

2018

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Allen, E. (2018) 'An investigation of pair-bonded Humboldt penguins (*Spheniscus humboldti*) in captivity: the differences between male-female pairs and male-male pairs', *The Plymouth Student Scientist*, 11(1), p. 5-23.

<http://hdl.handle.net/10026.1/14171>

The Plymouth Student Scientist
University of Plymouth

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An investigation of pair-bonded Humboldt penguins (*Spheniscus humboldti*) in captivity: the differences between male-female pairs and male-male pairs

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Abstract

Same-sex sexual and pair-bonding behaviours are seen in a wide range of species. This study compared opposite-sex and same-sex pair-bond behaviours, both affiliative within pairs, and agonistic between pairs and individuals, in Humboldt penguins (*Spheniscus humboldti*). This study also looked at where area preferences were within the enclosure to determine possible territory behaviour. A total of 200 minutes of footage of four pairs were analysed; two opposite-sex pairs, and two male-male pairs. It was found that there was no difference between agonistic behaviours ($P = 0.054$), but affiliative behaviours were seen significantly more in opposite-sex pairs ($P = 0.002$). More agonistic behaviours were performed in the morning than in the afternoon ($P = 0.040$). Both opposite-sex pairs had different enclosure preferences to the male-male pairs. As there are differences between same-sex pair and opposite-sex pair behaviours, explanations are explored concerning same-sex pair formation. Further research into this area is needed with opposite-sex pair comparisons to help discern the functions of these behaviours.

Keywords: Pair-Bond, Same-Sex Pairs, Humboldt Penguins, Social Behaviour

Introduction

Behavioural, as well as physiological and psychological knowledge, of as many species, in as many settings as possible, is vital to successful ex-situ conservation (Broom, 1992; Hutchins, Sheppard, Lyles & Casadei, 1995; Martin & Bateson, 2007; Wallace, 2000). Research is important and is actively encouraged by the World Association of Zoos and Aquariums (WAZA, 2005). One of the aims of captivity is to bolster and re-establish populations in the wild, so behavioural considerations must be made when keeping animals to ensure their survival when released (Wallace, 2000). Included in the study of behaviour should be their behavioural repertoire as an individual, but also how they socially interact and respond to the behaviours of their conspecifics (Bohmke, 1995). It may not be the expression of the behaviour, but instead the responses and consequences of it that are beneficial to the individual (Veasey, Waran & Young, 1996).

Social behaviour includes all ways that an individual may influence another (Deag, 1980; Sedden, 1991). It evolves because it has benefits (reduced risk of predation) that outweigh the costs (need for more resources) of group living (Alcock, 2013; Dockery & Reiss, 1999). Without study, it is not possible to know what is normal or abnormal in terms of social behaviour (Deag, 1980; Hill & Broom, 2009; Young, 2005). A behaviour witnessed in captivity that is seemingly abnormal, may just be part of the behavioural repertoire for the species. As well as this, environmental restrictions associated with captivity (Hill & Broom, 2009; Young, 2005; WAZA, 2005) mean that group size has varying effects on behaviour, welfare, and even success of breeding (Fabregas, Guillén-Salazar & Garcés-Narro, 2012; Price & Stoinski, 2007). The social structure that exists in a captive setting may not be adequate for the behavioural and social needs of the captive individuals (Price & Stoinski, 2007).

Humboldt Penguins (*Spheniscus humboldti*) are one of the 18 species of penguin in the world. In Chile, their populations fluctuate frequently and severely. In 1988 they were listed as a "Threatened" species on the International Union for Conservation of Nature (IUCN) Red List. They are now considered Vulnerable with an estimated, and declining, 32,000 mature individuals left in the wild (BirdLife International, 2016). Threats to this species include natural variability in weather (such as El Niño) and in the Humboldt Current, which is their main source of food (BirdLife International, 2016). Fisheries, habitat loss, and the possibility of future pollution and oil spillage risks (Duffy, Hays & Plenge, 1984) are some of the human impacts on this species (BirdLife International, 2016).

Penguins have the potential to be of the most educational value in aquariums because of public interest (Collins, Quirke, Overy, Flannery & O'Riordan, 2016), and education is an important part of any institution. However, due to their colonial living (apart from yellow-eyed penguins, *Megadyptes antipodes*; Darby & Sedden, 1990; Richdale, 1951), consideration must go into the groupings of these species in captivity. There are important social needs that must be met for the exhibition of their natural behaviours (AZA Penguin TAG, 2014). The Association of Zoos and Aquariums (AZA) has a Taxonomic Advisory Group (TAG) for many species kept in captivity. It is their recommendation that no less than ten penguins are kept in one enclosure. This allows adequate mate selection and the fulfilment of social interaction needs (AZA Penguin TAG, 2014). Marshall et al. (2016) state that the size and age of the colony, and the social interactions that occur between the individuals, has a strong influence on the breeding success of Humboldts in captivity. In the wild, due

to the ever-changing environment, birds must breed when conditions are most favourable, or risk possibly fatal consequences for themselves or their offspring (Ancel, Beaulieu & Gilbert, 2013). There is no reason to believe that this is not also the case in captive environments. The AZA Penguin TAG (Ellis, 2005) support the evidence that penguins in a captive environment require social interactions and stimulation from their colony to be able to reproduce successfully. A lack of colony may be detrimental to the penguin's welfare and ability to express normal behaviours (AZA Penguin TAG, 2014).

Within the colony, penguins form pair-bonds. This is often seen in animals that need cooperative parental care to successfully raise their young (Deag, 1980). An important part of pair-bonding is social grooming, seen primarily in birds and mammals, and birds also use vocalisations for recognition as part of their pair-bond (Deag, 1980). For example, the male king penguin (*Aptenodytes patagonicus*) learns its partner's song and uses it to find her amongst the other females when returning from foraging at sea (Pincemy, Dobson & Jouventin, 2010). Being colonial nesting birds, penguins often exhibit territoriality around their nesting site (Deag, 1980). Due to the usually small size of these nesting sites, aggression towards others is common (AZA Penguin TAG: Ellis, 2005). Preference to the area around the nesting site, to defend the site inhabited by the pair and the benefits it brings (Deag, 1980), may also strengthen the pair-bond.

