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Welfare implications of an enrichment program for two species of common aquaria fish

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Abstract

Public and academic interest into the welfare of captive animals is strong, and ever growing (Barrows, 2017). In accordance with this, standards of zoo and aquarium welfare science has thrived within recent years, with considerable resource spent assuring the lives of those in captivity are to an acceptable standard (Damasceno et al, 2017). Despite this, an understanding into the mechanisms which impact welfare, and the theoretical tools to assess it, require further specification (Barrows, 2017). This research assessed the welfare of two common aquaria fish, a population of regal tangs (*Paracanthurus hepatus*), and a population of yellow tangs (*Zebrasoma flavescens*). Concerns regarding the welfare of the two species were raised due to the prevalence of aggressive behaviour within the tabt6nk, leading to the application of an enrichment program, manipulating the number of feed locations from a single location to two distinct locations. Coding of behavioural variables indicated the success of the manipulation; however, further systematic replication is required to cement this link. The findings were applied to a framework of evolutionary game theory, culminating in a suggested alteration to the current theoretical tools to aid assessment of welfare.

Introduction

In the United Kingdom alone, it is estimated that 14% of all households have a home aquarium (Branson, 2008), contributing to a global industry worth around US$ 5-6 billion (Ploeg, 2007). Implications of the trade are vast and varied, ranging from providing financial security to coastal regions around the indo-pacific, and educating and inspiring people around the world, whilst simultaneously contributing to the destruction of some of the world's most diverse environments (Branson, 2008). There is an ever-expanding literature into home aquaria, in addition to increased public and government interest in the damaging impacts of the ornamental fish trade (Leal, Vaz, Puga, et al, 2016). Furthering an understanding into every element of the trade, from collection to the housing of aquatic life, has the possibility of far reaching benefits to welfare of captive animals.

Attempts to improve animal welfare centre upon three broad scientific approaches: (1) a functioning based approach, to promote good physical health and functioning at a biological level; (2), a hedonistic approach, to maximise positive 'affective states' and minimise negative states; and (3) a natural behaviour approach, to facilitate the presentation of the species' natural behaviour (Fraser, 2009). The different philosophies have led to differing assessment methodologies, from assessing mortality and disease (1), to indicators of fear and distress (2), and an emphasis on the presentation of natural living (3). Fish are the most highly utilized vertebrate taxa by humans, being captured as part of the global fishing industry, employed commonly as pets and also commonly used in scientific research (Leal et al, 2016). Having said this, fish are rarely afforded the same levels of welfare as other 'higher' vertebrates. At the heart of this debate, is a larger question, are fish sentient beings? Do they have the capacity to perceive and consciously experience painful stimuli and wellbeing? Philosophical views on this subject are diverse and have stretched back many centuries; from the likes of Hume and Bentham, who emphasised the qualitative similarities of subjective feeling between animals and humans, to Descartes, who likened animals as inanimate machines (Branson, 2008). The modern prevailing view took influence from philosophers such as Hume and Bentham, leading to the introduction of the first Animal Welfare Bill in 1822, known as Martins Bill.

Although fish are legally regarded as sentient beings (Animal Welfare Act, CCB 2006), the research literature remains inconclusive. Key's (2016) building on the work of Rose (2002), argued that fish lack the neuroanatomical complexity to warrant conscious experience, highlighting a lack of neocortex. Numerous authors have rejected this claim, dissecting Key's (2016) paper and stating that the structure-function analogy set forward and applied to the rest of the vertebrate kingdom is selective, over-simplistic and outdated (Brown, 2016). Many of the authors argue that the same neurological regions do not consistently represent brain functions across taxa, therefore the concept of pain may also be represented by other brain regions within fish. Balcombe (2016) stated that through application of Key's (2016) justification, fish would be deprived of not only consciousness and pain perception, but also learning, which is evidently not the case. It is evident that a greater understanding of both human and fish sentience is paramount prior to reaching a conclusion. Balcombe (2016) added that with science's fundamental inability to prove anything, precaution dictates that we should give the benefit of the doubt to the fish, for the state of the oceans and aquaria.

The modern philosophy towards fish welfare is one of precaution; combining elements of the hedonistic and natural behaviour approach with a pre-existing functional-based philosophy, creating an all-encompassing definition of welfare. Further sophistication has led to the development of the 'five freedoms', a list of 5 areas where welfare is compromised if not sufficiently managed: (1) hunger, (2) undue environmental challenge, (3) disease and injury, (4) behavioural restriction and (5) mental suffering (FAW, 1992). The five freedoms, although simple, provide a fundamental and timeless basis of animal welfare, that can be readily applied to the most recent of research (Webster, 2016). Having said this, attempting to quantify welfare is a challenging task; there is a tendency for researchers to anthropomorphise those animals under inspection. Behaviour must be carefully scrutinised with consideration to what is beneficial to the needs of the individual, and the wider species.

Animal husbandry and environmental enrichment have become an integral element to both aquaria and zoos, drawing influence from the likes of Skinner and Markowitz, employing operant conditioning techniques to aid welfare (Mellen & MacPhee, 2001). Enrichment can occur through alterations to the environment, feeding methods and training, all of which are common place in aquaria around the world (Damesceno et al, 2017). The introduction of a tailored enrichment program has been demonstrated to reduce stress, promote natural behaviours and decrease stereotypical, abnormal behaviour, providing a direct link to welfare (Corcoran, 2015).
Aggressive behaviour is a heteronomous set of behaviours which inflict or threaten to inflict damage on another member of the population (Villars, 1983). Aggression is highly prevalent within aquaria. It is detrimental to those individuals it is directed towards to the extent that it is the leading cause of injury within aquaria and, therefore, aggression is commonly targeted by enrichment programs (Bailey, 1999). Resource-defence theories attempt to understand aggressive behaviour in relation to ecological contexts, notably the available resource within an environment. Resources which may be guarded include: territory, shelter, mates, juveniles and food (Maher & Lott, 2000).

To maintain superiority within a social hierarchy in the wild, individuals must behave antagonistically towards competitors, demonstrating behaviours such as erecting the fins, chasing, posturing and biting (Branson, 2008). In some situations, a consequence of this behaviour is dominance, the prioritizing of access to available resources for an individual or group of individuals. Dominance of resource facilitates the likelihood of survival and succession of genes, representing an increase in evolutionary fitness. However, when considering aquaria, dominance and the exhibition of high levels of antagonistic behaviour is regarded as an undesirable set of behaviours, not in the best interests of a tank which contains a multitude of species. Dominance is uncommon between coral reef fish in the wild; however, due to the artificially induced space limitations and inability to escape, the behaviour is widespread in species such as surgeonfish, damselfish and parrotfish in captivity (Spotte, 1992). Social subordinates are subject to chronic levels of stress (Sloman & Armstrong, 2002), resulting in appetite inhibition (Kramer & Chapman, 1999), an inhibition of natural behaviours (Hoglund et al, 2005) and a growth reduction (Winberg, Nilsson & Olsen, 1992). This conflicts with all many of the previously mentioned five freedoms of animal husbandry.

Food is a highly motivating resource, central to survival and reproduction; therefore, closely linked to the presentation of aggression. Algae feeders develop territories to defend a patch of algae, demonstrating aggression to those who attempt to challenge for this resource. Certain fish are more proficient at locating distant food patches by incorporating spatial location knowledge, making using of cognitive mapping (Mazeroll & Montgomery, 1998). The ability to spatially navigate is dependent on the complexity of the environment which the species inhabits in the wild. Following this rule, lake dwelling fish would be far less proficient at spatial learning tasks than inhabitants of a coral reef, for example surgeonfish. Fish with complex spatial navigation skills can employ cognitive mapping to assess the quality of food patches, and distribute themselves equally amongst the available patches in accordance with the proportion of their profitability. This is known as an ideal free distribution.

