control condition (Mdn = 0.10, SD = 0.25) to the retreat condition (Mdn = 0.20, SD = 0.31; Z = -0.50, p = 0.616), but this difference was not significant as indicated by Wilcoxon signed rank test. In addition, despite the fact there was a retreat area in the back of the pool, the average percentage of rays in the visitor area remained significantly higher (Mdn = 0.18, SD = 0.13), than the average percentage of rays in the experimental area (Mdn = 0.08, SD = 0.07; Z = -3.64, p < 0.001) (Figure 12). This corresponds to the best-fit linear mixed model for proximity that showed a significant effect of experimental condition.

Comparison Between Regulated Interactions and No Regulation

Total aggression increased from the control condition and the retreat condition to the limited access condition. However, a Wilcoxon signed rank-test did not indicate a significant difference between the average aggression per minute between the control condition (Mdn = 1.80, SD = 1.38) and the limited access condition (Mdn = 3.20, SD = 3.65; Z = -1.88, p = 0.061), or a significant difference in average aggression per minute between the retreat condition (Mdn = 2.80, SD = 1.75) and the limited access condition (Mdn =3.20, SD = 3.65; Z = -0.05, p = 0.958). Negative reactions also decreased from the control condition to the limited access condition, but there was no significant difference in average negative reactions per minute from the control condition (Mdn = 1.60, SD = 1.08) and the limited access condition (Mdn = 0.20, SD = 1.61; Z = -1.53, p = 0.126). In contrast, average negative reactions per minute increased from the retreat condition (Mdn = 0.60, SD = 1.05) to the limited access condition (Mdn = 0.20, SD = 1.61; Z = -0.48, p = 0.629), but again, this difference was not significant. There was also no significant

difference between average positive reactions to interaction per minute in the control condition (Mdn = 0.10, SD = 0.25) and the limited access condition (Mdn = 0.00, SD = 0.40; Z = -0.99, p = 0.364) despite an increase in positive interactions from the control condition to the limited access condition. Similarly, there was significant difference between in the average number of positive reactions between the retreat condition (Mdn = 0.10, SD = 0.31) and the limited access condition (Mdn = 0.00, SD = 0.40; Z = -0.26, p = 0.796).

There was also no significant difference in average negative interactions from the control condition (Mdn = 0.50, SD = 0.53) to the limited access condition (Mdn = 0.00, SD = 0.51; Z = -1.92, p = 0.055). However, there was a significant decrease in average touch count per minute from the control condition (Mdn = 2.70, SD = 1.85) to the limited access condition (Mdn =0.20, SD = 2.31; Z = -2.10, p = 0.035) (Figure 13), which means both forms of interaction decreased when interaction was regulated, though only one was significant. In addition, there was a significantly greater percentage of rays proximate to the visitor area in the control condition (Mdn = 0.14, SD = 0.06) compared to the percent proximate in the experimental area in the limited access condition (Mdn = 0.07, SD = 0.03; Z = -3.60, p < 0.001) (Figure 14). There was also a significantly greater percent of rays proximate to the visitor area in the retreat condition (Mdn = 0.11, SD = 0.04) compared to the percent proximate in the experimental area in the limited access condition (Mdn = 0.07, SD = 0.03; Z = -3.34, p < 0.001) (Figure 14).

Comparison Between Ray Species

R. bonasus was involved in an overwhelming majority of visitor interactions over all three conditions of this study. Out of 1,477 total interactions recorded, 1,456 (98.57%) of them involved *R. bonasus*. In contrast, *H. say* was only involved in 30 interactions (0.02%), and *H. sabina* was only involved in 1 interaction (< 0.001%). In addition, there were significantly more

aggressions from *R. bonasus* (Mdn = 1.60, SD = 1.98) compared to the other three species in the touch pool combined (Mdn = 0.50, SD = 0.72, U = 7964, $p < 0.001$) (Figure 15), as indicated by Mann-Whitney U test. *R. bonasus* also had significantly higher proximity percentages to visitors in both observation areas (Mdn = 0.22, SD = 0.07) than the other rays combined (Mdn = 0.07, SD = 0.06, W = 3754.5, $p < 0.001$) (Figure 16). In terms of the effect of food on aggression, there was a significant increase in the average occurrence of aggression per minute for *R. bonasus* when food was present (Mdn = 4.15, SD = 2.25) than when food was not present (Mdn = 1.10, SD = 0.88; Z = -6.84, $p < 0.001$) (Figure 17). This increase in aggression was also observed with the other three “flat-body” species when food was present (Mdn = 0.80, SD = 0.97) than when food was not present (Mdn = 0.50, SD = 0.46; Z = -6.74, $p < 0.001$) (Figure 18).

IV. DISCUSSION

Visitor presence and behavior has a significant effect on both aggression and negative reactions towards visitors for rays housed in an aquatic touch pool. While the hypothesized effects of the experimental conditions were not observed, it did demonstrate that management decisions like providing retreat spaces or regulating visitor contact may not be effective in reducing the negative impacts of visitor interaction. This is especially true if visitors are providing food and may be potentially contributing to aggressive interference competition. In addition, while many touch pools are mixed species exhibits, the results show that species can behave significantly different from each other in terms of aggression and engagement with visitors.

This study provides support for the behavioral assessment of welfare for animals involved in visitor interaction programs for visitors, especially species that are exposed to frequent and intense physical contact. Additionally, it shows the importance of collecting empirical data to determine if perceived improvements to welfare are actually effective at achieving desired effects. As the science of animal welfare continues to advance, it is important to consider that while interactive experiences may be enriching for visitors, there is more research needed to demonstrate that positive welfare outcomes for animals can also be achieved.

Visitor density and interaction in touch pool experiences may be predictors for ray behaviors associated with negative welfare

Overall, there were several significant relationships between visitor behavior and ray behavior. It was expected that higher numbers of visitors and amounts of interaction, and more intense forms of interactions would result in higher rates of undesirable behaviors from the ray. This included behaviors such as aggression, startles, and withdrawals from visitor interactions. There was a significant positive correlation between the average number of touch interactions

per minute and the average number of aggressive ray behaviors per minute. This suggests that higher frequencies of interaction may lead to more aggression among the rays. There was also a significant positive relation between the average number of touch interactions per minute and the average number of negative reactions from rays. Negative reactions included startled responses to contact with visitors, as well as withdrawal from contact. This suggests that higher frequencies of interaction may also lead to more behaviors associated with fear and stress from rays. In a study conducted with zoo education animals, Baird et al. (2016) also found that a large amount of handling during interactive programs led to undesirable behavior among armadillos (*Chaetophractus vellerosus*, *Dasypus novemcinctus*, *Euphractus sexcinctus*, and *Tolypeutes matcus*), as well higher levels of fecal glucocorticoids in armadillos (*Chaetophractus vellerosus* and *Tolypeutes matcus*), African hedgehogs (*Atelerix albiventris*), and red-tailed hawks (*Buteo jamaicensis*). Thus, the amount of handling may have significant implications on zoo animal welfare, if higher amounts of visitor interaction can lead to greater instances of aggression and stress from animals involved in interactive experiences.

Negative reactions from the rays also had a significant positive correlation with both the average number of visitors per minute and the average number of negative interactions from visitors per minute. Large crowd sizes have also been linked to high occurrences of negative behavior in species housed in traditional exhibits (Carlstead et al, 1991; Sellinger and Ha, 2005; Fernandez et al., 2009; Soriano et al., 2013), and research has also demonstrated that intense behavior from humans can lead to stress and fear responses (Broom, 1986; Broom, 2001; Fernandez et al., 2009). In addition, Anderson et al. (2002) found that a higher density of visitors in a petting zoo was correlated to higher rates of both aggressive and stress-related behavior in both African pygmy goats (*Capra hircus*) and Romanov sheep (*Ovis aries*). This study

demonstrates that rays in a touch pool may be more likely to respond negatively towards visitors when there are large crowds and there is a higher frequency of interactions, regardless of whether the interactions are "gentle" or "rough." Though considering that negative interactions can occur, which may lead to injury among the rays as has been previously suggested (Casamitjana, 2004), this study also provides support that visitor interactions should be closely monitored as a part of any zoo or aquarium's management strategy for their touch pool.

The occurrence of positive reactions to visitor interactions suggests the potential for a positive relationship between rays and visitors

However, the results also showed a significant positive correlation between the average number of touch interactions per minute and the number of positive reactions from rays, suggesting that higher frequencies of interactions can also lead to more affiliative responses from the rays, not just stress responses. For example, positive reactions involved rays swimming up to visitors and assuming a stationary position with their heads pointed towards the surface in the direction of visitors' hands. There appeared to be no objective or goal to this behavior, other than providing a temporary halt to normal swimming patterns to engage in contact with visitors. This provides evidence that positive interactions are possible between human visitors and rays in a touch pool experience, which has not yet been demonstrated by any previous studies (Biasetti et al., 2020).

Yet because negative reactions were much more common in response to desirable "touch" forms of interaction, more research is necessary to determine 1) if certain types of "touch" behaviors are preferred by the rays and 2) if individual rays are more likely to respond positively or negatively to touch interactions. Research has shown gentle petting can lead to positive associations between animals and humans (Hemsworth, 2003). For example, Pederson et al. (1998) found that plasma cortisol levels were significantly lower in pregnant sows that

received gentle petting or stroking than sows that experienced neutral or negative handling, like being shocked by a battery operated goader/prodder. While it is common for zoos and aquariums to ask visitors to interact with animals in touch pools in a certain way (e.g., two-finger touch), there is no research evidence that suggests rays or other elasmobranchs prefer this type of interaction over others. Furthermore, individual differences within a species – such as temperament and previous experiences with humans – have the potential to influence how an animal responds to human interaction. For some individuals in a species, it possible that visitors can either be a source of stress or a source of stimulation (Sherwen and Hemsworth, 2019). However, while researchers have highlighted that an individual's temperament can have a great influence on zoo population management and social cohesion, there are few studies that have attempted to understand how an individual animal's temperament may affect its response to human visitors. In a study on Western lowland gorillas (*Gorilla gorilla gorilla*) living in a zoo environment, Stoinski et al. (2012) found a trend toward variation in response to crowd size as a function on individual personality ratings of extroversion, dominance, fearfulness, and understanding. Thus, research into understanding HARs at the individual level may be critical for the management of animals involved in close encounter experiences, including elasmobranchs.

The role of food competition in touch pools and its potential effects on elasmobranch welfare

The purpose of the two experimental conditions was 1) to limit where interactions could take place around the pool and 2) to limit the type of interactions that visitors could perform. It was predicted that these experimental conditions may increase the occurrence of positive reactions to contact from the rays while also reducing the negative behaviors from the rays. Interestingly, there was a higher percentage of positive interactions in the control condition

compared with to the retreat condition and the limited access condition. Yet because the total number of interactions decreased during the experimental conditions, there was a higher percentage of negative reactions in the retreat condition and the limited access condition compared to the control condition. Average aggressions per minute also significantly increased from the control condition to the retreat condition, and while it was not significant, average aggression per minute also increased from the control condition to the limited access condition. In terms of proximity, there was a significantly higher percentage of rays proximate in the visitor area than the experimental area where interactions were not occurring. Also, there was a significantly higher percentage of rays proximate to the Visitor Area in both the control condition and the retreat condition compared rays proximate to the Experimental Area in the limited access condition, when the interactions were being regulated.