Same-sex sexual and pairing behaviours are those that occur between opposite-sex individuals in the usual reproductive sense, but are instead seen between two same-sex individuals, despite the lack of reproductive context (Bailey & Zuk, 2009). This includes behaviours such as courtship, pair-bonding and attempted copulation. Despite early studies claiming it to be an abnormality (Solanki & Zothansiana, 2012) seen only as a result of captivity (Vasey & Sommer, 2006), this kind of behaviour is observed in wild animals across a broad range of mammals, birds, invertebrates and more (Bailey & Zuk, 2009). It may even surpass the levels at which heterosexuality is seen (Vasey & Sommer, 2006). MacFarlane, Blomberg, Kaplan and Rogers (2007) sum up what many studies try to answer: why are these behaviours seen if they are not directly contributing to the individuals' fitness? Evolutionarily, there are many speculations, but not one that explains all cases (Bailey & Zuk, 2009). Also still in dispute, is whether a same-sex pair occurs by preference (Bailey & Zuk, 2009). In terms of sexual selection, when given the choice, a male-female pair should always be the outcome (Vasey & Sommer, 2006). It is much more difficult to determine whether an animal is sexually-orientated towards members of its own sex, or whether it is merely forming the bond as part of a social need. This idea of a "Social Partnership", one of finding a companion regardless of sex, is supported by several studies (Bailey & Zuk, 2009; Vasey & Sommer, 2006; Zuk, 2006).

Some of the explanations for this phenomenon give insight into how it might be an adaptation. This includes "Social Glue" which is the formation of alliances and a deterrent of conflict, and "Practice" referring to mainly juvenile cases of same-sex pairings, in which they are gaining experience for future opposite-sex pairings. However, there are also explanations that discount the intention of this behaviour and view it as forced or accidental. For example, "Mistaken Identity", where an individual believes they are with a member of the opposite sex, can occur in species that are not sexually dimorphic. However, this would imply a trial-and-error strategy towards reproduction (Richdale, 1951). There is also the "Prison Effect", where

individuals are not able to access members of the opposite sex due to a biased sex ratio (Elie, Mathevon, & Vignal, 2011), and so pair with members of their own sex. Even "Infection" comes as a possible explanation, proposing that an illness heightens the likelihood of a same-sex preference. It is also possible that same-sex sexual behaviour has an impact on reproductive strategies that are yet to be understood (Ricucci, 2011).

In a study of captive stump-tailed macaques (*Macaca arctoides*), male-male sexual behaviour was found to be exhibited five times more than that of male-female sexual behaviour (Solanki & Zothansiana, 2012). In this case, the researchers put this down to dominance assertion and reconciliation between the individuals, rather than the social need that might be seen in other animals. However, whilst these behaviours were not due to preference, they still had important functions depending on the age of the individuals involved. Similarly, in wild orangutans (*Pongo pygmaeus abelii*; Fox, 2001), same-sex sexual behaviour was seen in fully wild individuals just once over the 9000 hours of footage in this study. They concluded it as a natural, yet rare, part of the behavioural repertoire. Interestingly, when it was performed by orangutans that were descended from re-released individuals, the same-sex sexual behaviour occurred in association with tension and aggression, whereas the completely wild orangutans exhibited the same homosexual behaviours, but with affiliative interactions (Fox, 2001). This could be due to behaviours being contextually altered in captive environments, but the behaviours themselves have natural roots.

MacFarlane et al. (2007) found that within birds, male-male sexual behaviour was more common with an increase of polygamy within the species. Female-female sexual behaviour was seen most commonly in that of monogamous species. However, many monogamous birds do not have clear sexual dimorphism, and so evidence for this in wild birds may be under-reported if the sexes of the studied individuals are not known (Elie et al., 2011). As well as this, many studies focusing on same-sex pairs look solely at sexual behaviours, and do not include pair behaviours such as mutual grooming. Elie et al. (2011) suggest that the behaviours that occur between same-sex pairs could be compared to opposite-sex pairs in more depth and across a wider range of species, possibly answering questions of function for same-sex sexual and pair-bond behaviour.

Same-sex penguin pair-bonding has been documented, including cases of Humboldt penguins, and the AZA Penguin TAG (2014) suggest that it is not harmful to the individuals. In a study of wild king penguins, over a quarter of the sample size were homosexually displaying couples, however, it was much rarer for the couples to actually become pair-bonded (Pincemy et al., 2010). They suggest that other publications have not reported on such behaviour due to the sex of the individuals in the study not being known. In a captive example, a male-male penguin pair at Baltimore Zoo fostered an egg and raised a chick for two consecutive breeding seasons (AZA Penguin TAG: Ellis, 2005). Most famously, two chinstrap penguins (*Pygoscelis antarcticus*) Roy and Silo at New York City's Central Park Zoo (Smith, 2004), were pair-bonded for over 6 years and successfully hatched and raised a chick. However, eventually, the pair did split, and one then paired with a female; this is also mentioned as normal in the AZA Penguin TAG (2014). As it is seen that they can separate and pair heterosexually, it is likely that the same-sex sexual and pair-bonding behaviour is not preferential, but has experience-related or social need benefits.

The aims of this study are to broaden the understanding and comparisons of pair-bonding behaviour in same-sex and opposite-sex pairs of captive Humboldt penguins. The objective is to discern whether there are differences between the pair-bonding, affiliative behaviours within the pairs, and agonistic behaviours towards others. From this, it may be better understood the function of these behaviours in this case.

Methods and materials

Ethical note

Before the observation videos were recorded, an animal ethics form and off-site risk assessment were completed for consideration by the Plymouth University Ethical Approval Board. Once consent was given, observations began. Alongside the ethical considerations, the welfare of the animals was considered, however no environmental changes or direct contact occurred with the subjects, so there were no implications as a result of this study.

Study site

The enclosure in this study that houses the ten Humboldt penguins, at the Oceanarium in Bournemouth, has 33m² of land area and a pool surface area of the same (11m x 3m). The pool volume is 198m³ and held 198,000 litres of filtered natural sea water, pumped in via a pipeline, as the Oceanarium is situated next to Bournemouth beach. There are twelve nest boxes in total, six on the left-hand side of the enclosure, and six on the right; these all have top access, and draining floors. The land area is covered in fine sand with large pebbles built up in certain areas, carex grasses and in-built floor drains (covered with large pebbles to avoid injury to the penguins). There are two underwater viewing windows which visitors can look through, as well as two viewing windows behind the enclosure. The enclosure itself is situated in an open-air aviary (6m high x 15m long x 8m wide, forming a half-cylinder) and is surrounded by a walkway, for visitor viewing. The penguins are in a mixed species exhibit with six Inca terns (*Larosterna inca*).