The primary focus of this study will be two species of surgeonfish (*Acanthuridae*); a population of yellow tangs (*Zebrasoma flavescens*), and of regal tangs, also known as palette surgeonfish, or blue tang (*Paracanthurus hepatus*). Both species are highly prevalent within the wild, regal tangs flourishing within the tropics, and yellow tangs distributed widely in the West of Hawaii, towards the East of Japan (Claisse, McTee & Parrish, 2009). Surgeonfish derive their name from the sharp 'scalpel' like spike near the base of their tail, which can be deployed when threatened or alarmed, warning away both predators and competitors (Toonen et al, 2011). Both species live in the Epipelagic zone, ranging from 2-46 meters in depth, usually found on exposed outer reefs (Toonen et al, 2011).

Surgeonfish either live solitarily, or within small groups. However, when feeding, individuals combine into large shoals of conspecifics and often other species, searching, locating and defending food patches, often exhibiting highly aggressive behaviour towards individuals who are biologically similar, and have a similar diet (Selkoe, Gaggiotti, Bowen & Toonen, 2014)**.** Huang et al, (2017) completed a mitochondrial genome of 10 close relativities of the regal tang (all from the *Acanthuroidei* family), finding the highest common identities of base composition to the yellow tang (87%). Both species are omnivores, with a large proportion of their diet consisting of the consumption of algae. This is a key element of the functionality of the reef ecosystem, avoiding suffocation of the coral and ensuring a diverse, healthy reef (Pitkin, 2001). Both species have long life spans in the wild, with the potential to exceed 30 years (Militz & Foale, 2017), however, this is significantly reduced in aquaria (8-12 years), where they may more readily catch disease (Parrish & Claisse, 2005).

Wild capture of both species had risen dramatically throughout the previous three decades, leading to a concern for both habitats and natural populations, especially within the West of Hawaii, where 400,000 individuals are collected annually (Callan, Burgess, Rothe & Touse, 2018). Both species are classified as level 0 of domestication, meaning that all individuals in aquaria are captured from the wild (Teletchea, 2016). Having said this, recent efforts to breed both species within captivity have been successful, Matthews et al (2017) documenting the first successful captive larval culture and metamorphosis of regal tangs, and Callan et al (2013) for yellow tangs. Captive breeding of these two species is in its infancy, with further development having the possibility of wide reaching positive implications to both the welfare of individuals, and the habitats in which they naturally reside.

The Biozone 6 tank (BZN6) at the National Marine Aquarium (NMA), in Plymouth, Devon, contains two schools of surgeonfish, 16 regal tangs and 19 yellow tangs, in addition to a series of other species of coral reef fish. An inflated level of aggression was observed in the tank, notably from the regal tangs, brought to the attention of our research team by the staff at the NMA. Concerned that levels of aggression would continue to increase to a level which threatened the health of the inhabitants of BZN6, staff at the NMA proposed the design of a tailored enrichment program to target welfare, providing the motivation for this research.

It was observed by NMA staff that aggressive behaviour spiked during feeding, inferring that the current medium of delivering food was detrimental to welfare, and ultimately the health of the two species of surgeonfish. It was therefore proposed that the number of feeding locations (the locations in which food is placed) should be manipulated. Prior to investigation, the surgeonfish were fed solely in one location, an entire graze block in the left-hand size of the tank. To manipulate this, the block will be split into two, and half placed on either side of the tank. It was inferred that a single week of manipulation (ABAB design) would not be sufficient time to override the existing foraging spatial associations. To account for this, the manipulation stage will consist of two weeks (ABBA design).

Surgeonfish naturally reside in highly complex environments, therefore are likely to have access to cognitive mapping (Mazeroll & Montgomery, 1998). As a result of this, it is expected that both species will adopt an ideal free distribution, through identification of additional feed patches and the application of the most economical strategy to maximise resource. Based on previous examples of enrichments (Corcoran, 2015), it is predicted that the separation of feed into two separate territories will promote natural behaviours (foraging behaviour, schooling behaviour), decrease adverse natural behaviour (aggression) and ultimately lead to a positive impact in the welfare of the targeted species. The conclusions reached in this research paper will be compared to the current understanding of the mechanisms impacting surgeonfish welfare, with the ultimate goal of generalizing the findings to further tanks containing aggressive surgeonfish around the NMA.

Methodology

Subjects
The primary focus of this research is the behaviour of two species of surgeonfish (*Acanthuridae*) present in BZN6. Within the tank there are 16 regal tangs (*Paracanthurus hepatus*) approximately 8-12cm in length, and 19 yellow tangs (*Zebrasoma flavescents*) approximately 5-8cm in length, for which data will be extracted. In addition to these two species, BZN6 also contains 5 further species: 2 porcupine pufferfish (*Diodon holocanthus*), 1 cleaner wrasse (*Labroides dimidiatus*), 15 white tailed humbugs (*Dascyllus aruanus*), 4 common clownfish (*Amphiprion ocellaris*) and 2 moorish idols (*Zanclus cornutus*).

Materials
BNZ6 has a volume of 7440L (accounting to a total system volume of 9000L), a width of 381cm, and a height of 84cm. The tank simulates a natural coral reef environment, including concrete rockwork, and three types of fake coral: plating, acroporas, and staghorn corals, with a base substrate of coral sand with a depth of 2-3mm. BNZ6

also contained a water pump to aid water movement ensuring sufficient aeration as well as providing a further enrichment tool. Temperature and water quality are maintained by means of filtration and regular examination. To aid measurements of spatial distribution, a 4x2 grid was imposed onto the glass on the tank using highly visible tape. The tank was subdivided into four columns each 95cm in width, and two rows, each 42cm in height, creating eight equally sized grids. To avoid impeding the view of the camera, sections of tape were placed only where the tank met the wall, with the exception of tape marking the central cross (see figure 1).
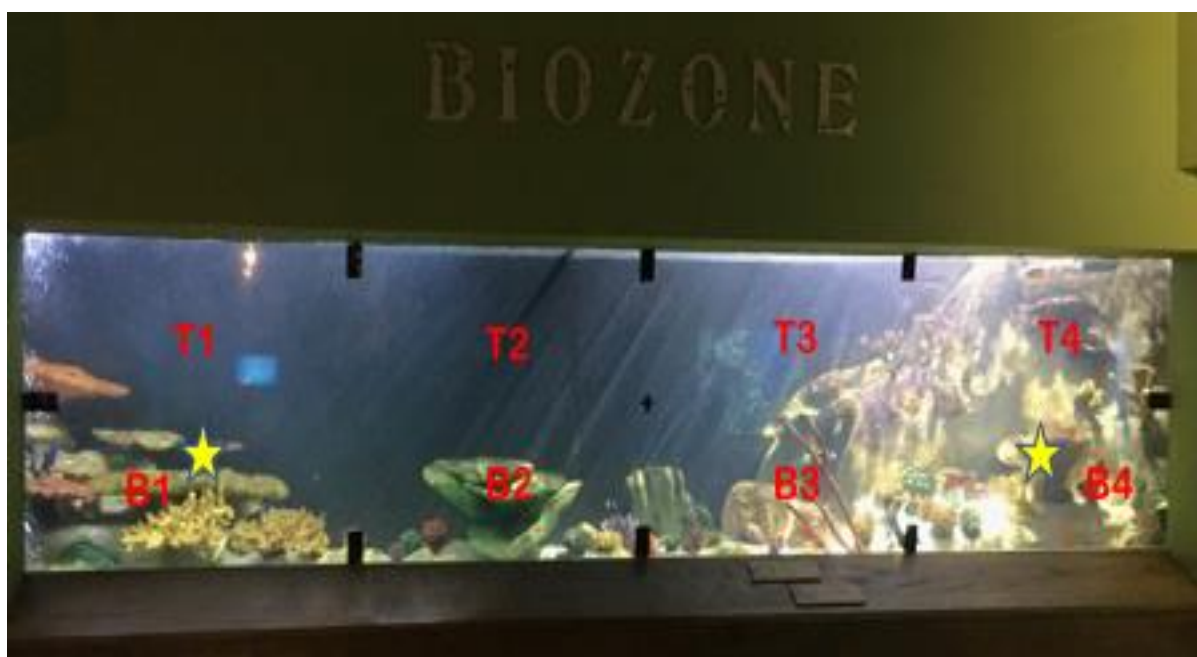

Figure 1: Image of BNZ6, demonstrating the placement of tape separating the tank into 8 equally sized grids. Locations of feed placement are represented by yellow stars.

