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It takes two to hatch: The role of cooperation and pair bonding in captive northern rockhopper penguins (Eudyptes moseleyi) breeding success

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Abstract

Penguins are among the three most threatened groups of seabirds, with the northern rockhopper penguin (*Eudyptes moseleyi*) being particularly at risk. Recognised as a distinct species only since 2006, *E. moseleyi* remains understudied, especially regarding the behavioural drivers of breeding success in captivity. To address this research gap, a study on the intra-pair behaviours linked to coordination (nest building, proximity up to three metres, nest switching) and bonding (mutual display, allopreening, proximity up to beak length), as well as sex differences in investment, was conducted on a population at Vienna Zoo (n = 13 pairs). Five pairs successfully hatched at least one egg, while eight did not. During incubation, females invested significantly more time in egg incubation, whereas males spent more time gathering nesting material and building nests across all breeding phases. These sex differences did not differ between successful and unsuccessful groups. Among bonding behaviours, proximity up to beak length was significantly longer in successful pairs. A Firth logistic regression identified three predictors of success: greater odds with increased time spent in proximity up to beak length and between beak length and three metres, and reduced odds for mixed-origin pairs (one zoo-born, one wild-born). No other demographic variables predicted success. These findings highlight the importance of intra-pair proximity for reproductive success and suggest that mixed-origin pairings may face coordination challenges. This study provides first detailed insights into breeding behaviour of *E. moseleyi* in captivity, emphasising the value of behavioural monitoring for welfare and conservation management.

Abstract - German Version

Pinguine zählen zu den drei am stärksten bedrohten Gruppen der Seevögel, wobei der Nördliche Felsenpinguin (*Eudyptes moseleyi*) besonders gefährdet ist. Er ist erst seit 2006 als eigenständige Art anerkannt, daher ist *E. moseleyi* bislang wenig erforscht, insbesondere in Bezug auf die verhaltensbezogenen Faktoren des Bruterfolgs in menschlicher Obhut. Um diese Forschungslücke zu verringern, wurde eine Studie zu innerpaarlichen Verhaltensweisen durchgeführt, die mit Koordination (Nestbau, Nähe bis zu drei Meter, Nestwechsel) und Bindung (gegenseitige Balz, gegenseitige Gefiederpflege, Nähe bis zu Schnabellänge) in Zusammenhang stehen, sowie zu geschlechtsspezifischen Unterschieden im Brutaufwand. Untersucht wurde eine Population im Tiergarten Schönbrunn (n = 13 Paare). Fünf Paare waren erfolgreich in mindestens einem Eischlupf, während acht Paare erfolgslos blieben. Während der Brutzeit investierten Weibchen signifikant mehr Zeit in die Inkubation, während Männchen über alle Brutphasen hinweg mehr Zeit mit dem Sammeln von Nistmaterial und dem Nestbau verbrachten. Diese geschlechtsspezifischen Unterschiede unterschieden sich nicht zwischen den Erfolgsgruppen. Von den Bindungsverhalten zeigte nur die Nähe bis zu Schnabellänge eine signifikant längere Dauer bei erfolgreichen Paaren. Eine Firth-Logistische-Regression ergab drei Prädiktoren für den Bruterfolg: erhöhte Erfolgswahrscheinlichkeit bei längerer Nähe bis zu Schnabellänge sowie zwischen Schnabellänge und drei Metern, und verringerte Erfolgswahrscheinlichkeit bei Paaren gemischter Herkunft (ein Zoo geborenes und ein wildgeborenes Individuum). Andere demografische Variablen sagten den Erfolg nicht voraus. Diese Ergebnisse unterstreichen die Bedeutung innerpaarlicher Nähe für den Bruterfolg und deuten darauf hin, dass Paarungen mit gemischter Herkunft Koordinationsschwierigkeiten haben könnten. Die Studie liefert erste detaillierte Einblicke in das Brutverhalten von *E. moseleyi* in menschlicher Obhut und betont den Wert verhaltensbiologischer Beobachtungen für das Tierwohl und Tierartenschutz.