This data suggests that the experimental conditions were not effective in achieving the predicted responses from the rays. However, there is a potential explanation for why this occurred. The linear mixed model analysis showed that feeding occurrence had a significant effect on both aggression and negative reactions to visitors. Further there was a significant increase in the average aggressions and negative reactions per minute when food was present, demonstrating that food presence can lead to more aggression and negative reactions from the rays. During the control condition, there were 14 recorded feedings. Then during the experimental conditions, feedings increased to 24 (the retreat condition) and 34 (the limited access condition). Thus, it appears that as feedings increased during each condition, the number of aggressions and the percentage of negative reactions also increased. Research has shown that elasmobranchs quickly learn the location of feedings areas (Sabalones, 2004), which may explain why a greater percentage of rays were proximate to the visitor area compared to the experimental

area during the retreat condition of this study. In addition, other studies of elasmobranchs in captivity have shown an increase in aggressive behaviors both before and during feeding events, which has been attributed to resource competition. For example, Murphy et al., 2019 found that blue-spotted ribbontail rays (*Taeniura lymma*) and blue-spotted maskrays (*Neotrygon kuhlii*) exhibited more aggression after training events, which may have been due to the anticipation of daily feeding, which occurred right after feeding. Intense competition can also lead elasmobranch to avoid feeding areas in response to more aggressive individuals, which has been observed in nurse sharks (*Ginglymostoma cirratum*) in which large individuals arrive first at the feeding area and use their strength to keep other sharks behind or beneath them (Sabalones, 2004).

Even in the wild, agonistic behaviors can also be observed among rays competing for food. Previous research at the Cayman Islands has shown that tourist feeding has altered the behavior of *Hypanus americanus (*southern stingray) to the point that, in comparison to rays from non-tourist sites incur more injuries and participate in intense interference competition (Semeniuk and Rothley, 2008). It has been suggested that aggression and competition between stingrays at tourists sites may have health implications for wild populations. This includes higher chances of injury and wound infection, greater energy expenditure, elevated metabolic rates, decreased food utilization efficiency, and impaired immune function (McNamara and Buchanan 2005; Ashley, 2007). These health risks also apply to captive populations, and this study demonstrates that the effects of food provisioning during interactive experiences with animals in touch pool exhibits should be further examined. Especially due to the fact that, at least in this study, food presence did not have a significant effect on positive reactions from rays to visitors.

Species-based similarities and differences in a mixed species touch pool exhibit

Due to differences in social traits among the ray species in this study, it was predicted that *R. bonasus* would be more likely to interact with visitors compared to the other three species in the touch pool (*H. sabina*, *H. say*, and *P. lentiginosus*). The results showed that *R. bonasus* overwhelmingly participated in more interactions with visitors, thus supporting the idea that more differences in species traits, such as sociality, can affect how different species interact with human visitors. Furthermore, this study also shows evidence that more social species of elasmobranchs may be better suited to interactive exhibits than non-social species, especially considering that over 98% of all interactions involved *R. bonasus*.

However, it was also predicted that *R. bonasus* would show less aggressive behaviors than the other species. This was based on the assumption that, being a naturally social species, *R. bonasus* would exhibit greater social cohesion compared to the other species, which are naturally solitary animals. Yet *R. bonasus* showed significantly more aggressive behaviors than the other three ray species in the touch pool combined, which may be explained by several different factors. For one, *R. bonasus* outnumbered the other species in the pool by a considerable margin. They form a larger social group than the other species, and thus they may be more dominant in the social hierarchy of the touch pool. It is possible that *R. bonasus* perceive human interaction as a resource, and the aggression is the result of competition over the opportunity to interact with visitors.

In addition, aggression among *R. bonasus* was significantly higher when food was present. Research has shown that position within social hierarchy influences food intake, and aggression is often performed by higher-ranking individuals in social groups to establish dominance over resources (Harwood et al. 2003). In moderation, aggression aids in maintaining stable social groups, but it can also lead to compromised welfare if individuals are repeatedly

attacked (Krebs and Davies 1997; Turnbull et al. 1998). Because food provisioning at touch pools is done from a concentrated source, any individual positioning itself closest to visitors is more likely to receive food. In a similar situation of concentrated tourist feeding done at the Cayman Islands, Semeniuk and Rothley (2008) observed that larger *H. americana* would display "pushy" behaviors, ramming themselves into tourists with food and pursuing other rays nearby. This size-dependent, dominant behavior has also been observed at other ray feeding attractions (Newsome et al. 2004), and similar behaviors were observed by *R. bonasus* in this study when food was present, both towards conspecifics and allospecifics. This suggests that interactions and feeding may not only have the potential to affect the welfare of *R. bonasus* in a touch pool exhibit, but also other species as well.

All three of the other species in JZG's Stingray Bay are thought to be naturally less-social elasmobranchs (Snelson et al., 1988; Corcoran et al., 2013). Research in the Cayman Islands has shown potential health costs posed by "novel-grouping" and competitive aggression over a food resource in a naturally solitary forager, i.e., *H. americana* (Semeniuk and Rothley, 2008). This is especially relevant to both *H. sabina* and *H. say*, as these species are related to *H. americana*. Similar to *R. bonasus,* aggression among the three species in the JZG touch pool was significantly higher when food was present, though not as prevalent as aggression among *R. bonasus*. This provides evidence for species-based differences in aggression among elasmobranchs in captivity, which has also been demonstrated in the aggressive dominance of *T. lymma* individuals over *N. kuhlii* individuals in a captive environment (Murphy et al., 2019). Therefore, in terms of elasmobranch management in the context of a touch pool exhibit, group composition and compatibility must be an important consideration. *R. bonasus* displays different species-based behaviors compared to *H. sabina*, *H. say*, and *P. lentiginosus.* Based on the

interaction data, there is the potential for *R. bonasus* to potentially participate in positive interactions with visitors. In contrast, the other three species rarely interacted with visitors. Yet considering the higher levels of aggression exhibited by *R. bonasus,* especially in the presence of food, these other species may be avoiding interactions as a learned response to *R. bonasus* dominant aggressive behaviors. Further research should be done 1) to examine the differences in *R. bonasus* behaviors in touch pools with feeding versus no feeding, 2) to investigate how often *H. sabina, H. say*, and/or *P. lentiginosus* participate in interactions with visitors when they are in a mixed species exhibit versus when they are not in a mixed species exhibit, and 3) to evaluate if non-social species are well-suited to interactive experiences in comparison to more social species.

Research Implications

Close encounter experiences with elasmobranchs in touch pools may provide an opportunity for visitors to engage in positive interactions with rays that provide visitors with an educational benefit while also contributing positive welfare outcomes for rays. However, from a management perspective, measures should be taken to alleviate crowd size, the frequency of interactions, and the occurrence of negative interactions from visitors due to the correlation with aggression and negative responses from the rays. In addition, while feeding elasmobranchs at touch pools can be an engaging experience for visitors, there is evidence that rays will engage in intense resource competition, which may also detrimentally impact welfare (Semeniuk and Rothley, 2008; Murphy et al., 2019). This is especially true in social situations where certain species or individuals may target less dominant animals when there is competition for food. Considering that such agonistic behaviors related to interference can result in higher rates of

stress and injury, it is possible that visitor feedings could adversely affect the welfare of elasmobranchs involved in interactive experiences with visitors.

Future Aims

The existing variation of interactive touch pool exhibits across AZA institutions should be further assessed. This includes information on touch pool environmental features that promote human and animal safety, as well as the types of behaviors observed from both visitors and elasmobranchs. More research is needed to understand if management strategies to regulate visitor interactions are effective at both reducing negative welfare outcomes, as well as promoting positive welfare outcomes. Emphasis on promoting elasmobranch behaviors associated with positive welfare is particularly meaningful, as providing enriching experiences and increasing the occurrence of natural behaviors for elasmobranchs can be a challenge. There is still a lack of knowledge concerning the natural behaviors of elasmobranchs, as the constraints of observing their behavior in an ocean environment is considerably difficult. Thus, the behaviors that are considered "natural" for elasmobranchs in captivity are considered subjective and heavily debated (Murphy et al., 2019). Consistent observation, assessment, and comparison of elasmobranchs across AZA institutions will provide a better understanding of captive elasmobranch behavior, and as result, lead to a better understanding of behaviors that signify positive welfare.

To further understand the role of interactive experiences on elasmobranch behavior, and their effect on elasmobranch welfare, other factors such as seasonality, reproductive output, and exhibit complexity should also be considered and assessed. The study took place during the fall and winter months, and it is likely that season affects behavior in terms of activity, thermoregulation, and social dynamics, as well as visitor density. Being able to identify

individuals and conduct social network analysis would be a useful tool in better understanding how interactive experiences impacts conspecific interactions. In addition, the study timeline occurred outside of the reproductive season of *R. bonasus*. Aggression among *R. bonasus* is likely to increase in the spring and summer months when mating occurs (Ajeman and Powers, 2011), which has the potential to impact welfare if visitor interactions are also contributing to other forms of aggression. Furthermore, individual preference for interaction should also be considered and evaluated, as it is likely that an individual's participating in interactions with visitors is influenced by factors such genetics, temperament, tactile attraction or aversion to human contact, previous experiences with visitors (Sherwen and Hemsworth, 2019), and in the case of touch pools that provide food, hunger.

Conclusion

As zoos and aquariums continue to promote visitor engagement while also striving to promote animal welfare, the impacts of interactive experiences on animal well-being must be further studied in any species involved in close encounter experiences. This study demonstrates that higher visitor numbers, higher frequencies of interactions, intense forms of interaction, and the provisioning of food may lead to higher occurrences of negative behaviors from elasmobranchs in a touch pool experience. These results support the use of behavioral analysis to understand how visitor density and interaction impacts the behavior of elasmobranchs when they engage in interactions with visitors, and in turn, how those interactions affect the conspecific and allospecific dynamics among elasmobranchs. The evidence suggests that regulating visitor density, interaction, and feeding opportunities has the potential to reduce behaviors associated with negative welfare. However, because the experimental conditions of this study did not achieve the predicted effects, likely due to food provisioning, touch pool experiences should be

evaluated in order to determine if the implementation of various management strategies is effective in achieving desired welfare outcomes.

Table 1: Names, sexes, ages, and species at the institution displayed by ZIMS. Names denoted with * were the individuals dispositioned to another institution during the retreat condition.

Name	Sex	Age	Species
Macaroni	Male	5 Years	Hypanus sabinus
Monroe	Male	11 Years	Hypanus sabinus
Big Momma	Female	12 Years	Hypanus sabinus
Marilyn	Female	11 Years	Hypanus sabinus
Ravioli	Male	5 Years	Hypanus sabinus
Sandy	Female	11 Years	Hypanus say
Dolly	Female	12 Years	Hypanus say
Gibson	Male	14 Years	Pseudobatos lentiginosus
Little John	Male	1 Year	Rhinoptera bonasus
Floyd	Male	1 Year	Rhinoptera bonasus
Unicorn	Female	1 Year	Rhinoptera bonasus
Carson	Male	2 Years	Rhinoptera bonasus
Andre	Male	2 Years	Rhinoptera bonasus
Elton John	Male	2 Years	Rhinoptera bonasus
Benni	Male	12 Years	Rhinoptera bonasus
Jacques	Male	3 Years	Rhinoptera bonasus
Shark Bite	Male	10 Years	Rhinoptera bonasus
Lightning McQueen	Male	12 Years	Rhinoptera bonasus
Bogart	Male	12 Years	Rhinoptera bonasus
Mr. Stratchins	Male	12 Years	Rhinoptera bonasus
Patches	Male	12 Years	Rhinoptera bonasus
Stevie Ray	Male	12 Years	Rhinoptera bonasus
Hudson Hornet	Male	10 Years	Rhinoptera bonasus
Sylvia Earle	Female	5 Years	Rhinoptera bonasus
Mariah	Female	12 Years	Rhinoptera bonasus
Gilly	Female	12 Years	Rhinoptera bonasus
Spock	Female	10 Years	Rhinoptera bonasus
Tattoos	Female	12 Years	Rhinoptera bonasus
Pinky	Female	10 Years	Rhinoptera bonasus
Petunia	Female	12 Years	Rhinoptera bonasus
Ruby	Female	3 Years	Rhinoptera bonasus
Finn	Female	2 Years	Rhinoptera bonasus
Vulcan*	Female	3 Years	Rhinoptera bonasus
Tupac*	Female	4 Years	Rhinoptera bonasus
Rocky*	Male	6 Years	Rhinoptera bonasus
Smalls*	Male	4 Years	Rhinoptera bonasus
Sunny D*	Female	2 Years	Rhinoptera bonasus
Biggie*	Male	4 Years	Rhinoptera bonasus

Table 2: Behaviors measured. Ethogram adapted from Murphy et al. (2019) and ad libitum sampling at institution.