The feeding schedule in BZN6 consisted of a combination of live (frozen) feed, flake feed, pellet feed, and grazing blocks. On Mondays, Wednesdays, and Fridays, at approximately 11.30AM, a Vitalis Grazing Block was placed into the left-hand side of the tank. This feed specifically targeted the surgeonfish, meeting the specific dietary requirements of the two surgeonfish species present in BNZ6, containing a combination of fish derivatives, algae, derivatives of vegetable origin, oils and fats, molluscs and crustaceans, vitamins, minerals. The tank was also fed an 11.30am chunk feed (live feed) on Mondays (squid), Wednesdays (prawn) and Fridays (mussel), in addition to Vitalis algae flake and pellet feeds at 11.30am on Tuesdays, Thursdays and Saturdays. To avoid contact human contact with the water, extended tongs were used to accurately place the grazing block into the desired location. Due to the public nature of the NMA, safety measures were taken to inform and ensure the safety of the public. Barriers were erected around the cameras, preventing collisions, name badges were worn at all times within the NMA, in addition to signage informing the public that the tank was being recorded. The sign read: 'This tank is being recorded for research purposes. However, feel free to carry on as you would normally. If you have any questions, please ask the people attending the cameras'. During initial informal observations, it became evident that the width of the tank was a barrier to accurate and reliable data collection by eye, with observers only able to

attend to one side of the tank at a time. The inclusion of two Canon Legria HF R806 video cameras mounted on tripods resolved this issue, providing an advantageous means for data collection. The use of video recording reduced the total area of which an observer had to scan by 50%, aided by the ability to pause, rewind and slow down recordings, assisting the coding of fast paced, ambiguous behaviour. The ideal camera location required that neither frame was overlapping, yet the entirety of one half of the tank had to be captured with as much detail as possible. In the case of this research, the left tripod and camera was placed at a distance of 150cm perpendicular from the tank, at a distance of 95cm from the left wall of the tank. The use of the spirit level and compass on the tripod ensured the recording angle remained constant. These measurements were duplicated for the placement of the right side, with both measures taken from ground level, to the centre point of the tripod.

Accuracy and specificity of observation is central to behavioural research, especially if there are multiple observers, as was the case with this research. In order to ensure that each observer was extracting the same data from the observed behaviour, a detailed and specific behavioural checklist was created (see table 1). Each behaviour was coded for both species of surgeonfish.

Table 1: The behavioural checklist – presenting the behaviours measured in this research, and their corresponding definitions.

Behaviour	Definition
Chase Behaviour	The definition of chase behaviour employed in this research was based on Perreault, Semsar & Godwin's (2003) characterisation of the behaviour. Essential to the categorization of a chase was that two (or more) fish must have had a sudden increase in velocity. The chaser fish must have moved towards the chasee (within an estimated body length) at an increased velocity, which in turn, caused the chasee to suddenly increase its own velocity away from the chaser (this can be at any angle away from the chaser). The species of fish which was chased (the chasee) was not recorded, therefore any species present in BZN6 can be the chasee. Data for the chase was extracted from the chaser fish which demonstrated the act of aggression.
	The central distinction between a short and extended chase is that when the chasee attempted to change course and velocity (in an attempt to flee), the chasing fish either stopped chasing (short chase), or continued to chase at high velocity, altering its path dependant on the chasee's direction of movement (extended chase). The chase was only extinguished upon the chasing fish decreasing its velocity, allowing the chasee to distance itself.

Spatial Distribution	The spatial distribution of a species refers to the location of the fish in regard to the tank and other individuals. A highly distributed arrangement of fish is exhibited by individuals spread evenly throughout the tank, with each individual located distally from all other individuals. A low spatial distribution resembled all individuals within close proximity, in one section of the tank. This measure of distribution is species specific, with each observation only measuring one species at a time, at specific points in time (sample points). The measure was recorded by counting each number of the species present in each section (grid) of the tank.
Chase Distribution	Chase distribution is a measure of the location from where a fish instigates a chase. This measure was recorded at the location where the chasing fish begins to increase velocity, recorded as a grid reference.
Foraging	Foraging behaviour is characterised by a repetitive pecking action in contact with the environment (Pitcher, 1993). For this behaviour to be deemed 'foraging', the fish was required to repeat the behaviour a minimum of two times (movement of body and contact with coral). In addition to this, the behaviour must have occurred a minimum of a fish length away from the location of the feed (when present). Foraging behaviour was not defined by the number of pecking movements (multiple pecks regarded as a singular act of foraging); nor is the behaviour dependant on location. For as long as the fish remains pecking, a singular act can facilitate the fish moving along a piece of coral. If the fish did not exhibit the pecking movement for 5 seconds after the previous pecking movement (if in the same location) or moved more than a fish length away without constant pecking, this marks the termination of the behaviour.
Schooling	Morgan's (2003) observation into the social organisation of blue tangs, characterised schooling as at least 10 fish swimming in a polarized fashion. As this observational study was conducted on a fringing reef in the wild, with a significantly higher prevalence of fish than in BZN6, the minimum requirement of fish will be dropped to 5. This polarization of movement as described by Morgan (2003) requires a uniformity in their direction and speed of travel (velocity), and requires each individual to be within close proximity of another fish (no more than 1 fish length away).

Design and Procedure
When conducting a behavioural study, a series of informal observations is an important precursor to quantitative recording of behaviour, familiarizing the researcher with the species of interest, and complementing an in-depth literature review (Martin & Bateson, 2007). Behaviour is a continual stream of movement; informal observation is central to separating these movements into distinguishable behaviours and in the formation of the behavioural checklist (see table 1). The chosen behaviours were not solely measures of aggression such as chase behaviour, but also measures of behaviour which may have been facilitating the increase in aggression, and therefore may provide vital information aiding understanding of the wider issues present in BZN6 (spatial distribution, foraging and schooling).

The application of systematic constraints through sampling and recording rules avoids biases towards the highly salient stimuli, better representing the true behaviours exhibited (Hernandez-Lloreda, 2006). All behaviours were recorded over a time period of 30 minutes, divided into 30 intervals, each of one minute duration. Sample intervals provide a means of time stamping the behaviour, facilitating the comparison of behaviour over time in relation to specific events, e.g. food entering the tank. Behaviours rare enough to enable counting of every exhibition will be recorded using continuous recording (all occurrences), which is considered to be the best method to represent a behaviour (Martin & Bateson, 2007). This sampling will be employed for chase behaviour, chase distribution, foraging behaviour and schooling. Scan sampling, the extraction of behaviour from momentary scans of the whole population at regular intervals, was employed for behaviours which are too prevalent to track all occurrences. Spatial distribution is a behaviour which is always present (the location of the organism within the tank), therefore a scan sample was the only viable option. Samples were taken on each minute mark, for the entirety of the 30-minute observation. This sampling method was employed only for spatial distribution.

Following the formation of the behavioural checklist, a series of preliminary observations were completed with the goal of testing the plausibility of the methods chosen, refining data extraction skills, and identifying any issue prior to the onset of formal testing. One issue encountered in the preliminary stage was the accuracy of a subjective measure of spatial distribution due to the extreme width of the tank. The use of video recording made it possible to create snapshots, count the exact number of fish in each section of the tank, and therefore eradicate the issue. A sound methodology contains inter-rater reliability for 20% of the recording sessions throughout formal observations (Clearinghouse, 2010). To comply with these standards, and to take into account the high number of different observers in this research, a measure of inter-rater reliability was conducted every third observation on each measure of behaviour (33% of observations). A ten-minute sample was coded for every behaviour by all four observers, commencing at the start of minute five, and ending at the end of minute fourteen. Regular testing facilitated the identification of discrepancies between observers allowing these to be resolved quickly and avoiding the entirety of observations being affected. In addition to this, observers rotated which behaviour they coded every recording session, ensuring that any observer bias was minimised.