Contents

1. Introduction

Penguins are among the three most threatened groups of seabirds (Dias et al., 2019) - as diving and flightless birds, penguins are highly adapted to marine life, making them particularly sensitive to oceanographic changes (Delord et al., 2021). Although they spend only around 20% of the year on land, this short time is very important in their life. They must find a nest site, mate, lay eggs, rear chicks and moult. Most penguin species share some breeding characteristics, such as breeding synchrony for annual reproduction on land, and alteration of sojourns on land and at sea between partners during the chick-rearing period (Ancel et al., 2013). While some penguin species have been extensively studied, others remain largely unexplored, resulting in a lack of information concerning these species.

The northern rockhopper penguin (*Eudyptes moseleyi*) is an endangered species. It inhabits two groups of isles in the southern Atlantic and Indian Ocean. In general, declines of penguin populations are caused by at-sea factors including climate change, shifts in marine ecosystems and overfishing (Cunningham & Moors, 1994; Trathan et al., 2015). However, there are also terrestrial factors that have an influence on the breeding success, such as the timing of breeding (Kemper et al., 2007), nesting density (Sherley et al., 2014) and the response to threats by breeding birds (Miyazaki & Waas, 2003). Due to the fact that northern rockhopper penguins have only been seen as a separate species to other rockhopper penguins since 2006 (Banks et al., 2006; Jouventin et al., 2006), the focus so far has been on the southern rockhopper penguins. Therefore, *E. moseleyi* has been poorly researched and understood. While general breeding biology has been described for wild populations (Booth & McQuaid, 2013; Delord et al., 2021; Steinfurth et al., 2019; Wilson et al., 2010), no study to date has systematically examined how specific affiliative and coordination behaviours relate to reproductive success in captive *E. moseleyi*.

The breeding success of rockhopper penguins on Gough Island, which is the main living area of *E. moseleyi*, is estimated at around 36% (Cuthbert & Sommer, 2004), which is low compared to the breeding success of other rockhopper penguin populations (between 35-61% at Falkland Islands (Pütz et al., 2001)). Wilson et al. (2010) conducted a study examining the effects of nest density and threat-response dynamics on a colony of northern rockhopper penguins. Nevertheless, research on reproductive success of both, wild and captive populations, and on the influence of social relationships on breeding behaviour remains limited.

1.1 Ex-Situ Conservation

Due to the endangered status of northern rockhopper penguins and the difficulty of observing them in the wild, studies on populations housed in zoos offer a unique opportunity to investigate their behaviour under controlled conditions. Such research not only contributes to conservation knowledge (Borecki et al., 2024; Marshall et al., 2016), but also supports evidence-based welfare and management decisions for captive populations (Melfi, 2009). Ex-situ management is a conservation intervention designed to halt and reverse species declines, ranging from captive breeding and release programmes to targeted research, all of which can prevent extinction and move populations closer towards recovery and sustainability (McGowan et al., 2017). Retaining sufficient genetic variation for both short and long-term sustainability is a major aim of ex situ programmes, where assessment of genetic diversity in both zoo and wild populations is essential for informed and effective conservation breeding and reintroduction (Hunter et al., 2025). At the same time, ex-situ conservation must ensure that species-typical behavioural and cognitive traits are preserved through enrichment and management strategies (Clark et al., 2023). To reinforce the long-term resilience of such efforts for endangered species, including the northern rockhopper penguin, conservation programmes therefore prioritise the establishment of demographically robust, genetically diverse reserve populations that can buffer against stochastic losses and provide a viable safety net alongside in-situ conservation (Witzenberger & Hochkirch, 2011).