Aggression Behaviors	
Chase	A ray actively swims after another ray, following close behind them as the other ray evades interaction.
Collision	A ray swims into another moving or stationary individual, disrupting swimming pattern or displacement.
Feeding Frenzy	A group of at least four rays actively change swimming pattern, direction, and speed to engage with a visitor or keeper presenting food.
Retreat	A ray changes their swimming speed and direction to move away from another individual by at least one body length.
Visitor Interaction Behaviors	
Feed	A visitor presents food to one or more rays in the pool.
Touch	A visitor reaches into the pool and makes non-forceful contact with part of a ray's body.
Grab (Negative)	A visitor uses one or more hands to seize a part of a ray's body.
Hit (Negative)	A visitor uses one or more hands to strike at a ray.
Lift (Negative)	A visitor moves one or more hands underneath the body of a ray to raise the ray towards the surface of the water.
Splash (Negative)	A visitor uses or more hands to hit the surface of the water when a ray is in close contact.
Other	Any behavior that does not fall under any other description.
Ray Reaction Behaviors	
Affiliative (Positive)	A ray slows its swimming speed to a more stationary state and engages in prolonged contact with visitor(s).
None	An individual does not have an outwardly change its behavior and has no noticeable reaction after contact with visitor.
Dip (Negative)	A ray angles its head and body to swim downward to avoid or withdrawal after visitor contact.
Startle (Negative)	An individual suddenly changes behavior as result of contact with visitor. This includes a sudden, drastic change in body posture, swimming speed and/or swimming direction.
Proximity Count	
Distant	A ray is out of reach from visitors at the side of the pool. Distant rays were recorded in all visible areas of the pool every minute.
Proximate	A ray is within arm's reach from visitors at the side of the pool. Proximate rays were recorded in all visible areas of the pool every minute.

Table 3: Results of best-fit LMM models derived from AIC for ray behaviors of aggression, negative reaction from rays, positive reactions from rays, and ray proximity to visitors (weight = 1.00). Significant predictors ($p < 0.05$) are shown in bold.

Variable	Predicator	Estimate	SE	t-value	P
Aggression	Intercept	-0.4947	0.7189	-0.688	0.494
	Experimental Condition	0.842	0.2982	2.823	**0.006**
	Touch Count	0.422	0.1201	3.511	**0.001**
	Feed Occurrence	2.770	0.4962	5.583	**<0.001**
Negative Reaction	Intercept	0.079	0.08721	0.908	0.36851
	Visitor Density	-0.079	0.02797	-2.831	**0.007**
	Negative Interaction	0.256	0.10169	2.519	**0.015**
	Touch Count	0.583	0.03019	19.327	**<0.001**
	Feed Occurrence	0.245	0.1081	2.267	**0.027**
Positive Reaction	Intercept	0.044	0.050	0.886	0.380
	Touch Count	0.104	0.016	6.513	**<0.001**
Ray Proximity	**Intercept**	0.168	0.012	14.141	**<0.001**
	Experimental Condition	-0.040	0.006	-7.15	**<0.001**
	Feed Occurrence	0.054	0.009	5.715	**<0.001**

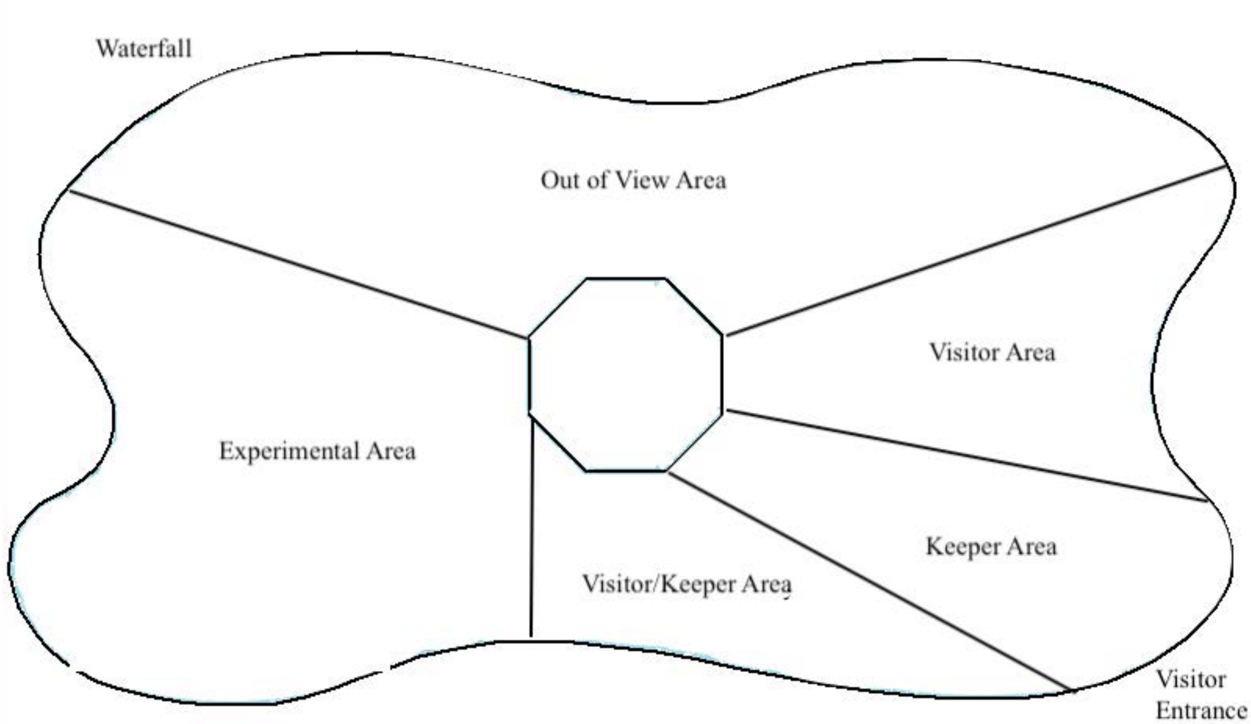

Figure 1: Jacksonville Zoo & Gardens' Stingray Bay Touch Pool. The Visitor Area shown above is where all-occurrence behaviors were recorded for the control condition and the retreat condition. It should be noted that during the control condition, visitors could access all four areas of the pool. Then, during the experimental conditions, visitors either were not able to access the Experimental Area at all, or they could only access to participate in controlled interactions. The Experimental Area shown above is where all-occurrence behaviors were recorded for the limited access condition. Ray proximity to visitors was recorded at every minute in the Visitor Area, Keeper Area, and Visitor/Keeper Area in the control condition. Ray proximity was recorded when the Experimental Area was added in the retreat condition and the limited access condition.

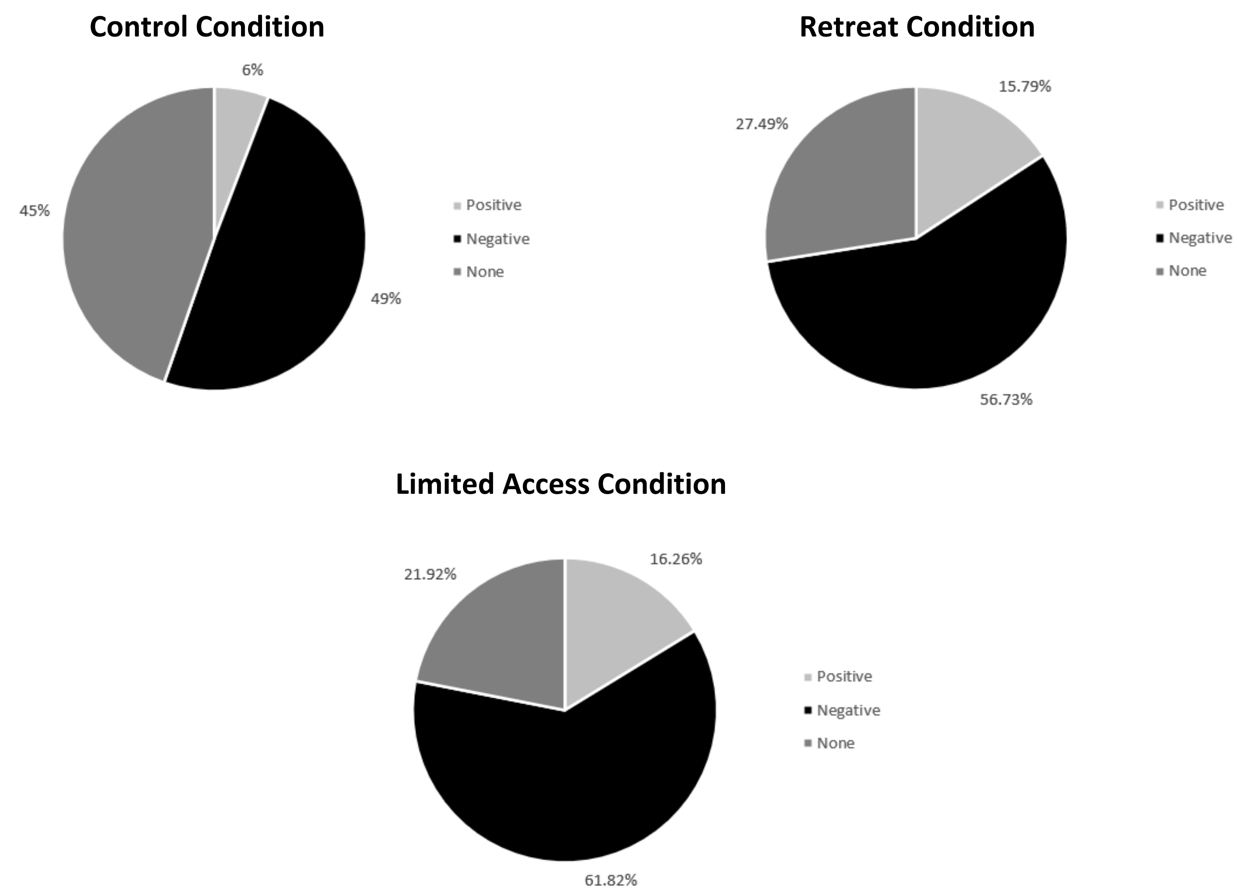

Figure 2: Reactions to visitor interaction by percentage of overall reaction type: positive, negative, and neutral.