Subjects

The Humboldt penguins at the Oceanarium are the newest addition to the aquarium. They arrived in July 2015 from Faunia, a zoo and botanical gardens in Madrid, Spain. There were ten penguins in total (eight males, two females) and the colony was established a year before this study took place. Since their arrival, three pair-bonds were recognised by the keepers. Two of those pair-bonds were heterosexual, the other was a same-sex pair. A further fourth pair, that was same-sex, was recognised soon after the end of this study (personal communications; A. Hopes, Head Penguin Keeper at the Oceanarium, Bournemouth), and so was included in all analyses.

Each penguin's age, sex, and relation to other individuals within the colony was known (see Table 1). The individuals had been sexed at their previous establishment, and so each penguin had a band on their flipper that meant they could be quickly and easily distinguished from the rest. Females had their bands on their right flippers, and males had theirs on their left; the heterosexual pairs had the same colour bands on the appropriate flippers for their sex.

The two opposite-sex pair-bonds were Pair 1, consisting of Pingu (F) and Augustin (M), and Pair 2 being Darwin (M) and Maria (F). The same-sex pair-bonds were between Diego (M) and Zorro (M) (Pair 3) and Chile (M) and Private (M) (Pair 4). That leaves two single males; Cobblepot and Peru (see Table 1).

All of the penguins received an Aquaminivit daily as a dietary supplement used for penguins, otters and seabirds. Each tablet contains several vitamins such as vitamin A and C, as well as iron, and other metabolites (International Zoo Veterinary Group, N.D.). As well as this, three of the penguins (Cobblepot, Darwin, and Pingu) all received a daily dose of meloxicam (Metacam; 1ml) for chronic arthritis, and glucosamine sulphate (375mg) as a joint lubricant (see Table 1). All medications were given with the morning feed, around 8:00am.

Table 1: Information on the penguin colony at the Oceanarium, Bournemouth

Pair	Name	Sex	Age	Relations within the colony?	Health Issues?	Daily Medications
1	Pingu	F	21	None	Chronic arthritis	Aquaminivit; Metacam; glucosamine sulphate
1	Augustin	M	13	None	None	Aquaminivit
2	Maria	F	12	None	None	Aquaminivit
2	Darwin	M	14	Brother of Diego	Chronic arthritis	Aquaminivit; Metacam; glucosamine sulphate
3	Diego	M	14	Brother of Darwin	None	Aquaminivit
3	Zorro	M	12	Father of Chile and Peru	None	Aquaminivit
4	Chile	M	6	Son of Zorro	None	Aquaminivit
4	Private	M	12	None	None	Aquaminivit
N/A	Cobblepot	M	12	None	Chronic arthritis	Aquaminivit; Metacam; glucosamine sulphate
N/A	Peru	M	7	Son of Zorro	None	Aquaminivit

Data collection

Twenty videos were recorded (using a Canon Legria HF R47 Camcorder) over the course of ten consecutive days in the post-nesting period. Defined by Marshall et al. (2016) as the period between the breeding season and their moulting period; in this case, this occurred in July 2016. Two videos were recorded per day, one in the morning (AM; around 9:50am), before the Oceanarium was open to the public, and one in the afternoon (PM; around 4:40pm), just before the Oceanarium would close. None of the videos were recorded within an hour of a feeding time. Each recording was 20 minutes long and looked at all the penguins at once. At the time of each recording, environmental conditions were noted such as temperature and humidity (using an Electronic Temperature Instruments digital hygrometer), wind speed (using a Technoline anemometer), pool temperature (using a Traceable water probe), and rain fall (using a simple rain gauge setup).

Once these videos were recorded, a total of 400 minutes of footage was available to be analysed for the penguin's behaviours. Out of these 400 minutes, 200 of them were analysed using Observer XT 13 (for which a Lab Risk Assessment Form was completed). These 200 minutes were made up of two five-minute observation periods from each 20-minute video. These were selected by Microsoft Excel's random

number generator function, whilst avoiding an overlap of the observational periods, and thus a replication of the same data. A number between 0 and 900 would be generated, and this would be the start time of the five-minute observation within the 20-minute video. The behaviours seen in these videos were scored using an ethogram adapted from Marshall et al. (2016), Sedden (1991), Richdale (1951) and personal observations of the colony used in this study (a shortened version can be seen as table 2). As well as this, the location of the penguins in the enclosure at any given time was recorded, including the pool (“Water Surface A”, “Water Surface B”, and “Underwater”; which is later converged to be “Water”), land (“Land 1”, “Land 2”, “Land 3”, and “Land 4”), and all 12 nestboxes (see Figure 1).

Data analysis

Data was extracted from the Observer software and collated in Microsoft Excel to be analysed in IBM SPSS Statistics 23 and Minitab 17. Not all of the defined behaviours were included in the statistical analysis; some were not performed over the observation period, and others were irrelevant to the aims of this study (such as “Out of Sight” and “Non-Social Behaviour”). The behaviours that were included in the analyses can be found in Table 2.