These principles are directly reflected in the management of northern rockhopper penguins in European zoos. The Vienna Zoo in Austria maintains a significant colony of around 15 breeding pairs with varying reproductive success and participates in the European Endangered Species Program (EEP) for *E. moseleyi*, coordinated by the European Association of Zoos and Aquaria (EAZA). This programme aims to secure a genetically and demographically healthy population while promoting positive animal welfare (EAZA, 2024). Ensuring the fulfilment of the psychological and physiological requirements of zoo animals can be challenging (Marshall et al., 2016), even more given the lack of knowledge regarding the species in question. Behavioural monitoring can therefore serve as a practical management tool, allowing zoos to identify compatible pairs, optimise chick adoption strategies, and ultimately enhance reproductive outcomes in endangered penguin populations.

1.2 Breeding Behaviour in Relation to Reproductive Success

Rockhopper penguins appear to be socially monogamous, form long-lasting pair bonds and have bi-parental care (Lera et al., 2023). Mate choice is crucial because both

parents share the responsibility of offspring survival during early stages of its life (Fargevieille et al., 2017). A study by Poisbleau et al. (2013) revealed that female southern rockhopper penguins paired with heavier or new mates exhibited reduced intra-clutch variation in egg mass and yolk androgen levels, traits linked to higher chick survival under sibling competition. This suggests that females may adjust their reproductive investment based on mate quality, supporting theories of condition-dependent mate choice.

There are many different interpretations of pair bonds, making it especially difficult to have a uniform definition across taxa due to differing behavioural traits (Bales et al., 2021). Pair bonds in birds are usually defined through behaviours such as high proximity, allopreening, mate guarding, vocalisations, cohabitation and courtship (Kenny et al., 2017; Sagar et al., 2002).

Stable pair bonds have been linked to higher reproductive success in several bird species (Prior, 2020). Intra-pair proximity is often used as a proxy for bond strength (Gabriel & Black, 2013; Galante & Margulis, 2022), and affiliative behaviours such as allopreening may function in reinforcing partnerships (Kenny et al., 2017; Kushlan, 2011; Spoon et al., 2006). The social function of allopreening in birds is less well understood compared to primates, where allogrooming is exchanged reciprocally between group members and is known to strengthen relationships (Silk et al., 2010; Tiddi et al., 2012). However, in the limited avian studies available, allopreening appears to function similarly, supporting pair retention and the maintenance of strong pair bonds (Bales et al., 2021). Behaviours that are associated with pair-bond strength, such as courtship behaviours, mutual duets, and allopreening, occur when pair members are close together (Bales et al., 2021), further establishing the importance of intra-pair proximity.

Parental cooperation is another key factor: during incubation and brooding, effective coordination between partners optimises energy allocation, supports offspring survival, and may strengthen social bonds (AlRashidi et al., 2010; Gillies et al., 2022). In captivity, where parents do not need to undertake long foraging trips, this coordination becomes particularly important for ensuring timely incubation switches, preventing egg cooling, and maintaining reproductive success (Lislevand et al., 2004; Sládeček et al., 2019), whilst the incubating parent is fasting (Gillies et al., 2022). For seabirds in the wild, the foraging grounds are often far away from the breeding site, and long bouts of fasting therefore allow the other parent to spend substantial time feeding at sea. When cooperation fails, it could lead to the depletion of body reserves in the incubation bird or could lead to breeding failure if the individual decides to abandon the nest in order to search for food (Jones et al., 2002). However, for seabirds in captivity, there is no

need for long foraging trips, and the non-incubating parent spends a lot of time near the nest, leading to the need of tight coordination between the parents, in order to understand when it is time to change the incubation or brooding duties (Sládeček et al., 2019). If the incubating individual leaves the nest before the arrival of the partner, it may leave to the cooling of the eggs and lead to unsuccessful hatching (Lislevand et al., 2004). Therefore, this “incubation switch” is important to time correctly, to increase the success of reproduction.