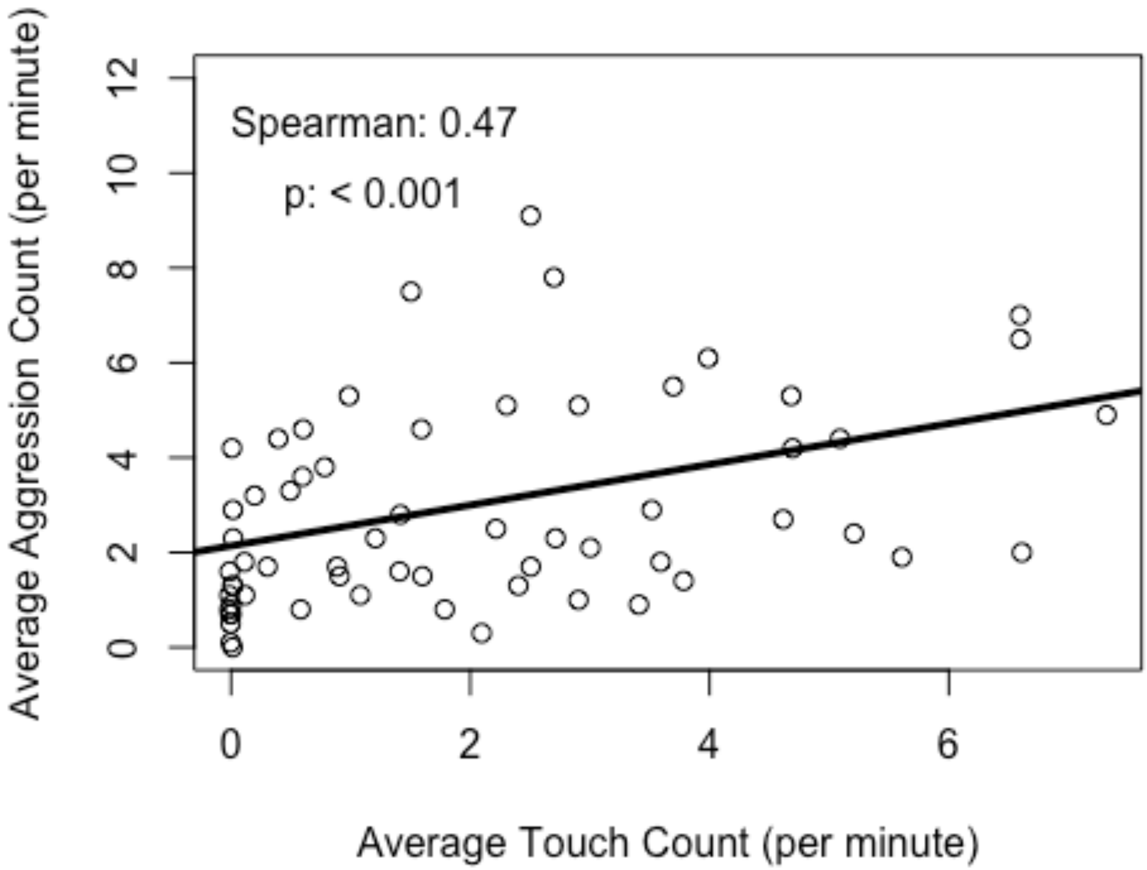

Figure 3: Correlation between average touch count (per minute) versus average aggression count (per minute) across all three conditions. Statistical relationship between the amount of touch interactions at the Jacksonville Zoo & Gardens touch pool (M = 2.03, SD = 2.03) and the amount of aggressive behaviors among rays (M = 3.01, SD = 2.54; $r(2) = 0.47$, $p < 0.001$) as indicated by Spearman's rank correlation coefficient (touch count SW = <0.001, aggression SW $p < 0.001$).

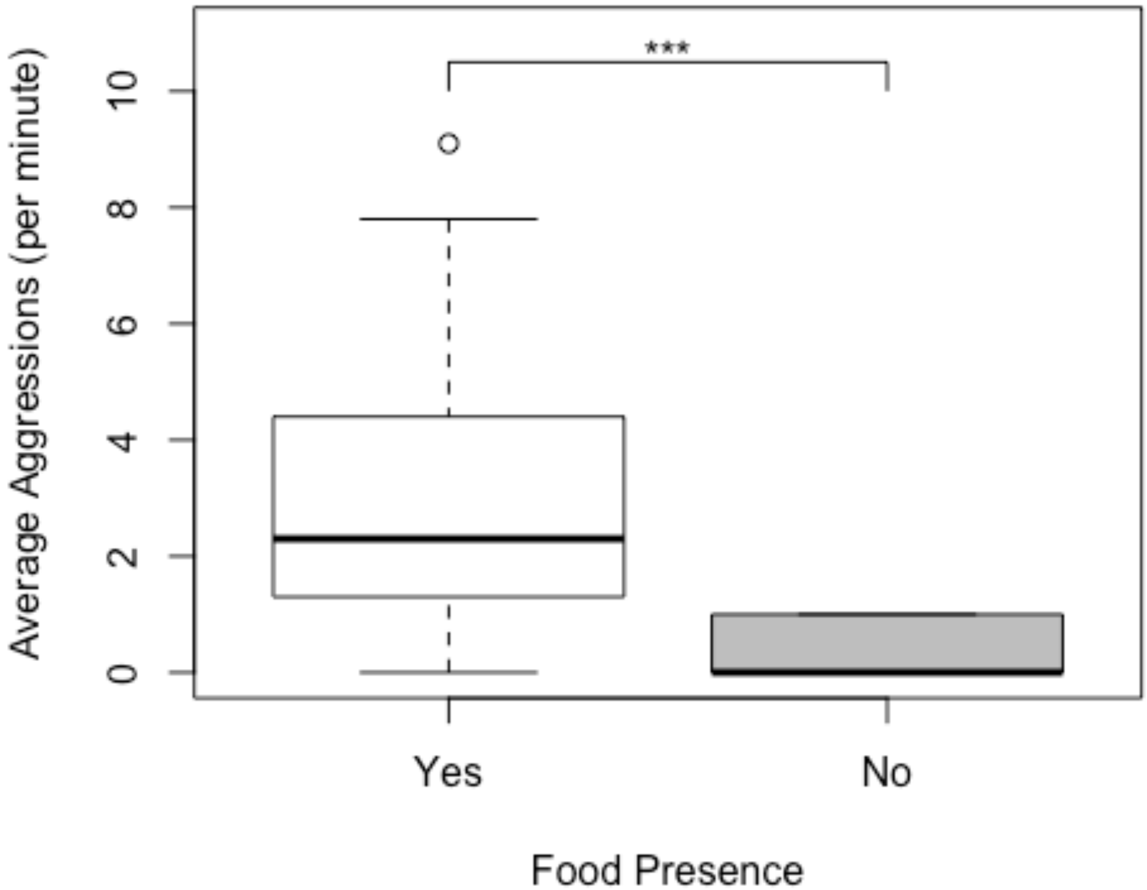

Figure 4: Difference in mean aggression (per minute) among all rays when food was present versus when food was not present. Statistical difference between the average amount of aggressive behaviors among rays when food was present (Mdn = 5.10, SD = 2.90) than when food was not present (Mdn = 1.60, SD = 1.24; Z = 6.84, p <0.001), as indicated by Wilcoxon signed rank test.

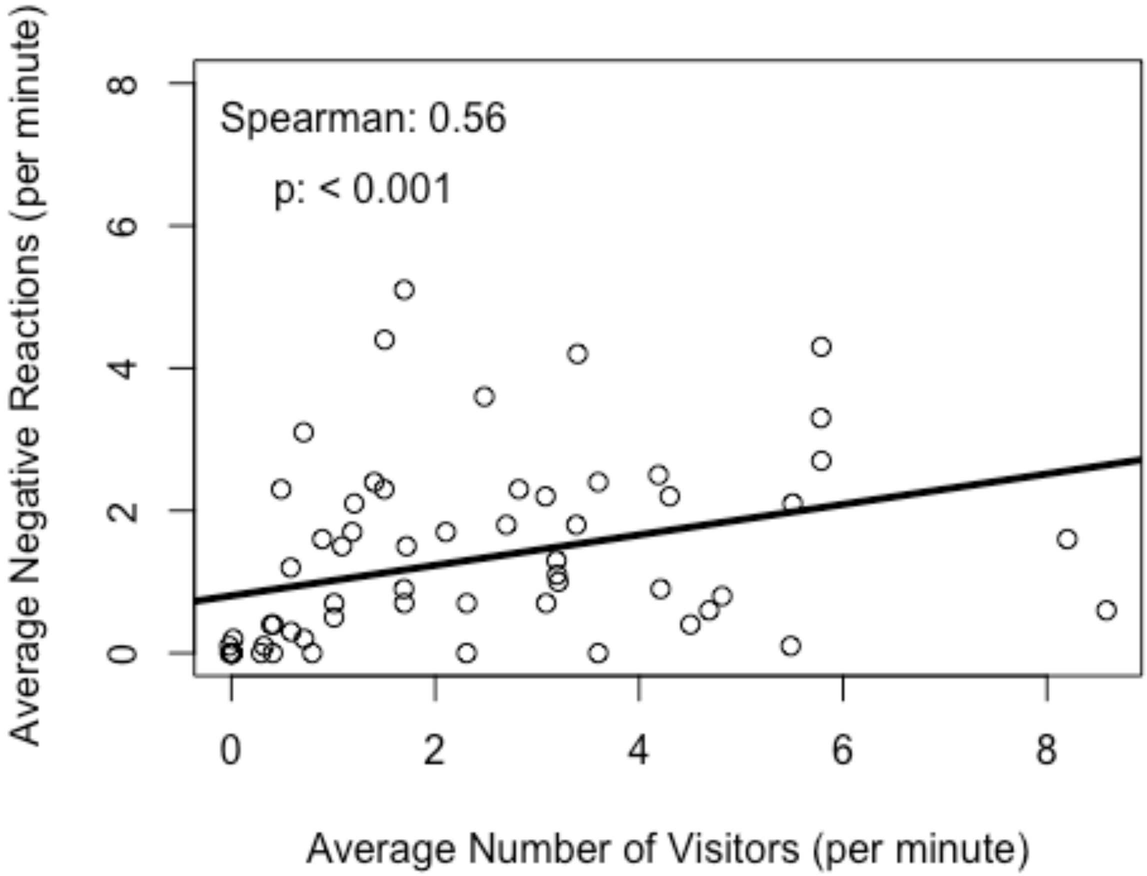

Figure 5: Correlation between average visitor density (per minute) versus average negative reactions from rays (per minute) across all three conditions. Statistical relationship between the amount of visitors at the Jacksonville Zoo & Gardens touch pool (M = 2.22, SD = 2.11) and the amount of negative reactions from rays (M = 1.28, SD = 1.30; $r(2) = 0.56$, $p < 0.001$) as indicated by Spearman's rank correlation coefficient (visitors SW = <0.001, negative reaction SW $p < 0.001$).