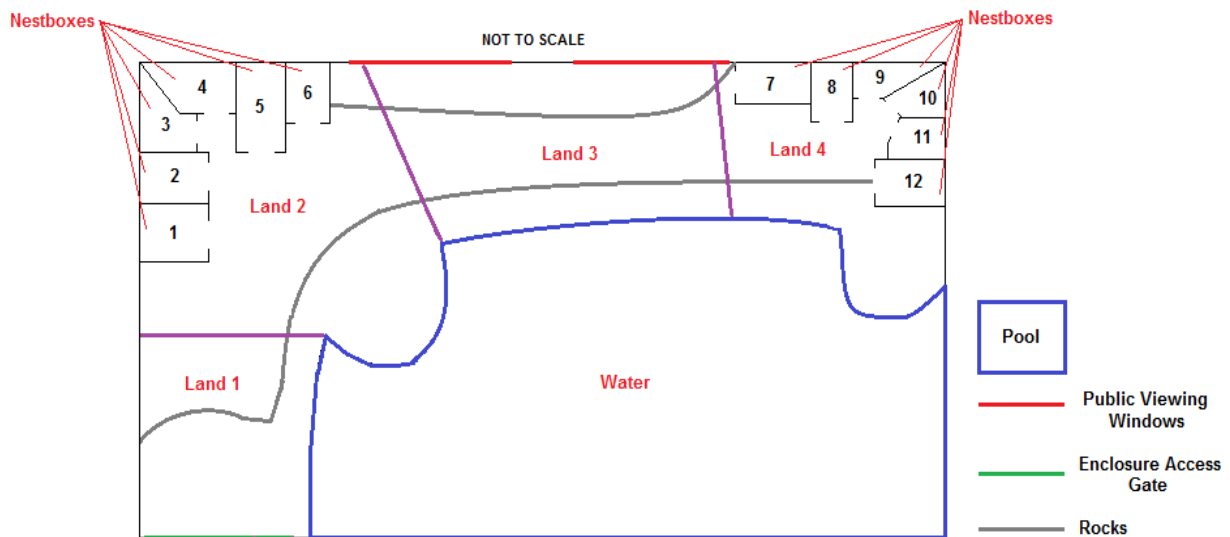


Figure 1: The enclosure layout of the penguin exhibit at the Oceanarium, Bournemouth. Included are the area sections used in the analysis to look at enclosure usage and preferences.

The agonistic and affiliative duration data was tested for normality. As the data was not normally distributed, a Generalised Linear Model (GLM) was used, with a Tweedie Log Link. Two GLMs were conducted including all pairs, and all AM and PM observations; one for agonistic behaviours, and one for affiliative. Also, a Bonferroni test was used for pairwise comparisons if an overall significance was found. A Mann-Whitney test was then conducted to discern whether there were affiliative or agonistic behavioural differences between the AM and PM observations.

Table 2: An ethogram of definitions for all the behaviours recorded and used in the statistical analyses.

Type	Behaviour	Description	Adapted From
Affiliative	Grooming	Preening can occur between pairs as a pair-bonding behaviour. May also include allo- or mutual preening, where two penguins preen each other simultaneously.	Marshall et al., 2016; Sedden, 1991; Richdale, 1951
	Mutual Trumpeting	Occurs between pairs. When the pair both lean forward and then upwards in synchrony whilst trumpeting. May commence with a variety of soft vocalizations and head shaking, building in intensity to the full display, often subsiding with similar actions. May be performed any time a pair are together.	Marshall et al., 2016; Sedden, 1991
	Beak Hitting	Occurs between pairs, often before or after another affiliative behaviour. The pair shake their heads so their beaks repeatedly hit together.	Personal Observation
	Sexual	In a pair, one of the individuals stands behind the other and beats their flippers onto the others back and sides, they may also rub the underside of their head on the recipients back and head.	Marshall et al., 2016
Agonistic	Sideways Stare	Penguin peers through a narrowed eye.	Marshall et al., 2016
	Alternate Stare	Penguin stares from one eye, then the other. It is given from both upright and prone positions in response to intrusion onto the nesting territory.	Marshall et al., 2016; Sedden, 1991
	Pointing	Bill is pointed to individual who is annoying the pointer. Penguin leans towards other, neck extended and may charge at other with beak agape.	Marshall et al., 2016; Sedden, 1991
	Beak Locking	Locking of beaks whilst tugging and grunting.	Marshall et al., 2016
	Dominance Mounting	Non-pair penguins only. One penguin tries to mount another that is not its mate.	Personal Observation
	Couple Territorial	Two pairs of penguins "face-off". Both pairs stand together facing the other pair, raising their heads from their feet whilst singing and grunting.	Personal Observation

Additionally, the enclosure count data was collated in Microsoft Excel, and the enclosure areas were condensed from 19 areas into six. These were "Land 1", "Land 2", "Land 3", "Land 4", "Water" (a combination of "Water Surface A", "Water Surface B", and "Underwater"), and "Nestbox" (a combination of all nestboxes). The nestboxes were combined as one area, despite three of the four pairs having established nestboxes, because the interest was not in which nestbox they were in, but how they apportioned their time to be within one at all. This data was then put in

to Minitab 17 and Chi-Square association tests were conducted to find differences of enclosure use within pairs. The data for the individuals in the pairs was then added together for between pair comparisons. As a final descriptive exploration of the data, bar graphs of the pair's enclosure use were made.

Results

The omnibus test from the agonistic duration data showed there were no significance differences between the pairs or between the days, although it did appear to be approaching significance (Likelihood Ratio Chi-Square = 13.839, $df = 7$, $P = 0.054$). However, the affiliative duration data was significantly different between pairs (Likelihood Ratio Chi-Square = 22.046, $df = 7$, $P = 0.002$). Post hoc tests showed that the significant difference (Wald Chi-Square = 19.638, $df = 3$, $P < 0.001$; see Figure 2) was between Pair 2 (Maria and Darwin) and Pair 4 (Chile and Private).

When testing the difference between AM and PM observations in regards to affiliative and agonistic behaviour, it was found that there was no difference in affiliative behaviours (Mann-Whitney $U = 674$, $N1 = N2 = 40$, $P = 0.167$). However, the amount of agonistic behaviour performed in the morning was significantly higher than was performed in the afternoon (Mann-Whitney $U = 663$, $N1 = N2 = 40$, $P = 0.040$; see Figure 3).

The individuals within Pair 1 and those in Pair 2, were each found to be significantly different to their respective partner in their enclosure preferences (Pair 1: Pearson Chi-Square = 18.440, $df = 4$, $P = 0.001$; Pair 2: Pearson Chi-Square = 29.194, $df = 4$, $P < 0.001$). In each of these pairs, one of the members suffers from chronic arthritis (Pingu in Pair 1, and Darwin in Pair 2), and in both cases, the significances were caused by the amount of extra time that the arthritis sufferer spent in the water in comparison to the healthy partner.