The experiment employed a reversal (ABBA) design, providing the means to demonstrate a functional relationship between the dependant variables (behaviours present on the behavioural checklist) and independent variable (location of feed). Measures of behaviour were equally apportioned to different observers to further avoid observer fatigue. Observer 1 extracted yellow tang chases, Observer 2 extracted regal tang chases, Observer 3 extracted spatial distribution data for both species, and Observer 4 extracted foraging behaviour for both species. Measures of behaviour were simply rotated for the following observation, culminating in each observer extracting data for each behaviour three times. Ethical implications of this research were considered throughout, with regular monitoring of aggression levels by both the researchers and NMA staff, who were on hand ready to stop the manipulation, following any evidence of aggression peaking at unacceptable levels (evidence of injury arising from attack).

Prior to the set-up of equipment around the tank, the immediate area was barricaded off and signage made readily visible. The two cameras were placed in their optimum locations, with the tank separated into 8 sections (as discussed in the 'materials' section). Following the setup of equipment, both cameras were set to record, starting at the same time. During the first week of observations, following the end of the tenth minute (dictated by the time stamp present on the camera), a whole grazing block was placed into the left side of BZN6 (for exact placement, see figure 1). The feed was placed between two pieces of coral, preventing the location of the feed from being manipulated by the inhabitants of BZN6. The feed was placed into the desired location using extended tongs to avoid the disruption of the fish through a hand entering the water. Following the introduction of the food at 10 minutes, a further 20 minutes of recording was taken before each camera was stopped at the same time. During the second and third week of manipulation, the process was repeated; however, the graze block was split into two (using a knife to size accurately), with half being placed in the left-hand side, and half placed in the right-hand side (see figure 1). The fourth week of observations reverted back to the procedure completed in week 1, with the entirety of the feed being placed in the left-hand side. Recordings were then uploaded to a communal shared drive, where each observer could independently download and extract the data attributed to them in that specific observation. If a visitor impeded the view of the tank, judged to have impacted the collection of data, that minutes data sampling for the variable in question was removed.

Results

This paper will contain only descriptive statistics, omitting the use of inferential statistics. Inferential statistics facilitate inferences to be drawn regarding a population when only a sample of this population has been taken and measured. This approach is adopted when researchers cannot access a whole population, for example if it is too large or not accessible. Descriptive statistics do not make inferences but purely demonstrate what the given data is showing, relying on visual inspection. In the case of this study, ease of access to the entire population of interest (the two species of tang in the BZN6 tank) warrants the omission of inferential statistics which do not adequately fit this research. In addition to this, inferential statistics have a tendency to group data together, so as to remove the 'noise'. However, because it applies to a given behaviour, it is this 'noise' which is of interest in behavioural studies and

therefore it should not be eradicated from our analysis. For this reason, the use of averages in data presentation will be avoided where ever possible, made use of only when an average is the only unbiased means of presenting data (when data has been omitted).

Inter-rater reliability
Before calculation of inter-rater reliability, a series of statistical tests were considered. Cohen's Kappa and Kendall's Coefficient of Concordance (W) are both robust and highly employed methods of measuring inter-rater reliability in observation studies (James, Demaree and Wolf, 1993). However, the data collected in this experiment (discrete numerical values measured on an interval scale) does not meet the requirements of either of these methods, with Cohen's Kappa requiring categorical data, and Kendal's W requiring ranked data. As a consequence, inter-rater reliability will be measured using percentage agreement, which is in itself highly robust and generalizable (Mitchell, 1979). Percentage agreement was calculated for each variable through analysis of a matrix of matched pairs/judges (J1/J2, J1/J3, J1/J4, J2/J3, J2/J4, J3/J4,). Each matched pair was assigned as either a match (1), or a mismatch (0) for every data point.

Table 2: The combined percentage agreement across all four weeks of observation, for all the measured variables

Variable	Species	Percentage Agreement (%)	Standard Deviation (SD)
Short Chases	Regal	57.7%	27.7
	Yellow	57.5%	26.8
Extended Chases	Regal	90.8%	12.2
	Yellow	92.9%	10.6
Short and Extended Chases Combined	Regal	74.3%	19.9
	Yellow	75.2%	18.7
Chase Distribution	Regal	87.0%	8.3
	Yellow	91.8%	5.4
Spatial Distribution	Regal	64.2%	13.2
	Yellow	80.4%	9.4
Foraging Behaviour	Regal	49.0%	25.9
	Yellow	59.4%	22.7

The number of agreed scores (1) were then combined and divided by the total number of units of observation that were rated, finally multiplied by 100, to provide a measure of agreement between raters for a specific extraction of data. For measures of spatial and chase distribution, this process was completed for each grid reference (T1, T2, T3, T4, B1, B2, B3, B4), with the corresponding measures averaged to provide percentage agreement between observers for the whole tank. Table 2 provides the percentage agreement and standard deviation of four observers for each variable, for each week of observation (collected on every third observation of the week).

The combined percentage agreement for all the observed variables across all four weeks of observation was 73.1%, with a standard deviation of 7.9. Figure 2 demonstrates how the differing weeks of observation compared in percentage agreement and standard deviation, demonstrating a clear increase in percentage agreement from weeks 1 to 4.
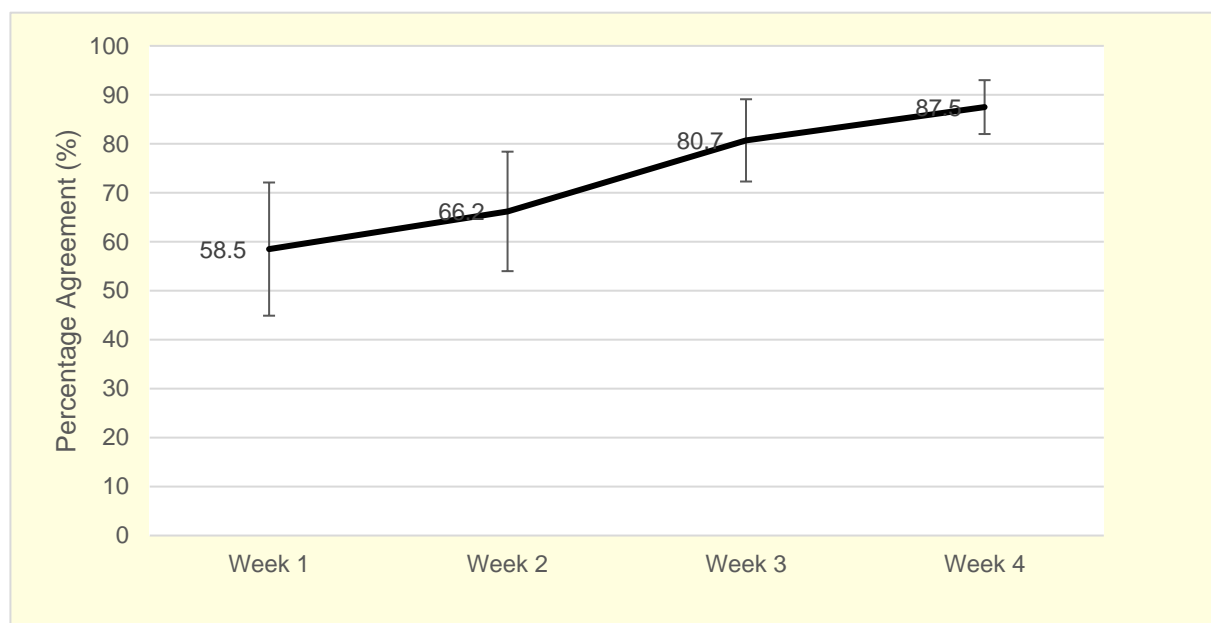
Figure 2: Combined percentage agreement of all measured variables across all four weeks of observation.

Schooling
The variable 'schooling' was presented rarely throughout the four weeks of observation. Failure to collect a significant number of behavioural responses significantly limits the confidence of the conclusions drawn from the data (Martin & Bateson, 2007). As a result, the variable has been omitted from analysis.