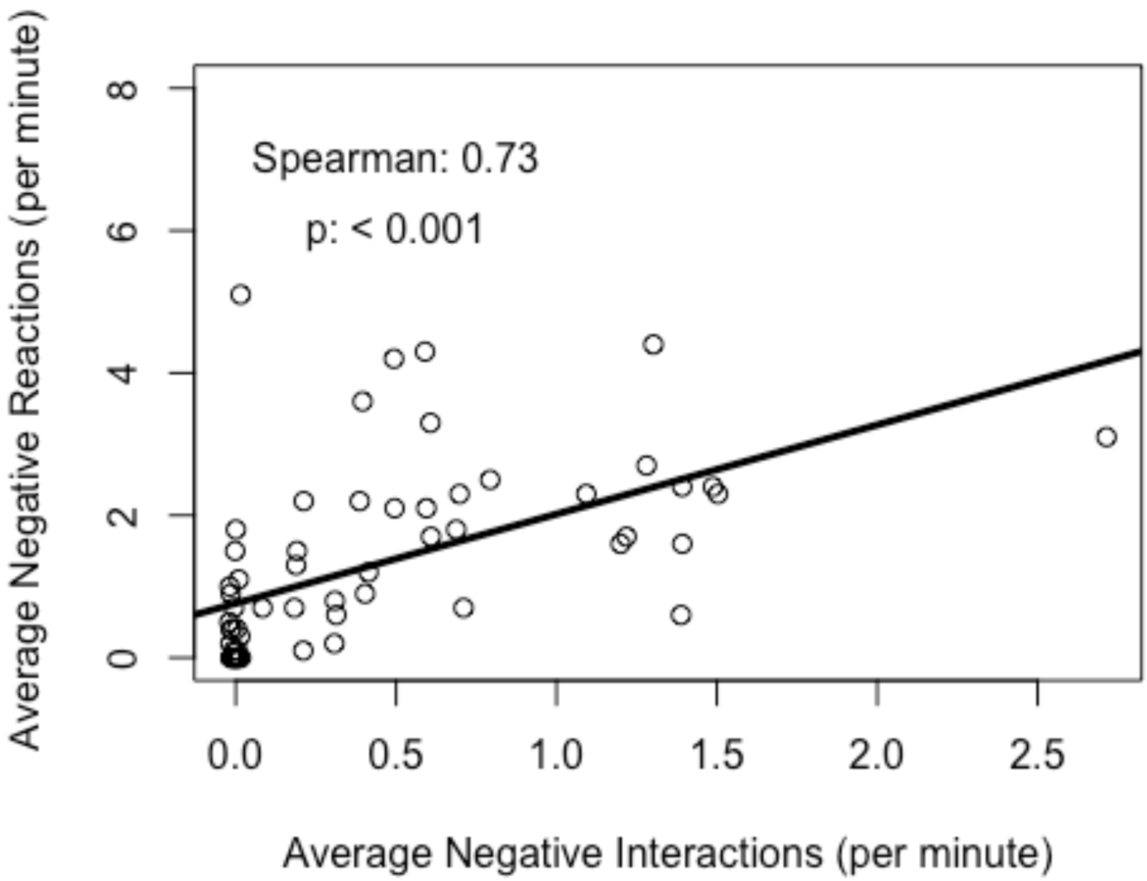

Figure 6: Correlation between average negative interactions from visitors (per minute) versus average negative reactions from rays (per minute) across all three conditions. Statistical relationship between the amount of negative interactions at the Jacksonville Zoo & Gardens touch pool ($M = 0.41$, $SD = 0.56$) and the amount of negative reactions from rays ($M = 1.28$, $SD = 1.30$; $r(2) = 0.73$, $p < 0.001$) as indicated by Spearman's rank correlation coefficient (negative interaction SW <0.001, negative reaction SW <0.001).

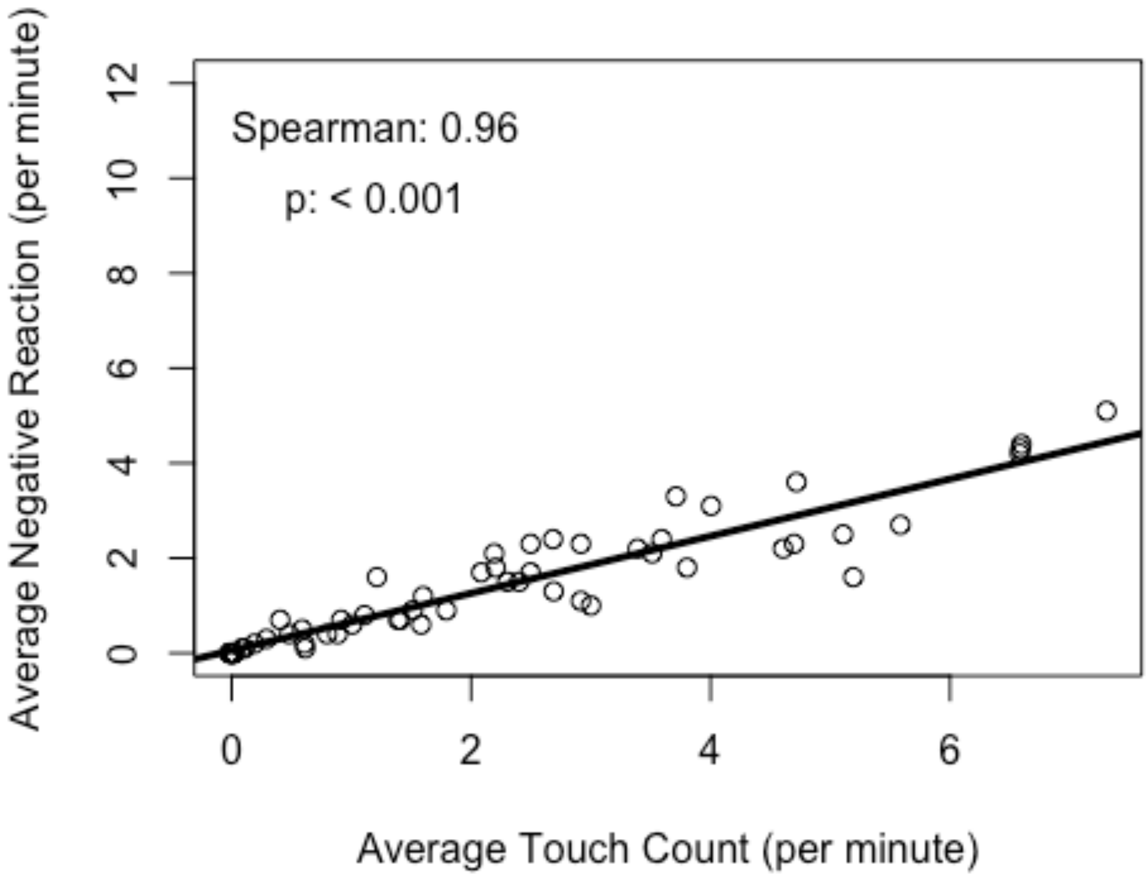

Figure 7: Correlation between average touch count (per minute) versus average negative reactions from rays (per minute) across all three conditions. Statistical relationship between the amount of touch interactions at the Jacksonville Zoo & Gardens touch pool (M = 2.03, SD = 2.03) and the amount of negative reactions from rays (M = 1.28, SD = 1.30; r(2) = 0.96, p <0.001) as indicated by Spearman's rank correlation coefficient (touch count SW <0.001, negative reaction SW <0.001).

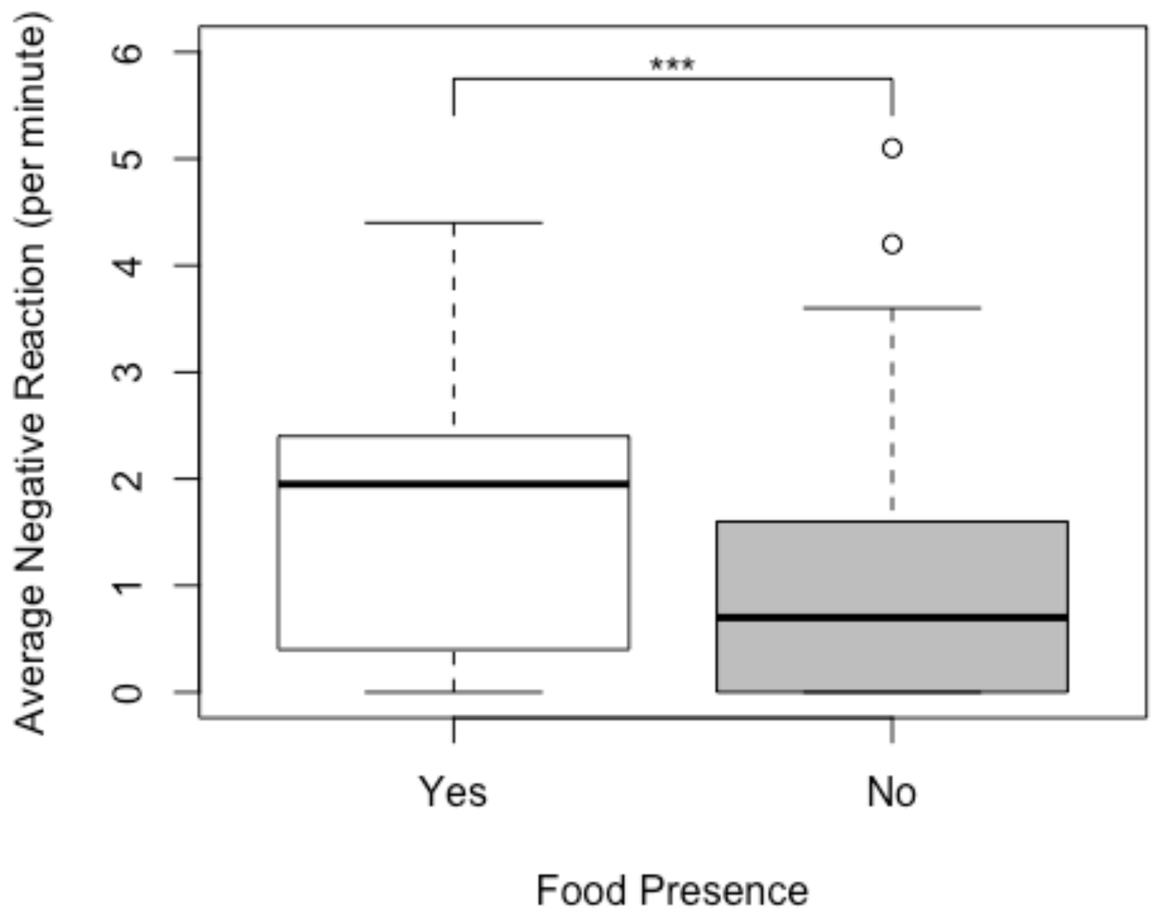

Figure 8: Difference in mean negative reactions (per minute) among all rays when food was present versus when food was not present. Statistical difference between the average amount of negative reactions behaviors among rays when food was present (Mdn = 1.95, SD = 1.33) than when food was not present (Mdn = 0.70, SD = 1.23; Z = -5.04, p <0.001), as indicated by Wilcoxon signed rank test.