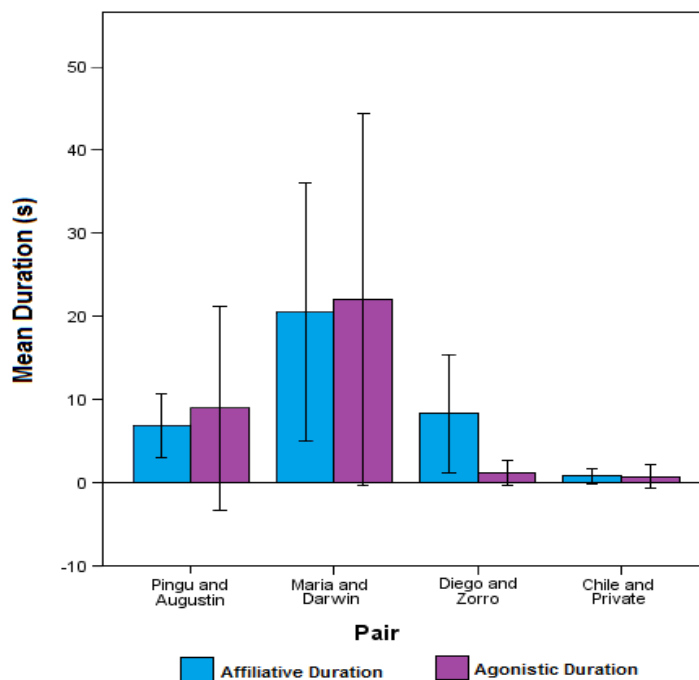


Figure 2: The affiliative and agonistic behaviour duration (in seconds) differences between all pairs across all observations,

For this reason, the same tests were run again, but with the “Water” count data removed. The result became non-significant for both pairs (Pair 1: Pearson Chi-Square = 2.550, $df = 3$, $P = 0.466$; Pair 2: Pearson Chi-Square = 3.995, $df = 3$, $P = 0.262$). Pair 3 and Pair 4 both had non-significant outcomes with the same test, even with the “Water” count data still present (Pair 3: Pearson Chi-Square = 6.676, $df = 5$, $P = 0.246$; Pair 4: Pearson Chi-Square = 4.772, $df = 5$, $P = 0.444$).

The heterosexual pairs, Pair 1 and Pair 2, showed a significant difference between their enclosure usage counts (Pearson Chi-Square = 13.653, $df = 3$, $P = 0.003$). The same-sex pairs, Pair 3 and Pair 4, were also significantly different (Pearson Chi-Square = 20.860, $df = 4$, $P < 0.001$), however, this difference was skewed by “Nestbox” usage. Pair 4 did not have an established nestbox at the time of this study, and so would have spent little of their time in one. For this reason, the test was

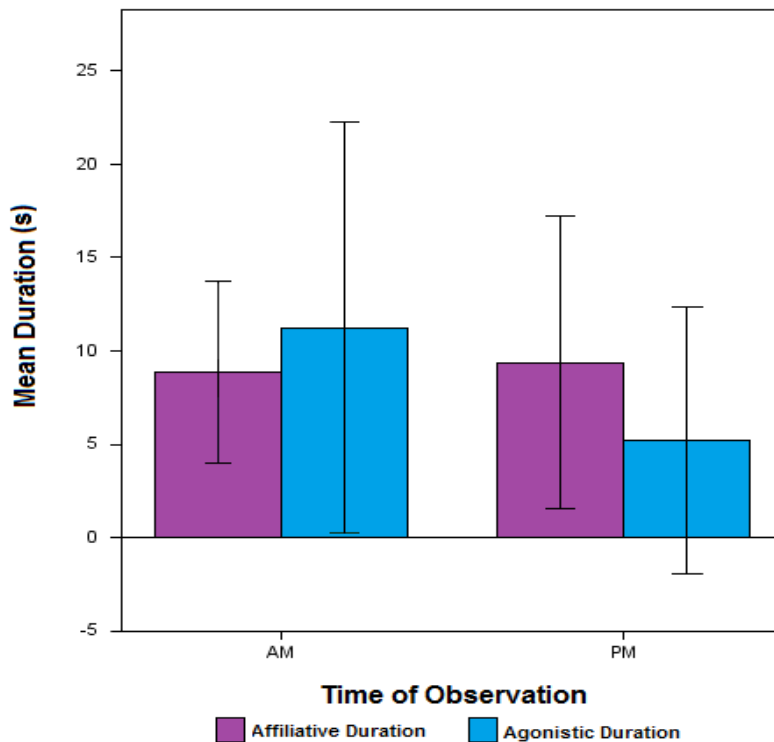


Figure 3: The affiliative and agonistic behaviour duration (in seconds) differences between morning and afternoon observation times, including Standard Error bars.

conducted again with the “Nestbox” count data removed, and the result showed there was no difference in the enclosure use between these pairs (Pearson Chi-Square = 0.922, $df = 3$, $P = 0.404$) (see Figures 4 to 7 for pair enclosure usage, including all enclosure areas). Between both opposite-sex pairs, and both same-sex pairs, there were significant differences in the way the enclosure was used. Pair 1 and 2 spent significantly more time in “Land 2” than Pair 3 or 4. Whilst Pairs 3 and 4 spent significantly more time in “Land 4” than Pairs 1 or 2 (Pair 1 against Pair 3: Pearson Chi-Square = 94.812, $df = 5$, $P < 0.001$; Pair 1 against Pair 4: Pearson Chi-Square = 102.219, $df = 5$, $P < 0.001$; Pair 2 against Pair 3: Pearson Chi-Square = 54.137, $df = 5$, $P < 0.001$; Pair 2 against Pair 4: Pearson Chi-Square = 111.024, $df = 5$, $P < 0.001$).

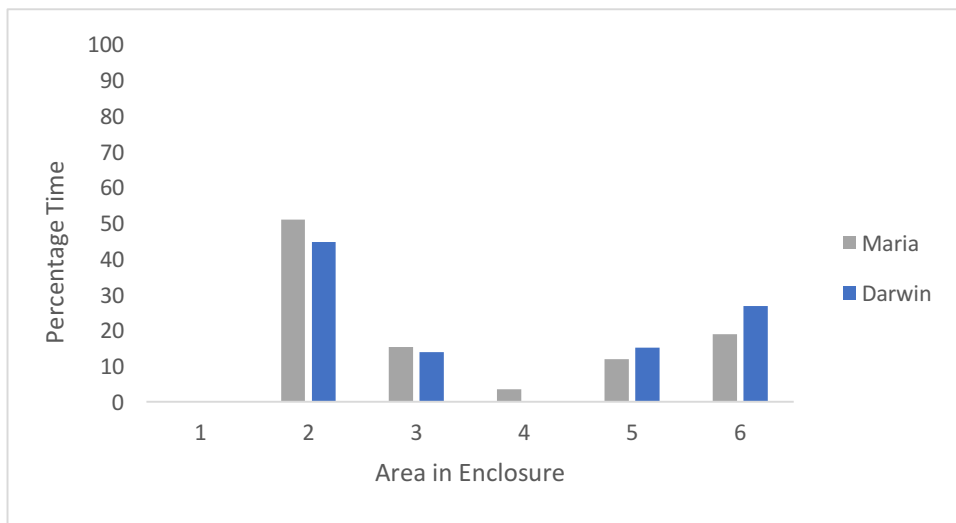


Figure 4: Pair 2's percentage time in each enclosure area over all observations. Areas: 1 = Land 1, 2 = Land 2, 3 = Land 3, 4 = Land 4, 5 = Water, 6 = Nestbox.