In rockhopper penguins, incubation duties are typically shared between both partners and require close coordination. Studies on southern and eastern rockhopper penguins have shown that incubation is divided into alternating shifts, often beginning with a jointly shared period followed by longer shifts taken individually by each partner (Dehnhard et al., 2011). Males may perform shorter foraging trips and frequently return to relieve their mates, suggesting a degree of behavioural flexibility and responsiveness in incubation scheduling (Pütz et al., 2018).This level of coordination is thought to be a strong indicator of pair bond quality and has direct implications for reproductive success. However, how such coordination manifests in northern rockhopper penguins, particularly under captive conditions, remains poorly understood.

But not only the coordination of incubation switching is important. Another possible driver of reproductive outcome is the division of labour during nest building. Hahn et al. (2021) found that nest building entails substantial investment from both partners, and may have important fitness consequences, as the relative female contribution in jackdaws (*Corvus monedula*) to transporting material and time spent together in the box were associated with measures of reproductive success (lay date and egg volume). Coordination therefore is a process involved in multiple aspects during the breeding season in birds.

1.3 Established Pair Bond

Many birds pair for life, but they may split up (“divorce”) and search for a new partner to increase their breeding success or nest site quality (Dubois & Cézilly, 2002). However, studies found that there is a reduction of reproductive success due to mate change in the first season after divorce (Forslund & Larsson, 1991; Gousy-Leblanc et al., 2023). This has also been found in little penguins; long-term fidelity benefits some, however others might adapt through alternative reproductive strategies, especially in fluctuating environmental conditions (Chiaradia, 1999). Sanchez-Macouzet et al. (2014) even found that pairs of blue-footed boobys (*Sula nebouxii*), which have been together for longer, established their clutches five weeks earlier in the season, produced 35% more fledglings, regardless of age and reproductive success. The

mechanisms towards a successful relationship maintenance and bi-parental care, such as mimicry, synchrony and body posture are important factors that may influence the pair-bond (Roth et al., 2021). In captive Humboldt penguins, a link between the duration of pairing was the best predictor of intra-pair proximity, leading to the conclusion that the establishment of a strong pair bond takes time, and pairs that have been together for longer demonstrate stronger pair bonds based in proximity maintenance (Galante & Margulis, 2022).

1.4 Adoption Practices

Rockhopper penguins produce a two-egg clutch, exhibiting extreme egg-size dimorphism where the first laid A-egg is significantly smaller and rarely produces fledged chicks compared to the B-egg (Stein & Williams, 2013). In the wild, the A-egg is typically laid a few days after the female returns from migration, at a time when her body is still recovering and nutrient stores are limited. As a result, the A-egg is smaller and contains less yolk and fewer hormones which are vital for early chick development and competitive ability (Poisbleau et al., 2015). The B-egg is laid a few days later, benefits from improved maternal condition, is larger, better provisioned, and usually hatches first due to reversed hatching asynchrony. This advantage allows the chick from the B-egg to dominate in sibling rivalry, often resulting in the neglect or death of the chick from the A-egg, a phenomenon known as “obligate brood reduction”. In the Vienna Zoo, zookeepers have previously observed that the pairs with two successful hatchlings often invest more energy in the stronger chick, leading to the death of the other. Therefore, when a pair has two successful hatchlings, one chick is transferred either to a pair known for successful rearing or to a pair that was successful in the past but have not succeeded in the current season. This is a management method aimed at ensuring the survival of all hatched chicks. This “adoption” is typically successful, with the pair raising the adopted chick it as if it were their own. Nevertheless, this situation could result in a younger pair remaining together even when they are unable to effectively reproduce, as they continue to care for a chick, which might prevent them from separating to seek a better suitable partner within the colony. Thus, studying the social interactions between bonded pairs of northern rockhopper penguins is of importance.