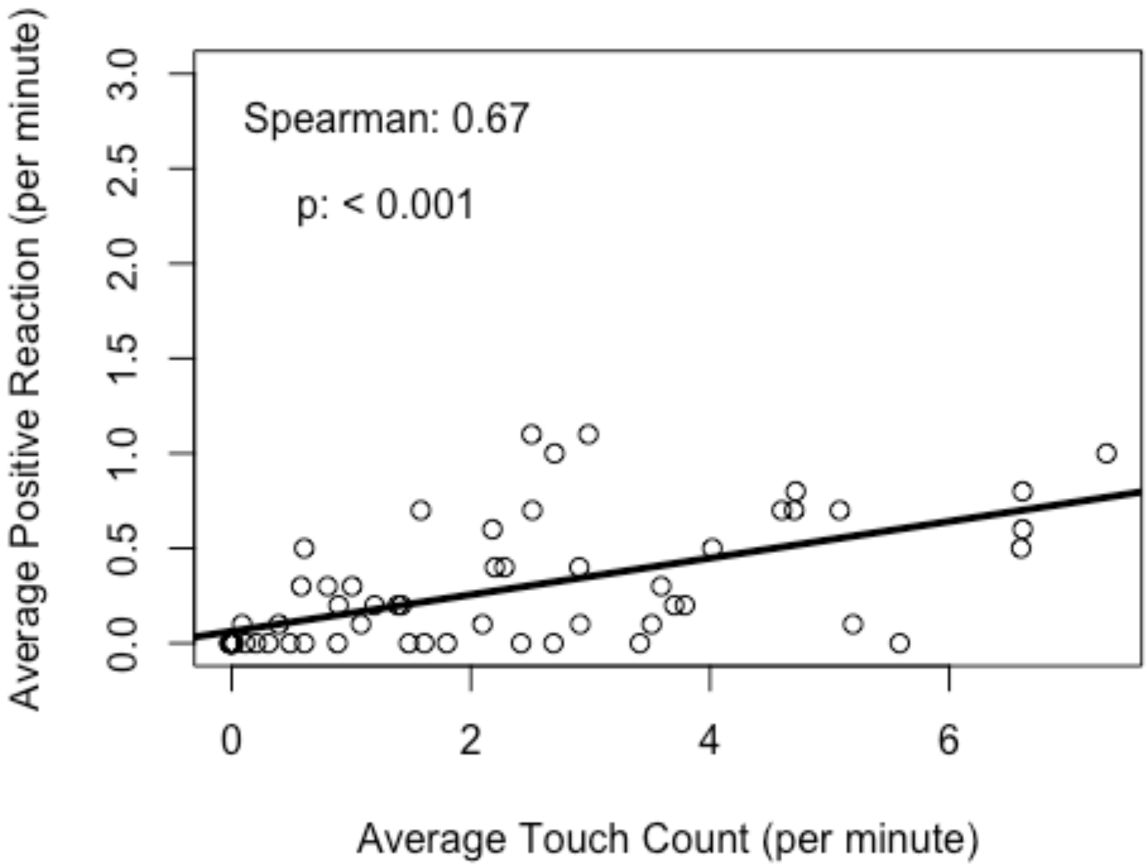

Figure 9: Correlation between average touch count (per minute) versus average positive reactions from rays (per minute) across all three conditions. Statistical relationship between the amount of touch interactions at the Jacksonville Zoo & Gardens touch pool (M = 2.03, SD = 2.03) and the amount of positive reactions from rays (M = 0.26, SD = 0.33; $r(2) = 0.67$, $p < 0.001$) as indicated by Spearman's rank correlation coefficient (touch count SW <0.001, positive reaction SW <0.001).

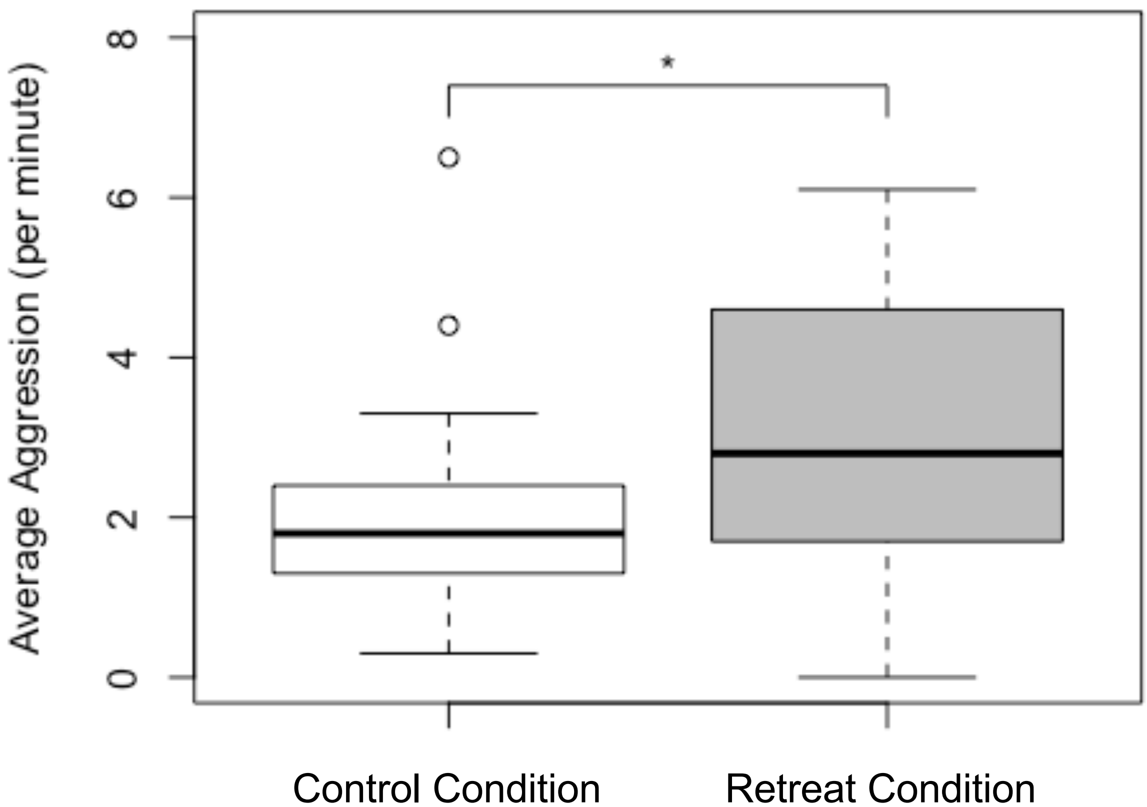

Figure 10: Difference in mean aggression (per minute) among all rays during the control condition versus the retreat condition. Statistical difference between the average amount of aggressive behaviors among rays during the control condition (Mdn = 1.80, SD = 1.38) and the retreat condition (Mdn = 2.80, SD = 1.75; Z = -2.09, p = 0.037), as indicated by Wilcoxon signed rank test.

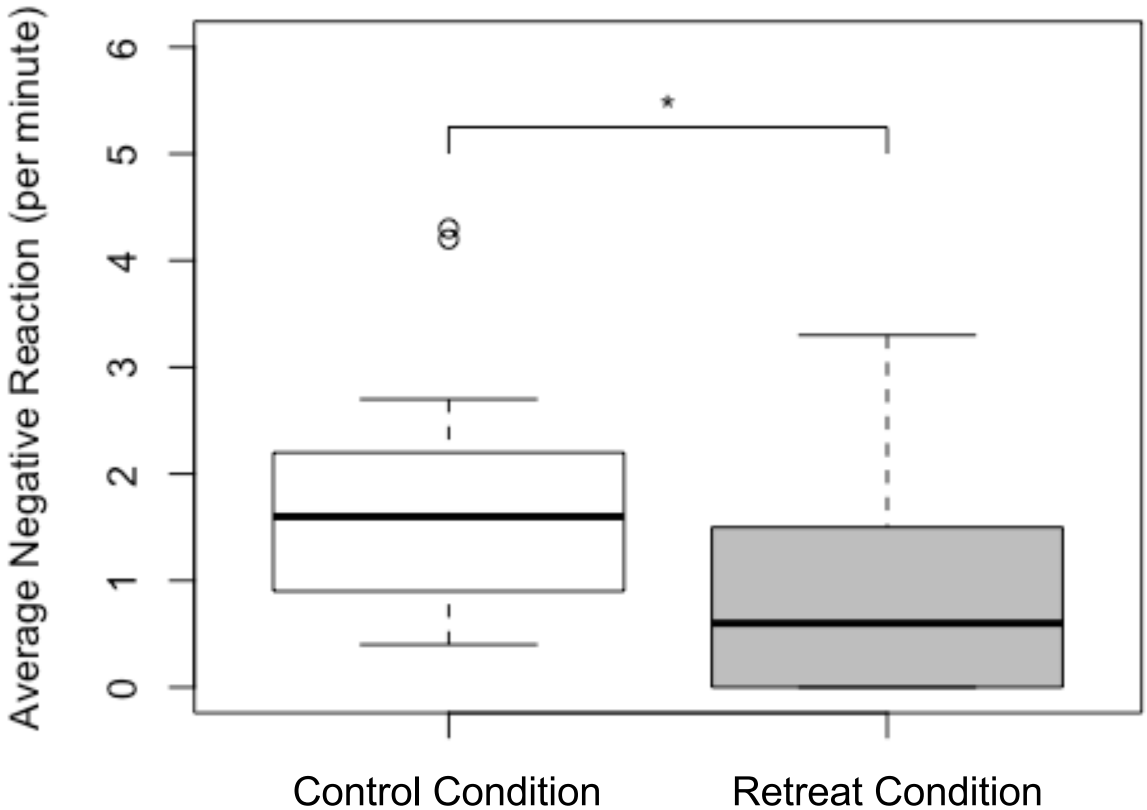

Figure 11: Difference in mean negative reactions (per minute) among all rays during the control condition versus the retreat condition. Statistical difference between the average amount of negative reactions among rays during the control condition (Mdn = 1.60, SD = 1.08) and the retreat condition (Mdn = 0.60, SD = 1.05; Z = -2.19, p = 0.030), as indicated by Wilcoxon signed rank test.

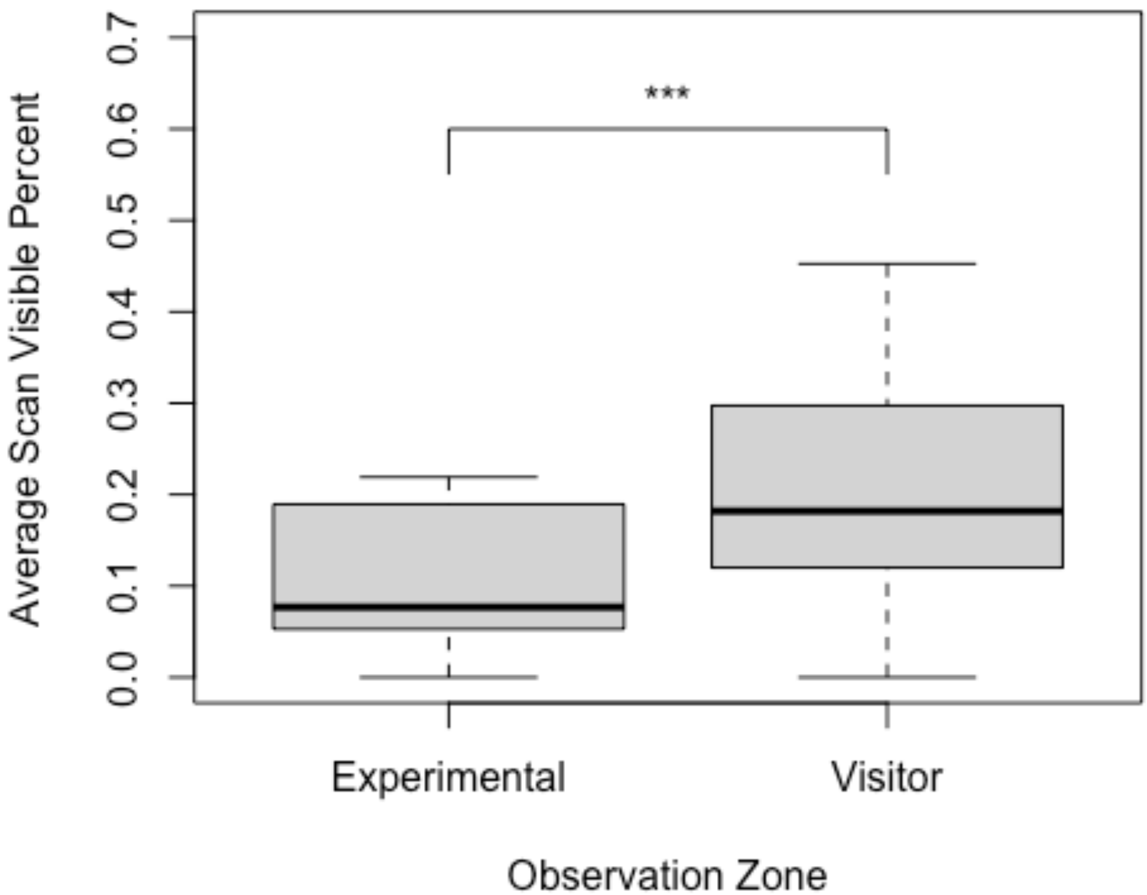

Figure 12: Difference in mean percentage of rays in visitor area versus experimental area during the retreat condition interval scans. Statistical difference between the average amount of rays in visitor area (Mdn = 0.18, SD = 0.13) and experimental area (Mdn = 0.08, SD = 0.07; $Z = -3.64$, $p < 0.001$), as indicated by Wilcoxon signed rank test.