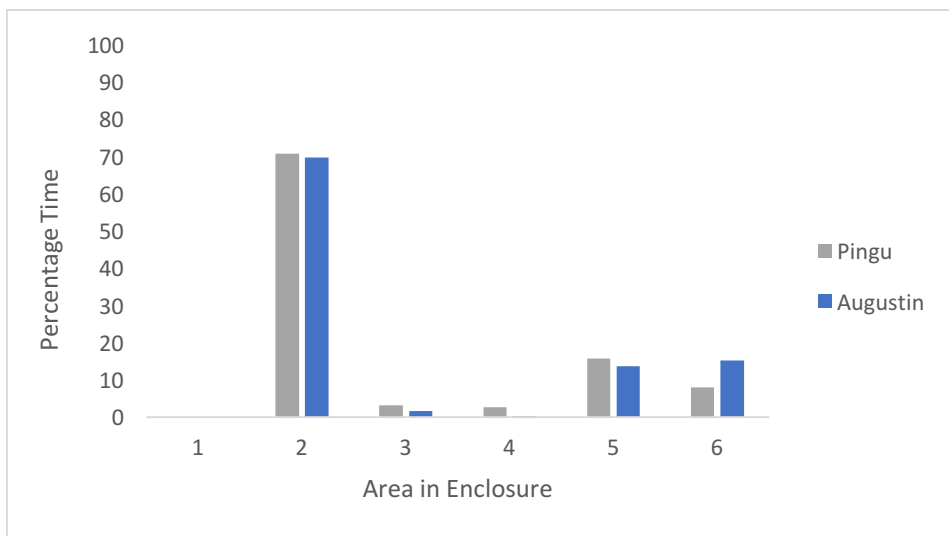


Figure 5: Pair 1's percentage time in each enclosure area over all observations. Areas: 1 = Land 1, 2 = Land 2, 3 = Land 3, 4 = Land 4, 5 = Water, 6 = Nestbox.

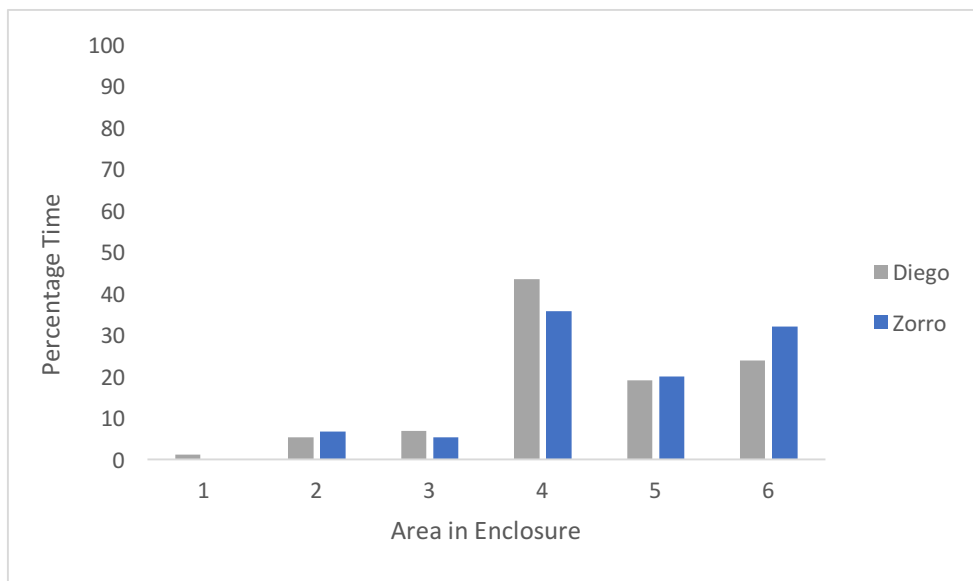


Figure 6: Pair 3's percentage time in each enclosure area over all observations. Areas: 1 = Land 1, 2 = Land 2, 3 = Land 3, 4 = Land 4, 5 = Water, 6 = Nestbox.

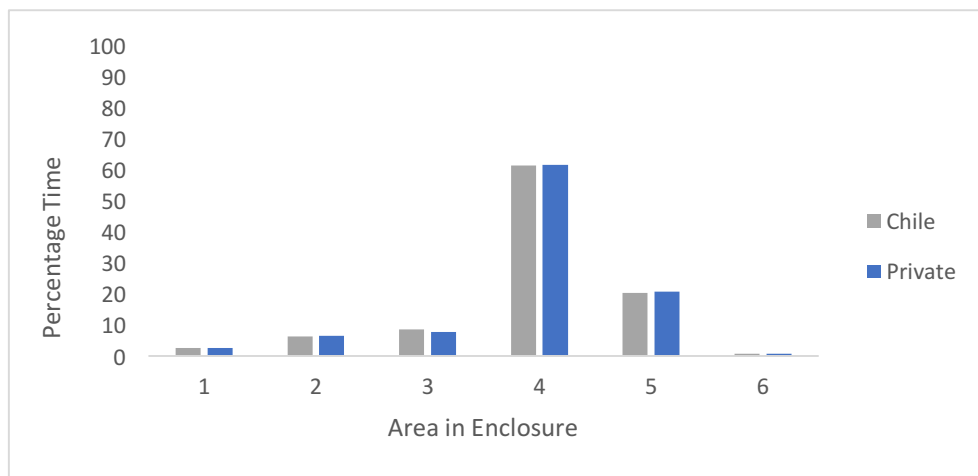


Figure 7: Pair 4's percentage time in each enclosure area over all observations. Areas: 1 = Land 1, 2 = Land 2, 3 = Land 3, 4 = Land 4, 5 = Water, 6 = Nestbox.