Furthermore, while chick adoption at the Vienna Zoo has proven effective in improving survival of second chicks, little is known about whether adoptees display the same behavioural coordination as naturally successful pairs, or whether adoption may mask underlying pair incompatibilities. Understanding these dynamics could help refine adoption practices and ensure that only well-coordinated pairs are selected as adoptees.

1.5 Study Aims

In the Vienna Zoo colony, some northern rockhopper penguin (*Eudyptes moseleyi*) pairs perform better than others, which may be due to differences in pair bond strength, compatibility or breeding strategies related to birth status. This study defines reproductive success as the successful hatching of at least one egg within the analysed breeding season.

The aim of this study is to investigate how behaviours associated with pair bond maintenance and coordination relate to reproductive success in captive northern rockhopper penguins during the 2024 breeding season. Usually, pair bonds assume a long-term relationship across multiple breeding events. In the context of this study, “pair bonding” refers to temporal mating behaviours in a pair, including the coordination in nesting behaviour, and affiliative interactions such as mutual preening, mutual displays and close proximity (Fargevieille et al., 2017; Roth et al., 2021). Further, whilst an established pair bond commonly implies breeding across multiple years (Bales et al., 2021), in this study it is defined as a pair that has remained together for at least two years. Due to limited data on pair duration, only the previous year could be used to assess whether a given pair met this criterion.

By exploring these aspects of social behaviour and coordination, this study aims to enhance a deeper understanding of breeding behaviour in *E. moseleyi* and contribute to improved welfare and management practices in captive endangered penguin populations.

1.5.1 Hypotheses

It was hypothesised that successful pairs would show higher levels of behavioural coordination and bonding behaviours than unsuccessful pairs. Furthermore, the role of partner history was examined by comparing newly formed pairs with established pairs. It was expected that established pairs would display more affiliative behaviours, thereby increasing their likelihood of reproductive success.

2. Methods

2.1 Study Site

The study was conducted at the Vienna Zoo, Vienna, Austria (Tiergarten Schönbrunn). As of December 31, 2022, the zoo housed a colony of 59 northern rockhopper penguins (*Eudyptes moseleyi*) in a shared enclosure with king penguins (*Aptenodytes patagonicus*). As participant in the European Endangered Species Programme (EEP), the zoo has been managing this species under controlled, conservation-oriented conditions. The penguin habitat includes both land and water areas and is designed to support naturalistic behaviours such as courtship, nesting, and incubation.

The enclosure is fully indoors, separated into three sub-enclosures (A, B and C), where all sections are connected via the water pool. Section A was designed to closely replicate the natural habitat of northern rockhopper penguins. The enclosure was built of solid rock, with different hight levels, to represent stony cliffs. Before the nest sites were permanently build into the enclosure, the zookeepers placed temporal nest sites unto the floor. Then, they assessed which nest sites the penguin pairs preferred, and at which distance to each other they were comfortable to breed. Once the zookeepers assessed the ideal location of the nesting sites, they build permanent nests into the enclosure out of stone. The enclosure now contains 26 permanently fixed nest sites that are aerated by a hole in the side. Individuals can choose freely which nest to select. Normally, only the sections B and C are accessible. However, during the breeding season, sections A and B are separated from section C by an underwater grid, preventing king penguins and non-breeding northern rockhopper penguins from entering (Fig. 1). For the first few weeks, yearlings and all potential breeding individuals remain in sections A and B, forming a transitional period. Once pairs have formed or individuals are visibly courting, the remaining unpaired individuals are relocated to section C, where they stay with the rest of the colony until the end of the breeding season.