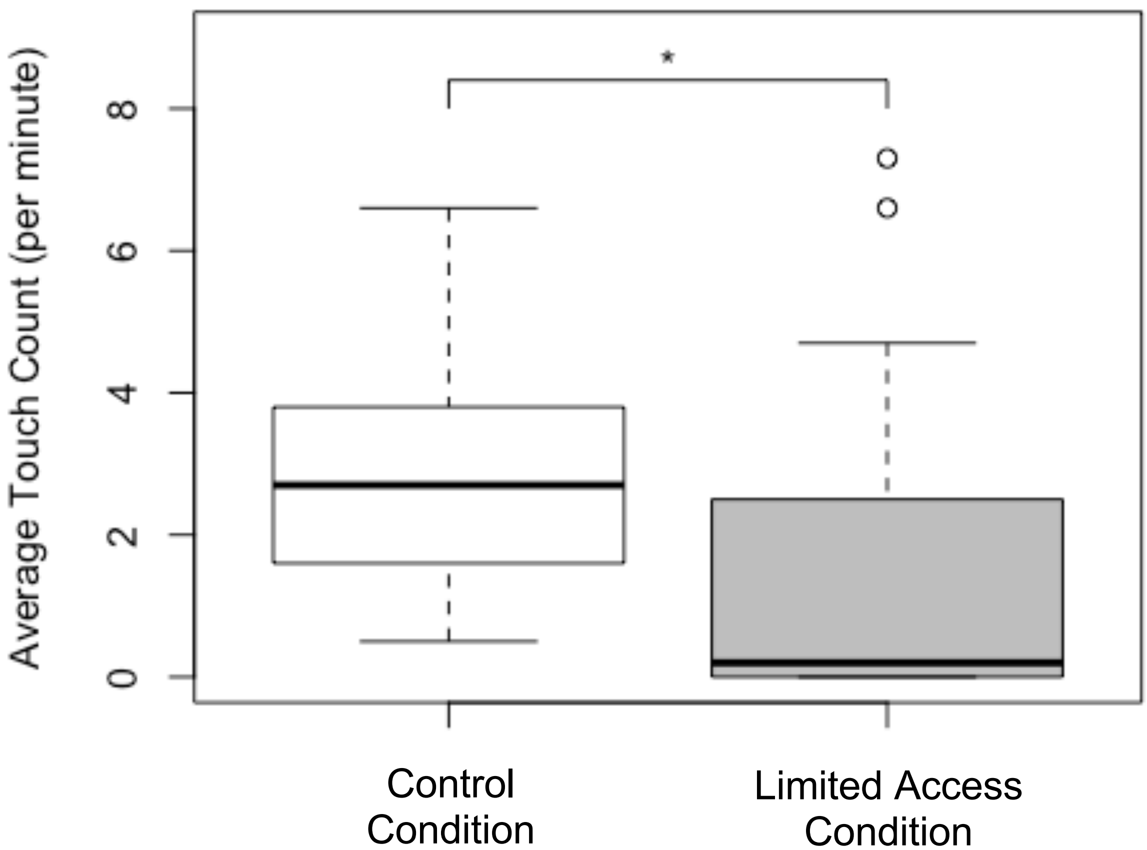

Figure 13: Difference in mean touch interactions (per minute) from visitors during the control condition versus the limited access condition. Statistical difference between the average amount of touch interactions from visitors during the control condition (Mdn = 2.70, SD = 1.85) and the limited access condition (Mdn = 0.20, SD = 2.31; Z = -2.10, p = 0.035), as indicated by Wilcoxon signed rank test.

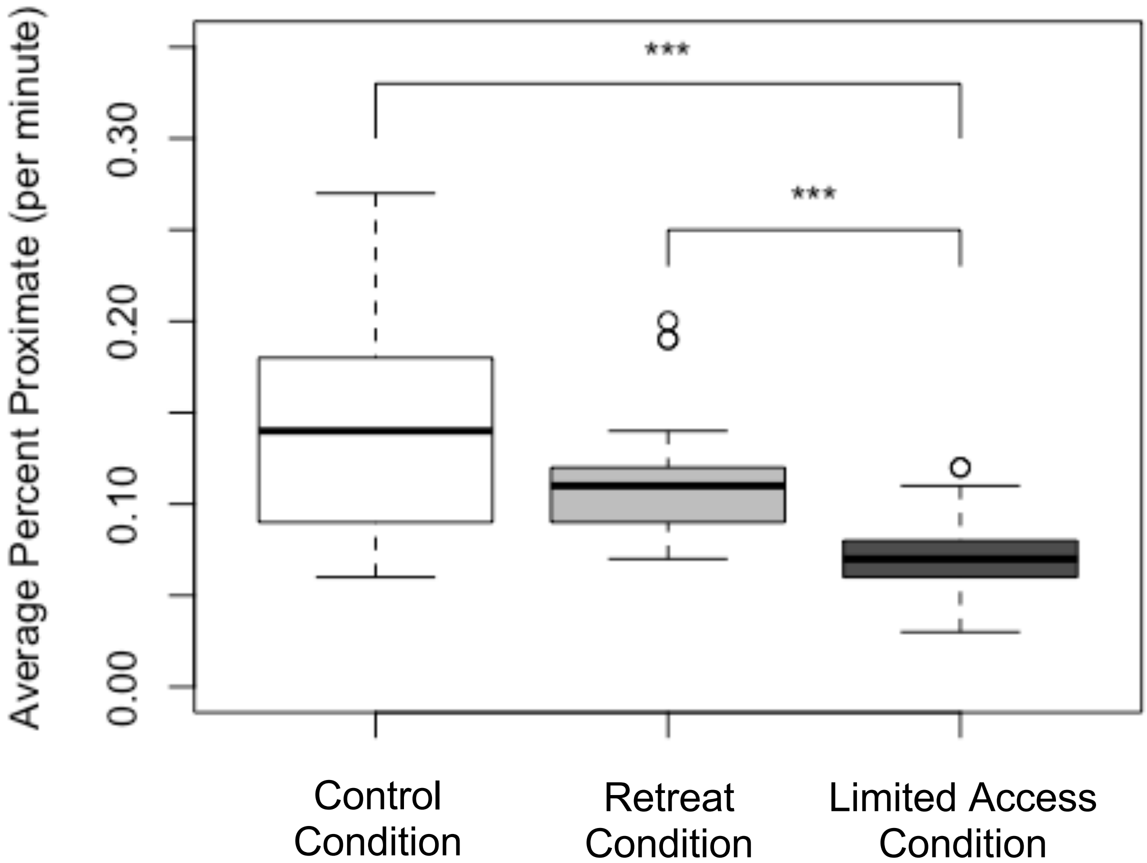

Figure 14: Difference in mean percentage of rays proximate in area of observation during the control condition, the retreat condition, and the limited access condition. Statistical difference between the average amount of rays proximate to visitors during the control condition (Mdn = 0.14, SD = 0.06) and the limited access condition (Mdn = 0.07, SD = 0.03; Z = -3.60, p <0.001), as indicated by Wilcoxon signed rank test. Statistical difference between the average amount of ray proximate to visitors during the retreat condition (Mdn = 0.11, SD = 0.04) and the limited access condition (Mdn = 0.07, SD = 0.03; Z = -3.34, p <0.001)

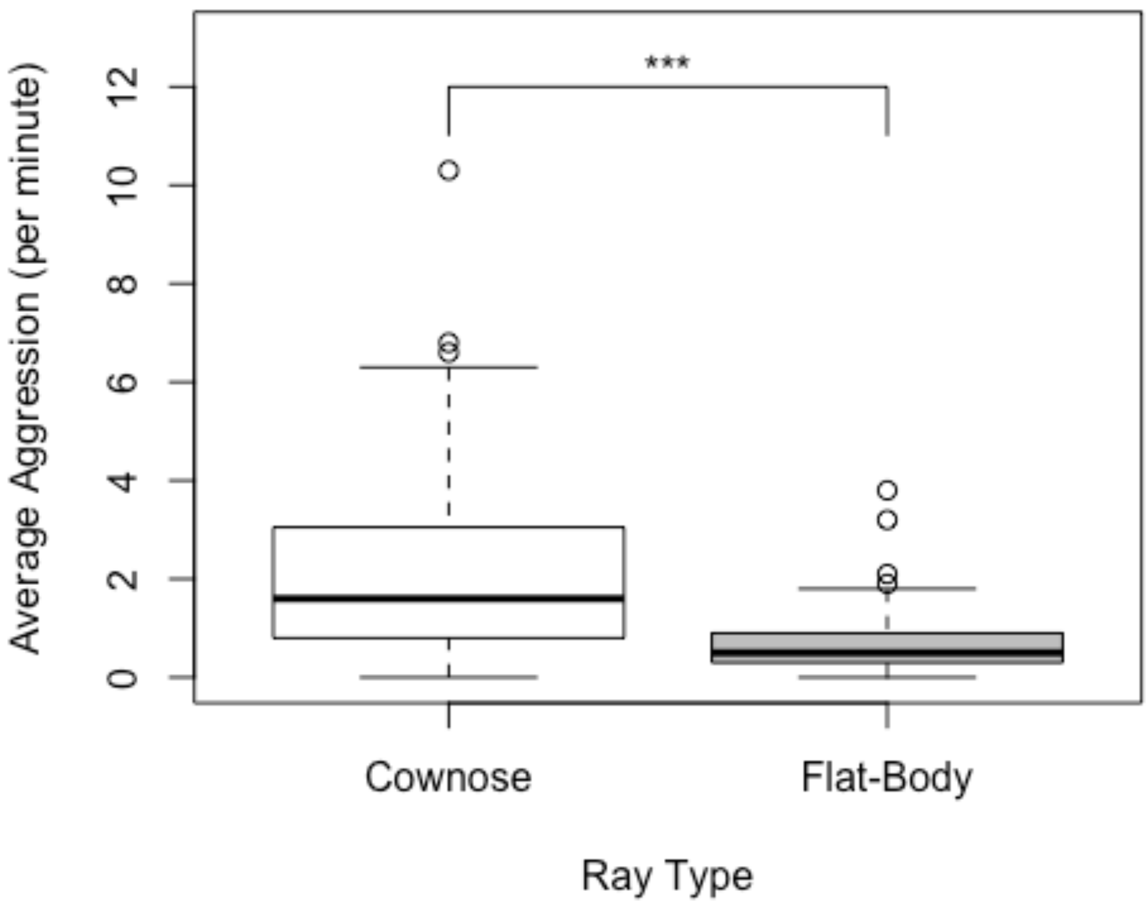

Figure 15: Difference in mean aggressions (per minute) between *R. bonasus* and the other species in the Jacksonville Zoo & Gardens touch pool. Statistical difference between the average amount of aggressive behaviors from *R. bonasus* (Mdn = 1.60, SD = 1.98) and the other three species combined (Mdn = 0.50, SD = 0.72; W = 7964, p <0.001), as indicated by Mann-Whitney U test.