Chase behaviour
Chase behaviour in week 1 was considerably higher for regal tangs, than it was for yellow tangs. Following an increase in chase behaviour in week 2 for both species, there was a large decrease in week 3, followed further a further decrease in week 4 in both species (See figure 3).
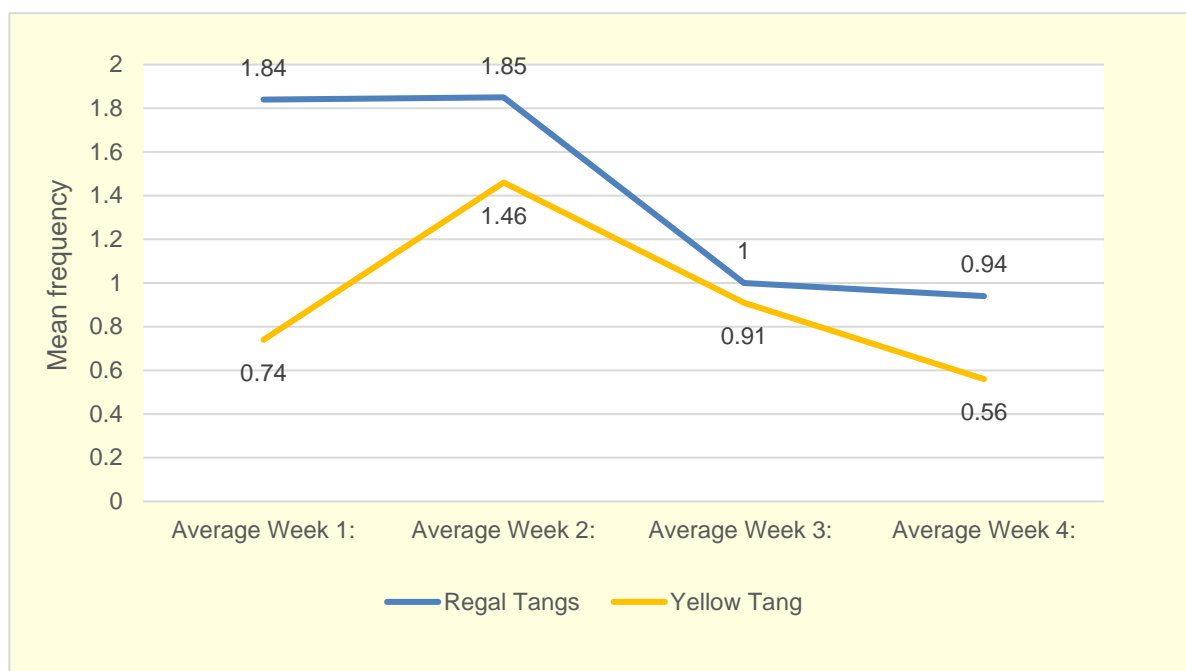
Figure 3: Mean frequency of chase behaviour (extended and short), for both species of surgeonfish, across all four weeks of observation.

Chase Distribution

As demonstrated in figures 4 and 5, Both species predominantly aggressed in the left-hand side of the tank (grids B1 and B2) for all four weeks of observation.
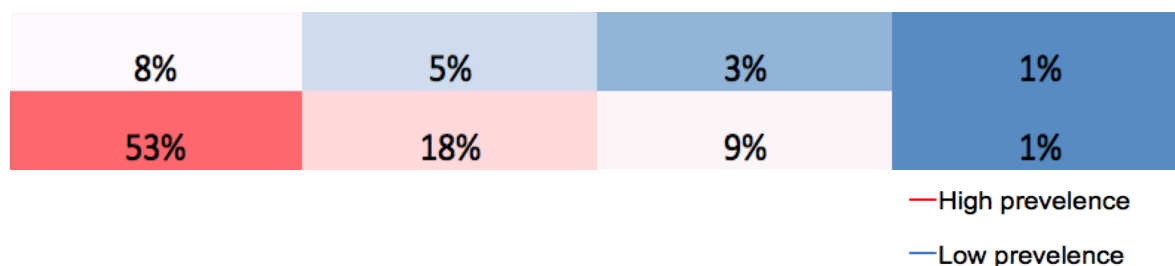

Figure 4: Distribution of regal tang chase behaviour, averaged from all four weeks of observation.
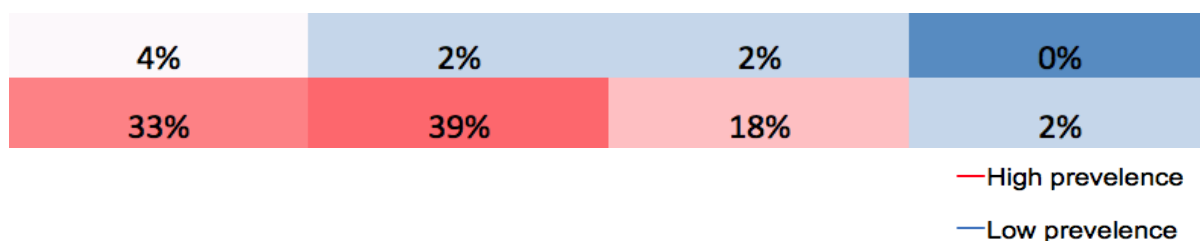

Figure 5: Distribution of yellow tang chase behaviour, averaged from all four weeks of observation.

Throughout the 4 weeks of observation, regal tang chase distribution remained predominantly in the left-hand side of the tank (B1, B2, T1, T2). However, as demonstrated in Figure 6, although still more prevalent in the left-hand side, the distribution of yellow tang aggression within the right-hand side is shown to increase from week 1 to 4.
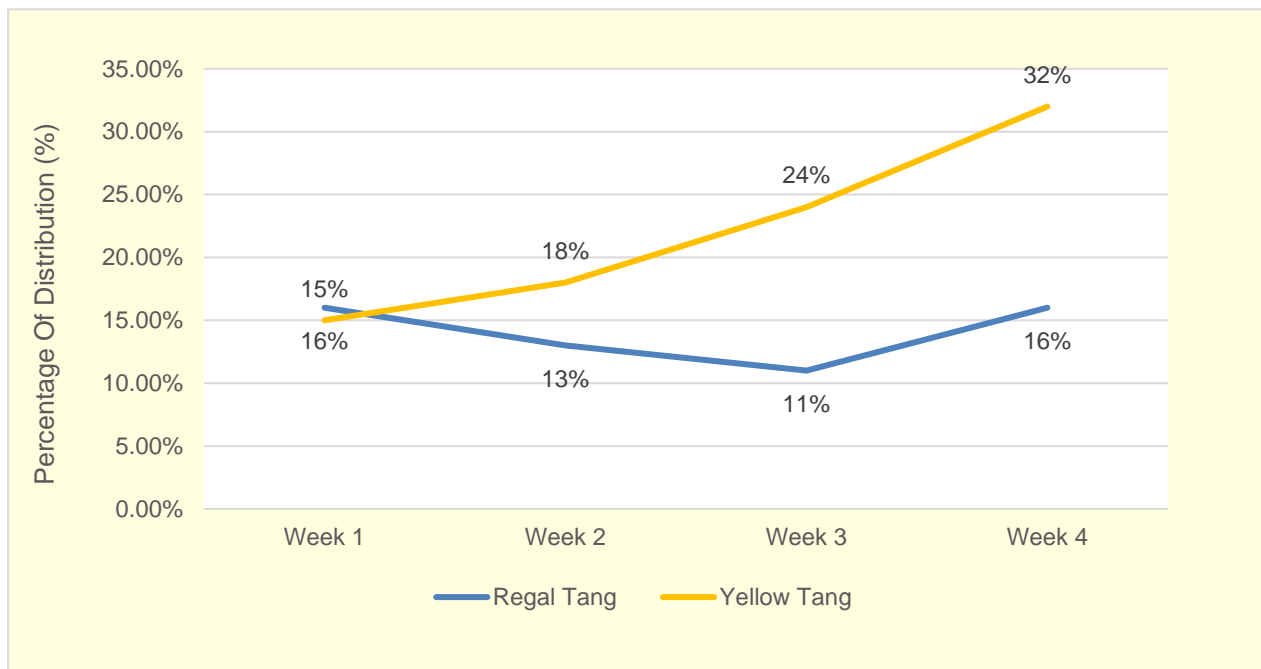

Figure 6: Percentage of chase behaviour distrbuted in the right hand side of the tank (B3,B4,T3,T4), across all four observations.