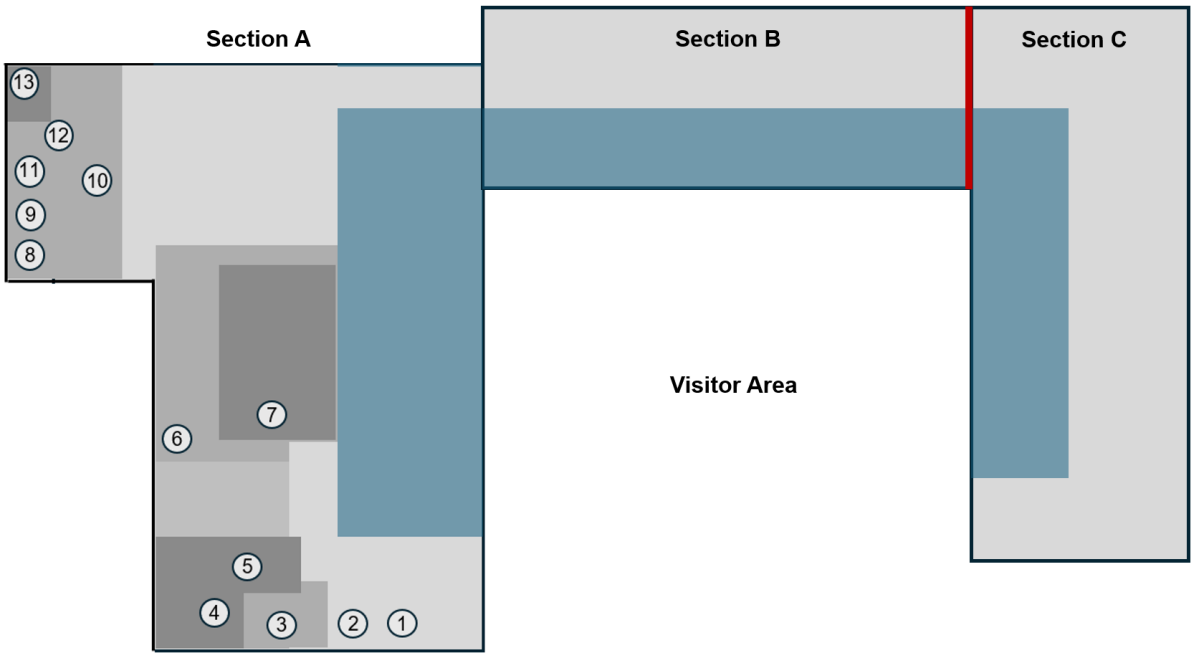

Figure 1: Layout of the enclosure sections A, B and C for E. moseleyi and A. patagonicus at the Vienna Zoo. Proportions are estimated (not to scale): Section A is enlarged for clarity of the breeding site, whilst Section C is only partially shown and reduced in size. Blue areas represent water, and darker grey shades indicate elevated areas in Section A. Numbered circles represent the occupied nest sites by pair ID. The red line marks the grid separating Sections A and B from Section C during the breeding season of E. moseleyi, restricting breeding pairs to Sections A and B, while the rest of the colony and A. patagonicus remain in Section C.

All individuals in the whole enclosure are fed daily at 11:30 AM, except on Tuesdays, when a fasting day is implemented. This is done to represent the natural behaviours of *E. moseleyi*.

Nesting material is provided by the staff, consisting of mainly sticks and dried leaves, given to the penguins throughout the whole season. Section A is monitored by the zoo staff during the breeding season by two cameras installed on the ceiling, keeping most of the nests in view.

2.2 Subjects

The *E. moseley*i colony consisted of 15 breeding pairs during the 2024 season, with 13 pairs (26 individuals) selected for analysis. Demographic information, including age data and birthing location, was derived from breeding records in the studbook. The studied individuals comprised both wild-born (n = 12) and captive-born penguins (n = 14). Individuals ranged in age from 2 to 24 years (M = 16.31 years, SD = 7.71 years). The exact age of the eleven oldest penguins is unknown, as they were brought to the

zoo for rehabilitation in 2000, when their age was estimated to be one year. Therefore, for analytical purposes, their current age was set to 24 years.