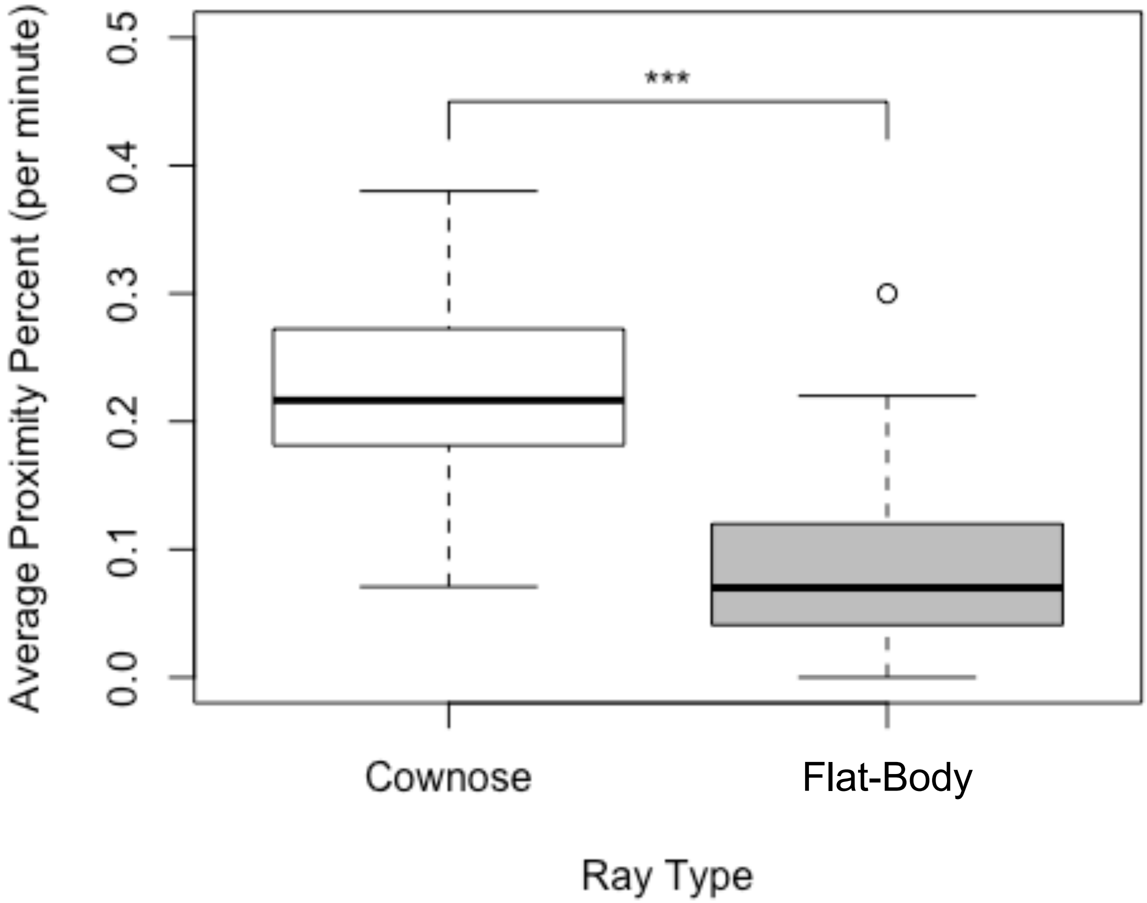

Figure 16: Difference in mean percentage of rays proximate in the observation area between *R. bonasus* and the other species in the Jacksonville Zoo & Gardens touch pool. Statistical difference between the average amount of rays proximate to visitors between *R. bonasus* (Mdn = 0.22, SD = 0.07) and the other three species combined (Mdn = 0.07, SD = 0.06; W = 3754.5, p <0.001), as indicated by Mann-Whitney U test.

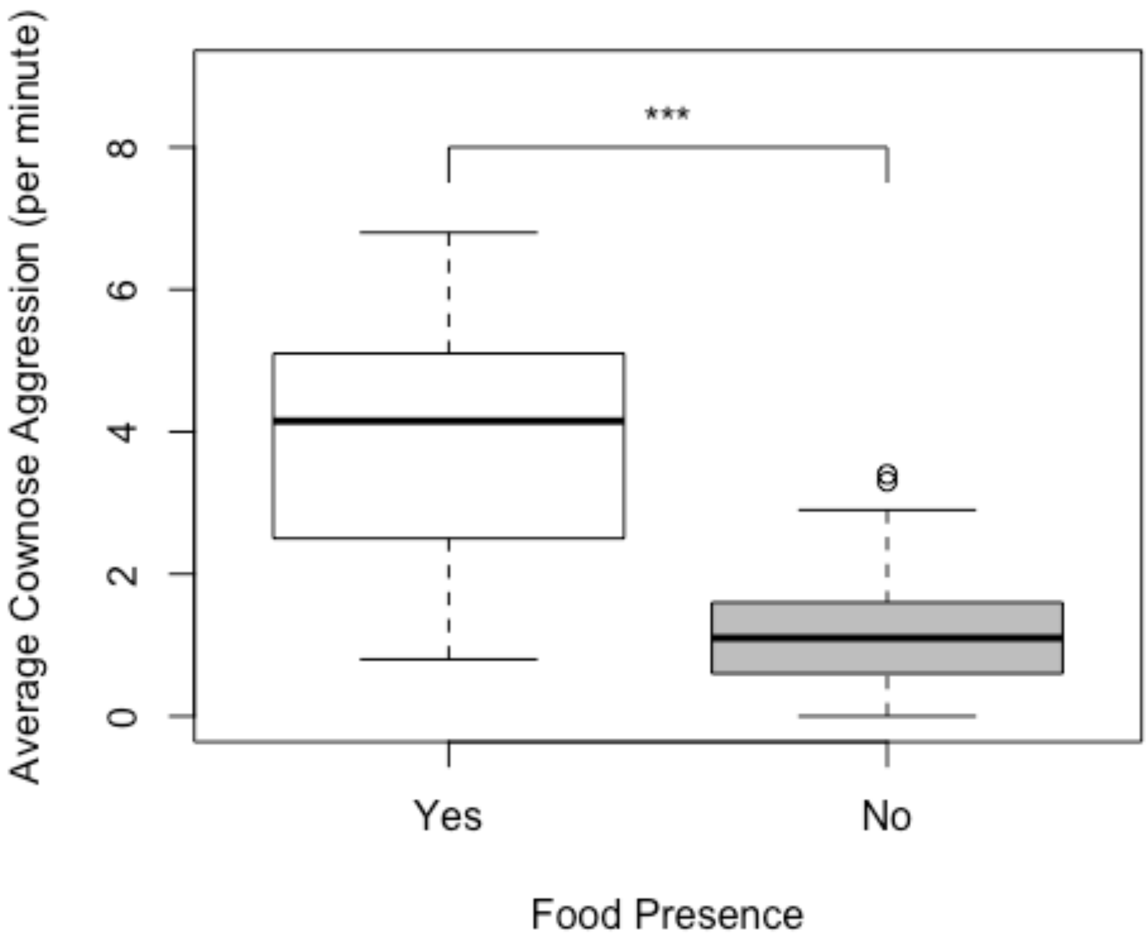

Figure 17: Difference in mean aggression (per minute) among *R. bonasus* when food was present versus when food was not present. Statistical difference between the average amount of aggressive behaviors among rays when food was present (Mdn = 4.15, SD = 2.25) than when food was not present (Mdn = 1.10, SD = 0.88; Z = -6.84, p <0.001), as indicated by Wilcoxon signed rank test.

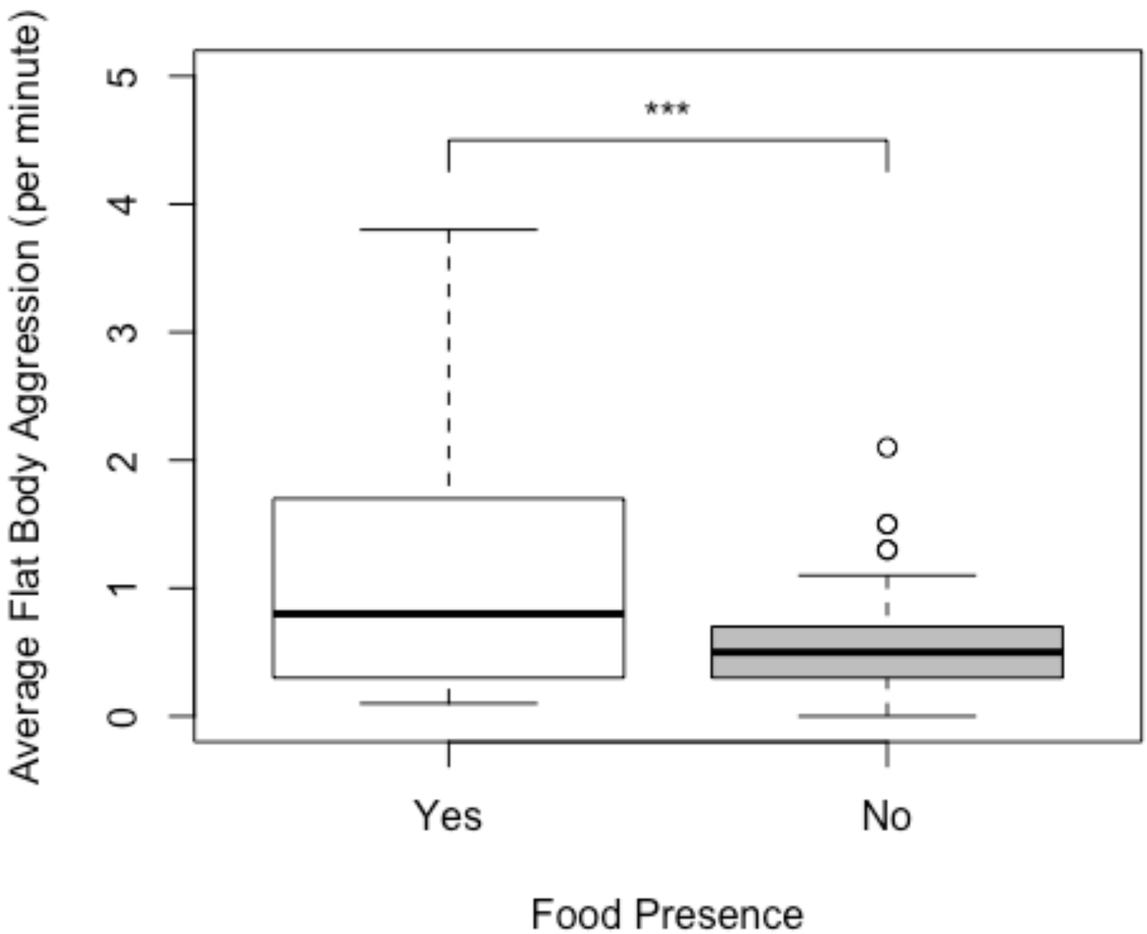

Figure 18: Difference in mean aggression (per minute) among flat-body species when food was present versus when food was not present. Statistical difference between the average amount of aggressive behaviors among flat-body rays when food was present (Mdn = 0.80, SD = 0.97) than when food was not present (Mdn = 0.50, SD = 0.46; Z = -6.74, p <0.001), as indicated by Wilcoxon signed rank test.

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