Spatial Distribution
A similar pattern is observed in the spatial distribution of both species. Figures 7 and 8 present the spatial distribution for each species across all four weeks of observation. Comparisons of the two demonstrate how the population of regal tangs are more heavily distributed in the left-hand side of the tank, notably in B1, whereas yellow tangs are more evenly distributed throughout the tank, although still with an emphasis on grids B1 and B2.
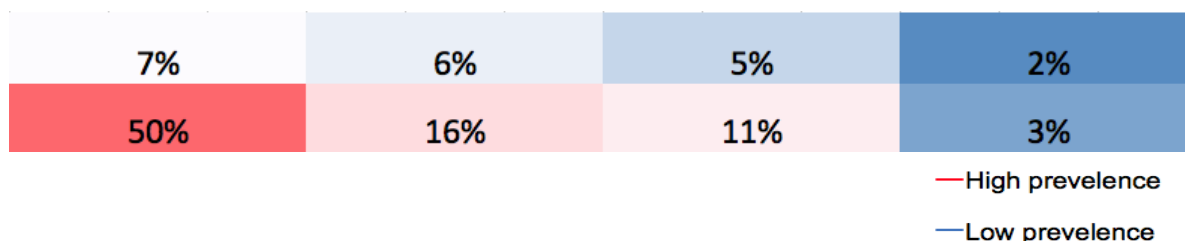

Figure 7: Spatial distribution of regal tangs - average from all four weeks of observation.

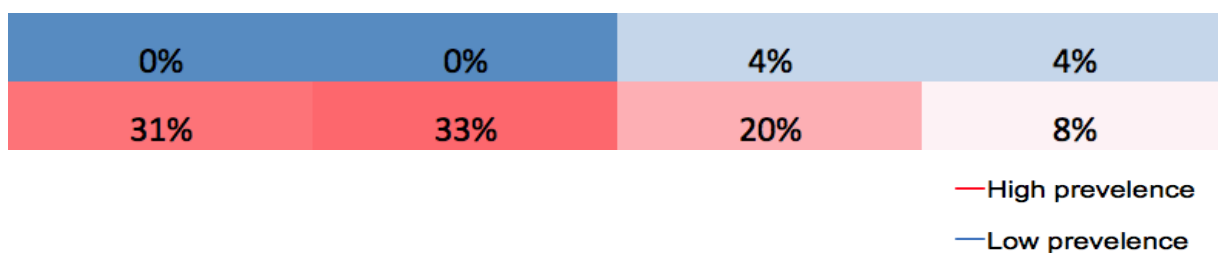| 0% | 0% | 4% | 4% |
| 31% | 33% | 20% | 8% |

—High prevelence
—Low prevelence

Figure 8: Spatial distribution of yellow tangs – average from all four weeks of observation.

Figure 9 represents the change in distribution over the four weeks of observation for both species. Regal tang spatial distribution remained predominantly in the left side of the tank, whereas the yellow tang distribution increased throughout each week, culminating in a distribution primarily in the right-hand side of the tank.
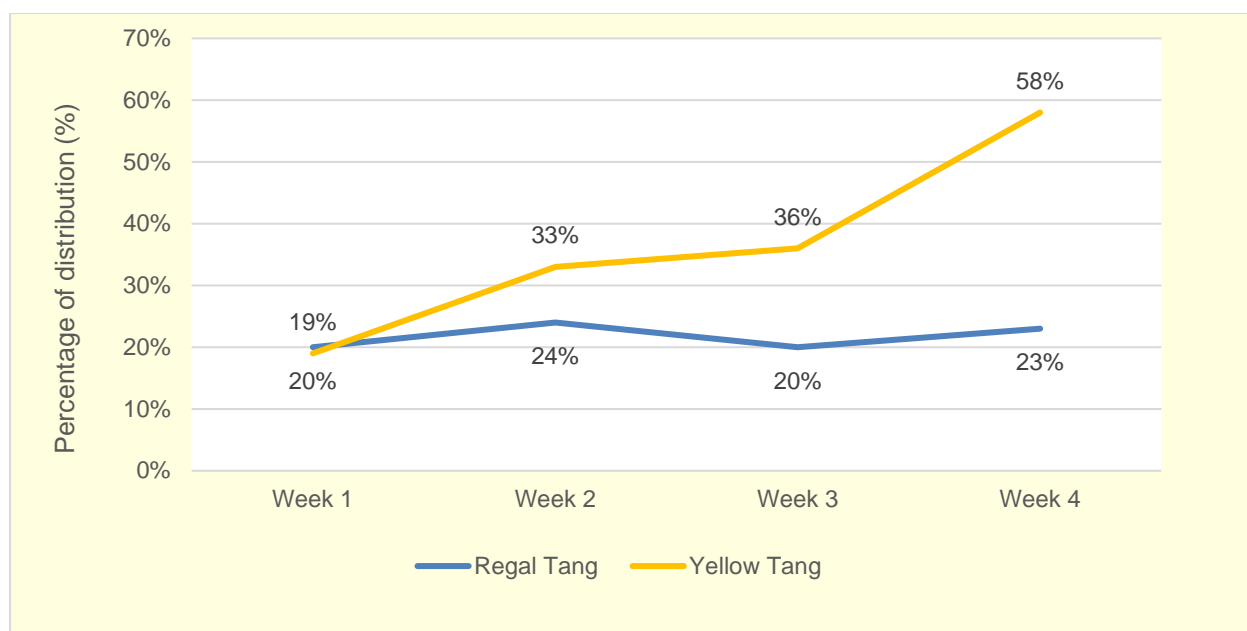

Figure 9: Percentage of regal and yellow tang spatial distributed in the right-hand side of the tank (B3, B4, T3, T4).

In addition to changes between observations, the changes within an observation are also of interest. Figure 10 represents the percentage of total distribution present in grids B1 and T1, for both species, across all 4 weeks of observation. Grids B1 and T1 represent the far left-hand side of the tank, where feed had been placed prior to manipulation (resident feeding location). Initial observation of Figure 10 clearly demonstrates that regal tang distribution is higher in grids B1 and T1 than yellow tang distribution throughout the observation period. There is an increase in both species distribution in grids B1 and T1 following the food being placed in this location (end of minute 10), followed by a gradual decrease during post-feed. Closer observation reveals that there are spikes where distribution in B1 and T1 increases,