Discussion

Same-sex and opposite-sex pair differences

The duration of affiliative behaviours was significant between Pair 2, an opposite-sex pair, and Pair 4, a newly established same-sex pair, but not between any of the other pairs. It may be that because there are no significances between Pair 3 (another same-sex pair) and either Pair 1 or Pair 2, that the significance seen between Pair 2 and 4 is an anomaly. Elie et al. (2011) found that there was no significant differences in affiliative behaviour between same-sex and opposite-sex pairs, so a bigger sample size for both pair-types may show that no true significance exists, and that it is just an artefact of this study.

Interestingly, there was no significant difference in agonistic behaviours between any pairs. However, from Figure 2, it can be seen that at least one heterosexual pair performed a lot more agonistic behaviours than either same-sex pair, who were agonistic for a small proportion of the overall data period. What is key, is that sometimes the opposite-sex pairs exhibited just as little agonistic behaviours as the same-sex pairs. For this reason, it does not show as significantly different, but again, it is likely that with a bigger sample size, a significance may be seen.

From these results, showing that only affiliative behaviours were less in same-sex pairs, it may be possible to conclude that the same-sex pairs formed for the benefits that pair-bonding brings, such as increased territory defence. If agonistic behaviours were also significantly less in same-sex pairs, then it could be attributed to the “Social Partnership” theory, just in a lesser degree than that which opposite-sex pairs exhibit.

AM and PM comparison

Morning (AM) and afternoon (PM) comparisons saw no significance between affiliative behaviours. However, it was seen that there was significantly more agonistic behaviour in the AM observation periods, than there was in the PM ones. Medications and pain killers were administered with the morning feed as previously stated, and can have a side effect of drowsiness (Karaman, 2015). It may be that the pain killers did not take effect until later in the morning, and continuing throughout the day. When they did take effect, it is possible that they made it so the individuals that were usually in some sort of pain (from arthritis), were pain-free and so were able to carry out a more normal behavioural repertoire. As this effect was not present in the AM observations, this may be the cause of the higher agonistic behaviours seen. All agonistic behaviours were directed at individuals outside of the pair that the exhibitor was in.

Enclosure preferences

The data used for the pair enclosure preferences was frequency counts, rather than duration. When the individuals in Pair 1 and Pair 2 were found to have significantly different enclosure preferences, it was because of the “Water” area usage of one individual in both pairs. As suggested in the Results section, this may have been due to the member of the pair suffering from arthritis (Pingu in Pair 1, and Darwin in Pair 2) using the pool more as pain-relief for their joints (as described similarly for humans in Evcik, 2015). When the “Water” count data was then removed, and the significance disappeared, it showed that, within the pairs, the individuals used similar enclosure areas to each other. There was, however, a significant difference seen between the pairs. Pair 1 and 2 spent more time in “Land 2” than Pair 3 and 4. This was expected, as both Pair 1 and Pair 2 have a nestbox that is situated within “Land 2”, so it is not

unusual that they spend more time near their nestboxes. Despite this similarity, the pairs still used the enclosure differently to each other, with Pingu and Augustin (Pair 1) not using “Land 3” and “Nestbox” as much as Maria and Darwin (Pair 2). This may be due to an overlap of territories, with Pair 1 potentially being more dominant than Pair 2, and so to avoid conflict, Pair 2 had to rest in “Land 3”, further from their nestbox.

There was no significance in enclosure area preference within the same-sex pairs but between them, Pair 3 had significantly different enclosure preferences to Pair 4. Mostly this was due to nestbox usage. Pair 3 had a nestbox in “Land 4”, however Pair 4, did not have an established nestbox at all, and so rarely entered any of them. They still spent a lot of their time around “Land 4” along with the other same-sex pair. When nestbox usage count data was removed, the result became non-significant. This means that Pair 3 and Pair 4 shared similarities in their enclosure preferences.

Between pairs, both Pair 1 and Pair 2 had different enclosure preferences to Pair 3 and Pair 4 (see Figures 4 to 7). Due to the location of the nestboxes of these pairs (excluding Pair 4), this is expected. Pair 1 and Pair 2 both had nestboxes in “Land 2” and Pair 3’s nestbox was in “Land 4”. It is not clear from this study whether the enclosure usage between the pair is different because of the nature of their pair-bonds (same-sex or opposite-sex). It is perhaps a territorial defence that inhibits either pair-type from travelling to the other side of the enclosure, to where the other pair-type resides; as a discrimination against the other type. On the other hand, it could be chance that the opposite-sex pairs have chosen to nest on the other side of the enclosure from the same-sex pairs. Further study could show that opposite-sex pairs have an aversion to same-sex pairs, or, indeed, the other way around.

Implications and conclusions

As mentioned earlier, there are many theories that try to understand the function, or reason, for same-sex sexual and pair-bonding behaviours. From this study, it is likely that there is not just one function for this phenomenon, but more a combination of theories. The “Social Partnership” theory suggests that there is a need for companionship in monogamous species (Bailey & Zuk, 2009; Vasey & Sommer, 2006; Zuk, 2006). In the wild, being paired has survival benefits (Elie et al., 2011; Zuk, 2006) for the individuals, but also any offspring produced get the benefit of parental care from two adults. For this reason, regardless of the sex of the other individual, a pair-bond is a natural survival tactic, which, considering the evidence, is not suppressed in captivity. The captive setting may give rise to more same-sex sexual and pair-bonding behaviours because of the constraints within the environment. Captivity is usually not able to mimic perfectly the environment that would be found in the wild, and this includes group size and composition. As Elie et al. (2011) suggest, mate choice is severely reduced due to lack of dispersion and bias sex ratios that are a constraint of captivity. As a result, individuals form atypical same-sex bonds more frequently than might be observed in the wild (Ricucci, 2011).

Pincemy et al. (2010) discuss the possibility that the same-sex pair-bonds are formed between younger males, thus supporting the “Practice” hypothesis for same-sex sexual and pair-bonding behaviour. However, in this study, three of the four males involved in the male-male pairs were over ten years old, and so it is unlikely that the pair-bond was formed to gain experience. Furthermore, one of the males in Pair 3 had previously been in a successfully breeding opposite-sex pair-bond, producing two (known to this study) chicks, Chile and Peru, who also reside in this colony. This

again suggests that experience was not the likely reason for the formation of these pair bonds, as one of the members was already previously successful. As well as this, two of the colony members, Darwin and Diego, are brothers. One of these brothers is in an opposite-sex pair, whilst the other is in a same-sex pair. Given that they are the same age, and likely had similar experiences as juveniles, it is unreasonable to believe that an experience-based same-sex pair-bond may be needed for one brother, but not the other.

Macfarlane et al. (2007) found that polygamous species showed more male-male same-sex sexual and pair-bonding behaviour, and monogamous species showed more female-female same-sex sexual and pair-bonding behaviour. Following this, as Humboldt penguins are known to be a monogamous species, male-male pair-bonds would not be expected. It is likely that the pair-bonds seen in this study then, may have formed through a type of "Prison Effect" from a bias sex ratio. Pincemy et al. (2010) concluded from their study of wild king penguins, that the sex ratio bias could be a likely explanation for the same-sex displaying pairs that they observed. In this study, the sex ratio was biased towards males and the introduction of females may break these pair-bonds. Elie et al. (2011) showed that the male-male pair-bonds in their study were strong and stable. Even when new females were introduced to the group, the same-sex pair-bonds remained. Similarly, at the Oceanarium shortly after this study concluded, six juvenile Humboldt penguins (three of which were female) were introduced to the colony making the sex ratio less male-bias (from a 0.8 male-bias, to a 0.688 male-bias). From personal communications (A. Hopes, Head Penguin Keeper at Oceanarium, Bournemouth) it is understood that even with the introduction of new females, both male-male pairs remained pair-bonded. It may be possible to conclude then, that the male-bias sex ratio was not the reason for the formation of the same-sex pair-bonds observed. On the other hand, there is the potential that the established male-male pair-bonds did not break because of the introduced juvenile females' inexperience; a same-sex pair-bond would be more beneficial to them, than a pair-bond with an inexperienced female. In support of this, Elie et al. (2011) found that if a male was already in an established relationship (even if it was with another male), their motivation to become paired with an introduced female was decreased. Whilst it may be a combination of theories, overall it seems the same-sex pair-bonds are forming to meet a need for companionship with another individual, regardless of their sex, much like in the "Social Partnership" theory.

Limitations

The way this study was carried out could be improved upon if conducted again, and from these improvements, different results could be seen. As this study used recorded footage to score the behaviours of the individuals, there is a possible loss of visibility of all the animals at once, meaning that important behaviours relating to the study could be missed (Martin & Bateson, 2007). When the penguins were in their nestboxes, they could not be seen, and so any pair-bonding behaviours that occurred between the pairs whilst they were in them was lost. As another example, Humboldt penguins have no sexual dimorphism; the bands worn by the penguins are on a certain flipper depending on the sex of the individual, for easy identification (Martin & Bateson, 2007). If the individual's band was not visible, identification could be difficult, especially as the quality of the video was not ideal. If a similar study were to be conducted, it may be worth having several vantage points for filming, in the hopes of increasing the accuracy of the results. There was, however, only one observer and behavioural scorer, which eliminated any inter-observer bias that may

have occurred (Martin & Bateson, 2007), but this does also mean that there was no inter-observer reliability. The continuous recording was beneficial in knowing exact durations, instead of it all being frequency counts (Martin & Bateson, 2007). It is possible that an individual could have exhibited the behaviour more in terms of frequency, but less in terms of duration, so possible further studies could look at both durations and counts, as there may be different results depending on the type of data used in the analysis.

The main drawback of this study was the sample size. Not just within the colony itself, having two pairs in each category of pair-type, but also because only one site was studied, in only one time period of the year. Due to this, these results cannot be reliably generalised for a wider understanding into same-sex sexual and pair-bonding behaviours. Whilst the data here may have included interesting results, an increase in sample size would greatly improve the reliability of the study. More studies in colonies at other zoos and aquariums, with longer observation periods and in different seasons, would give more insight into these types of behaviours, and wild studies could give even greater detail. However, properly sexing each penguin beforehand is vital as these behaviours may be over- or under-reported as suggested by Elie et al. (2011).

Finally, a more comprehensive and species specific ethogram could benefit this study. Many of the behaviours included were adapted from other penguin studies, but not all penguins behave in the same way. For example, the ethogram used by Sedden (1991) for yellow-eyed penguins was part of a study on wild individuals; this ethogram could include behaviours that are only seen in the wild, or miss out behaviours only seen in captivity. Furthermore, yellow-eyed penguins are the least colonial species of penguin (Darby & Sedden, 1990; Richdale, 1951) and they do not show true colony breeding areas like other penguin species do (Darby & Sedden, 1990). To use their behavioural repertoire to study the behaviour of Humboldt penguins could be detrimental to the results of the study. In addition to this, there are aggressive behaviours seen in other species of penguins that are not seen in any other species (such as Adélies; Richdale, 1951). A range of behavioural studies need to be conducted on all species individually, to improve knowledge of behavioural repertoires. From this, any further studies involving these species will be more reliable.

Further research

This study, whilst containing flaws, further opens questions about the functions and possible social benefits of pair-bonds in penguins. Samples across different seasons of the year, especially in the breeding season, could greatly improve knowledge and understanding of why same-sex sexual and pair-bonding behaviour occurs. Future studies should also attempt to test the strength of male-female pairs. It was mentioned that male-male bonds can be strong and, once established, potentially favourable over opposite-sex pairings (Elie et al., 2011). Does this mean that heterosexual pair-bonds have the same or more strength, as they are able to exhibit these behaviours with the reproductive context that the same-sex pairs lack?

Following this, whilst the AZA penguin TAG (2014) specify to keep at least ten penguins in the same enclosure for adequate mate selection, they do not specify the ideal group composition. Research into ideal colony sex ratios could greatly improve the welfare of captive penguins.

It would be beneficial for the research in this area to be split into easily studied sections. One assessing how similar the behaviours are between the two pair-types; going into detail about which behaviours same-sex pairs exhibit more or less than opposite-sex pairs. Another section could look into whether previously same-sex pair-bonded individuals had higher success rates when it came to reproduction and chick-raising.

There is much more that can be understood from further research. It is important that as many colonies (both wild and captive) in as many settings (changes in group size, composition, and even enclosure design) are studied, to fully understand the social and reproductive benefits of same-sex sexual and pair-bonding behaviour.

Acknowledgements

Thank you to the Oceanarium in Bournemouth, for allowing me to conduct my study on their penguins, and to all the staff, past and present, whilst I was there that helped to set me on the right path. I'd like to thank my advisor Sarah Collins for her help over the months, without her, this would not have been possible. Also Jane Ackerman (as well as the entirety of the Eco-Loans team), who helped so much with the behavioural software, and spent her valuable time helping me out.

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