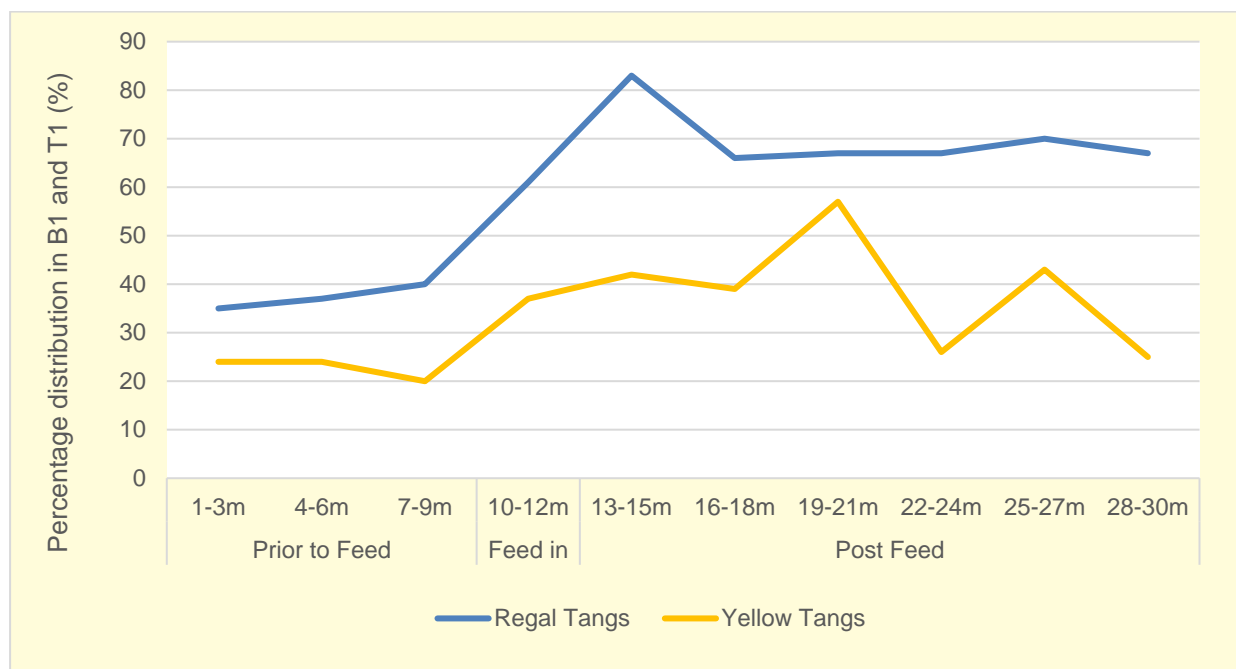in minutes 13-15 (83%) for regal tangs, and later in the observation, minutes 19-21 (57%) for yellow tangs.

Figure 10: The average percentage spatial distribution present in grids B1 and T1 over the 30 minute observation, across all four weeks of data. (m=minutes).

A number of suggestions were formulated to explain why the regal tang distribution did not increase in Weeks 2 & 3. One of these was the possible effect of territorial behaviour exhibited by two other species present in BZN6, namely the two moorish idols (*Zanclus cornutus)* and two porcupine pufferfish (*Diodon holocanthus*). To investigate this suggestion, spatial distribution was sampled throughout all four weeks for both species (Figure 11, and Figure 12). The same methodology was employed as previous measures of spatial distribution, however only taking six sample points from each 30-minute observation (at the end of minutes 1, 2, 14, 15, 21 & 22).

Foraging Behaviour
Foraging behaviour in both species of surgeonfish increased in the first week of manipulation (Week 2), however, following this, there was a decrease in Weeks 3 and 4 (even when the manipulation was reversed).
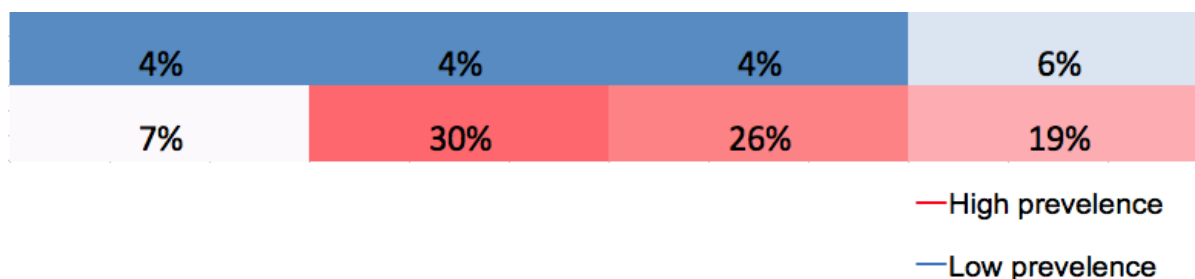

Figure 11: Spatial distribution of two moorish idols, averaged across all four weeks of observation.

As demonstrated in Figure 13, foraging behaviour was much more prevalent in yellow tangs, compared to the regal tangs in all four weeks of observation.
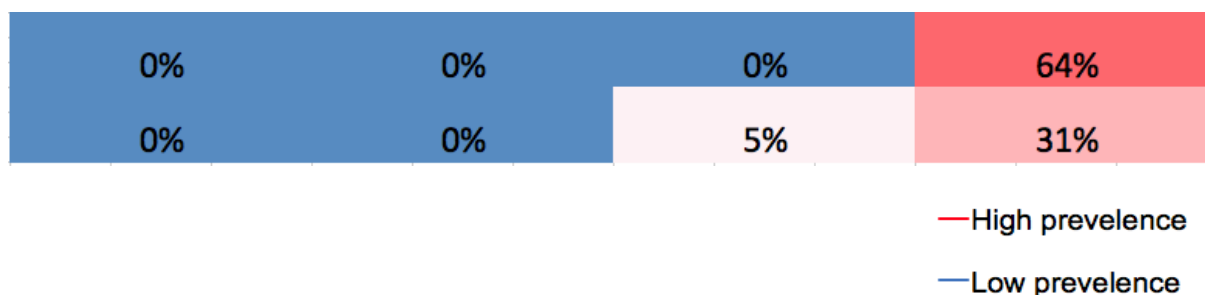

Figure 12: Spatial distribution of two porcupine pufferfish, averaged across all four weeks of observation.
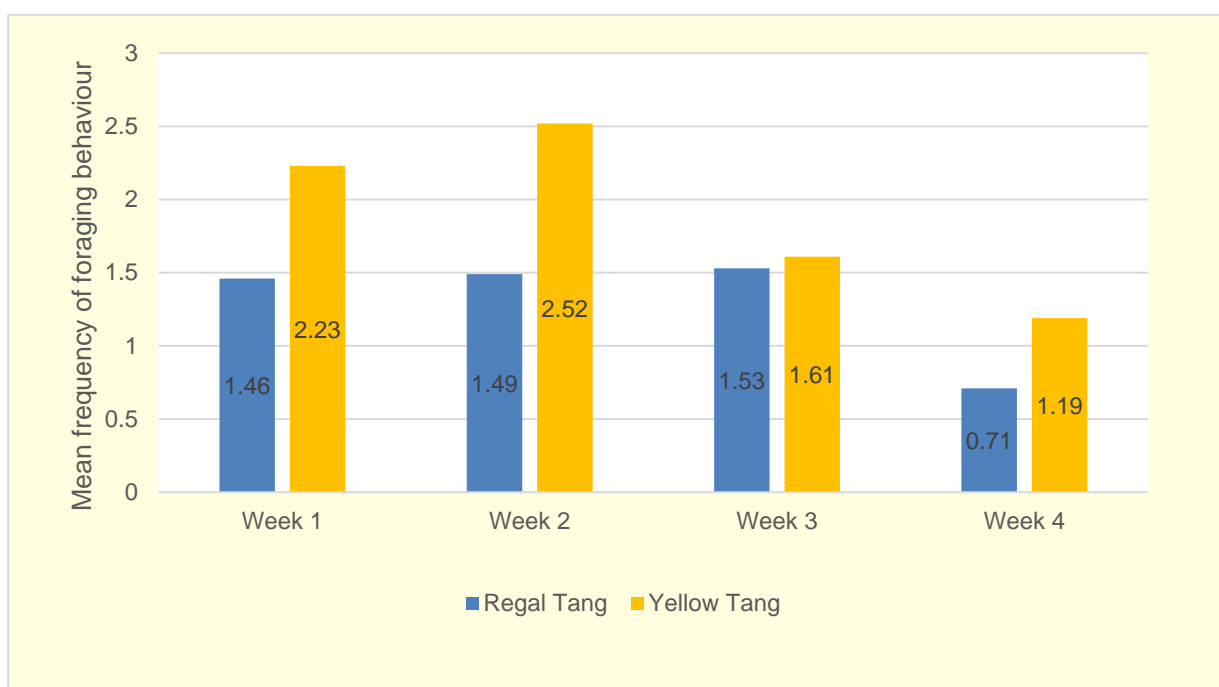

Figure 13: Mean frequency of foraging behaviour in both species of surgeonfish, over all four weeks of observation.

Discussion

The primary aim of this research was to reduce the levels of aggression exhibited by two species of surgeonfish in BNZ6 tank. Despite evidence of aggression decreasing from weeks one to four, it cannot be concluded that there is an exclusive, causal link between feed location and exhibitions of aggression. This is because there was no observed reversal of variables in the fourth week of observation, where the feed was returned to a single location. However, it can be concluded that the manipulation of feed location was a catalyst for change, which is inferred to be highly beneficial to the inhabitants of the BNZ6 tank.

Elements of the research complied with what was to be expected from the knowledge accumulated following a literature review into the behaviour and nature of surgeonfish. Chase behaviour, a measure of aggression, decreased following manipulation of the feed (Figure 3), complying with research of a similar nature (Corcoran, 2015). This indicates that feed is a catalyst to aggression, underlying the importance of the experiment to the inhabitant's welfare. In addition to this, distribution of chases closely aligned with overall distribution (see figures 4, 5, 7 and 8), suggesting that spatial distribution is also critical to aggression.

However, the collected data highlighted a series of unexpected discrepancies. For example, during the manipulation of the feed in week two, it was predicted that chase behaviour would decrease, not increase (figure 3), due to the separation of the two population's territories. In addition to this, reversal of the feed locations failed to reverse behavioural measures. Chase and foraging behaviour decreased further in week 4 (figure 3 and 13), despite feed being placed entirely in the left-hand side of the tank, contradicting expectations. It was predicted that spatial distribution for both species (in weeks two and three) would align to the feed patches profitability, forming an ideal free distribution, however, the regal tang distribution remained predominantly in the left-hand side of the tank (figure 9), once again contradicting expectations.

When discerning the reasons for the observed behaviour, one has to consider the possibility that one or both of the species failed to perceive the second feed patch. Previous research literature emphasises the sophistication of surgeonfish's spatial orientation skills (Mazeroll & Montgomery, 1998), questioning the credibility of this explanation. Another explanation is that the formation of a new food-location association was inhibited. As the associative strength of CS_L (left feed location) is at the asymptotic value through repeated prior exposure, additional learning in CS_R (right feed location) is impeded, known as blocking (Rescorla & Wagner, 1972). However, this approach fails to explain why the yellow tang distribution increased throughout the observations, leading to the conclusion that even if a blocking effect was impactful, it is most likely not a primary mechanism of influence.

The dispersal of certain phenotypes amongst populations can be modelled by the application of Evolutionary Game Theory (EGT). When applying game theory to evolution, the 'evolutionary stable strategy' (ESS) becomes the 'solution' to the game. Lawlor & Maynard-Smith (1976) first posed the question: 'If two species compete for two renewable resources, will they coevolve so as to make continued coexistence more likely?'. To answer this question in regard to this experiment, one has to determine the payoff value (benefit to Darwinian fitness) for each option, either to fight, or to display and retreat. This is further modelled in Hawk-Dove Game (Maynard-Smith, 1982). If both species choose to fight, it can be assumed that the cost in terms of injury or fatality is higher than the payoff (temporary access to feed/territory), resulting in a net loss in Darwinian fitness. If one or both of the populations chooses to display/flee, and coexist cooperatively, neither species experiences a loss in Darwinian fitness, and both coevolve as specialists on different resource patches (Lawlor & Maynard-Smith, 1976). It is inferred that the two species of surgeonfish present in BZN6, following an initial conflict, applied an ESS leading to the displacement of the subordinate population (yellow tangs), coexisting in different territories/patches. To support this speculative viewpoint, evidence will be drawn from both the data collected, and corresponding research literature.

Beeching (1992) demonstrated how body size is a strong predictor of hierarchal relationships, with the majority of aggression inflicted on individuals who are 75% of the body size of the attacker. The regal tangs exceed the yellow tangs in body size, and therefore are likely to be the dominant species. An increase in chase behaviour in week two (figure 3), accords with the suggestion that environmental change (manipulation of feed) led to an initial conflict. The displacement of the yellow tangs (subordinate fish) from the resident territory (B1) to the right-hand side of the tank, can be observed in figure 9. In addition to this, figure 10 demonstrates how the regal tang population fed in the resident territory (B1 & T1) earlier in each feeding session (13-15 mins), compared to the yellow tangs (19-21 mins), further demonstrating the subordination of the yellow tang population. Following the displacement of the yellow tangs, chase behaviour decreased for both species, which suggests the design of a new social hierarchy in which coexistence was found to be more beneficial than competition (an EES). Post hoc analysis of two further species present, the moorish idol, and the porcupine pufferfish, demonstrated that their distribution was highly skewed towards the right side of the tank (See figures 11 and 12). A possible antagonistic relationship between these two species and the regal tangs may have contributed to the observed spatial distribution.

There are elements of the collected data which do not align with an EGT prediction. Following the reversal of manipulation in week four, a return to the pre-existing social hierarchy was expected. It is suggested that following the adoption of an EES, there was insufficient time (only three observations) to revert back to the original hierarchy. In addition to this, foraging behaviour decreased following the application of a new social hierarchy, indicating a decrease in welfare. It is proposed that following the separation of territories, a perceived increase in resource negated the need for foraging. It is imperative to emphasise that although the application of EGT principles fits with the observed data, one can only infer the link, which at this stage is highly speculative; further investigation is essential to cement the link.

Systematic replication is a process of repeating an experiment multiple times whilst varying a different aspect of the study with each replication (O'Leary, Rosenbaum & Hughes, 1978). This process can provide substance to the speculative assumptions regarding the impact of the moorish idols and porcupine pufferfish on regal tang distribution (through removal of one species at a time), in addition to providing robust evidence of the predictions of a hierarchical relationship between the two species of tang. Along with this, the NMA were also recommended to implement a long-term AB trial, assessing the continuing impacting of the new hierarchical dynamic on welfare, with the goal of implementing a permanent alteration.

Current theoretical tools designed to determine welfare (the five freedoms) fail to encapsulate the complexity of a social dynamic within a population of captive animals. The five freedoms approach is to target each species in isolation, failing to adopt a holistic approach to welfare. It is suggested that the population's ability to reach a collective, mutually beneficial ESS, is a pre-requisite to welfare and should be further considered in concordance with the five freedoms.

An issue encountered with this methodological design was the inter-rater reliability (table 1). Although the combined percentage agreement exceeded 70% (73.3%), the benchmark for inter-rater reliability (Clearinghouse, 2010), certain variables

consistently failed to reach 60% agreement (short chase behaviour and foraging behaviour). Future adaptions of this research would benefit from a more specific, objectively defined measure of these two behaviours. In addition to this, figure 2 demonstrates how inter-rater reliability increased throughout the four weeks of observation, providing strong evidence for the presence of observer drift. Although unavoidable, the effect of observer drift can be negated if there is a prolonged preliminary observation stage, ensuring that any change in measurement occurs prior to the onset of formal testing. Furthermore, future research of this nature should also consider using a method/device to hold the graze blocks in place, preventing the fish from manipulating the location and thus compromising the trial.

Conclusion

This research project was undertaken because inflated levels of aggression were identified within two species of common aquaria fish in the BZN6 tank at the National Marine Aquarium. The primary goal was to gain an insight into the factors which influence levels of aggression, and the relationship between certain behavioural tendencies and welfare. The findings presented suggest that there is a relationship between placement of feed and levels of aggression, the result of a complex socio-environmental dynamic arising from a confrontation for a resident territory (B1) and the displacement of the subordinate species (yellow tang). This relationship aligned with Evolutionary Game Theory explanations of interspecies confrontation (Maynard-Smith, 1982).

It would be fruitful to pursue further systematic replication to fully understand the exact mechanisms impacting the complex socio-environmental relationship within BZN6. As a result of this research, the NMA have been recommended to implement a longer term AB trail, with the ultimate goal of a permanent alteration which would increase the welfare of the animals under their protection. This research also raises important questions regarding the theoretical tools employed for the measurement of welfare, with the suggestion that a more holistic approach, as employed through a measure of evolutionary stable strategies, better represents the welfare of the tank community.

Acknowledgements

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