Pairs were categorised based on their pairing history: established pairs, which had previously bred in 2023 and continued pairing in 2024, and new pairs, that formed for the first time in 2024 (see Table 1 for demographic information).

Table 1: Demographic characteristics of the northern rockhopper penguin subjects included in this study (n = 26, forming 13 pairs).

Pair ID	Age	Sex	Birth status	Established pair
1	24	Male	Wild	Yes
1	16	Female	Zoo-born	Yes
2	24	Male	Wild	No
2	3	Female	Zoo-born	No
3	15	Male	Zoo-born	No
3	24	Female	Wild	No
4	24	Male	Wild	No
4	2	Female	Zoo-born	No
5	24	Male	Wild	Yes
5	24	Female	Wild	Yes
6	24	Male	Wild	Yes
6	24	Female	Wild	Yes
7	14	Male	Zoo-born	Yes
7	13	Female	Zoo-born	Yes
8	18	Male	Zoo-born	Yes
8	14	Female	Zoo-born	Yes
9	7	Male	Wild	Yes
9	24	Female	Wild	Yes
10	2	Male	Zoo-born	Yes
10	11	Female	Zoo-born	Yes
11	7	Male	Wild	No
11	5	Female	Zoo-born	No
12	16	Male	Zoo-born	No
12	5	Female	Zoo-born	No
13	24	Male	Wild	Yes
13	15	Female	Zoo-born	Yes

Each penguin was identified using their unique wing band colour combination. The bands, which are single- or multicoloured, allowed individual recognition. For captive-born penguins, band colours indicate genetic relationships, with offspring sharing colours from their parents. Generally, the placement of the colour band on the left or right flipper indicates the penguins sex (left = female, right = male). However, since this

practice was not always strictly followed, precise individual identification required verifying the exact colour combination rather than relying solely on band placement.

Pairs were removed from the breeding enclosure (Section A and B) for two reasons during the season: (1) if they had not laid any eggs by the time the first chicks from another pair hatched, or (2) once their chicks were two weeks old and had been separated from them for safe monitoring by zoo staff. At the end of April, the grid dividing Section A and B from Section C was removed, even if some pairs had not yet hatched their eggs.

2.3 Data Collection

Data were collected from February 15th to April 31st, 2024 (11 weeks). Video recordings were made using two GoPro-Black9 cameras mounted outside the enclosure, facing the nests in Section A. Each camera recorded a total of 37 hours between 7 AM and 5 PM, primarily on weekdays due to higher visitor traffic on weekends. Cameras were set up two to three times per week and recorded for 1-3 hours per day, resulting in approximately 3.5 hours of video data per week. Filming days were randomly selected, while ensuring a relatively even distribution across weekdays, taking into account that Tuesdays were fasting days, which could influence behaviour. Daily activity patterns were considered; therefore, roughly equal recording times were allocated to morning (7 AM to 12 PM, 20 hours total) and afternoon (12 PM to 5 PM, 17 hours total) sessions.

Supplementary video material from the cameras inside the enclosure totalling around 700 hours, was provided by the zoo staff.

2.4 Video Selection

The breeding season was divided into three phases for each individual pair, following the natural breeding cycle of rockhopper penguins: pair formation (courtship and mating), incubation (starting with the first egg laid), and brooding (beginning once the first egg hatched). To allow for consistent behavioural comparisons across breeding phases and pairs, selection criteria were applied to define the minimum required footage per phase (see below). If a pair had a particularly short courtship period and laid eggs early, resulting in less video material during pair formation, supplementary recordings from the zoo cameras on other days were used. Most pairs remained in Section A and B for 11 weeks. While the duration of the pair formation phase varied widely between pairs, incubation typically lasted 4-5 weeks, and brooding always lasted 2 weeks.

Based on this information, minimum thresholds were set for each phase per pair: ≥ 5 hours for pair formation, ≥ 15 hours for incubation (the longest phase), and ≥ 5 hours for brooding. In the end, the video material per pair ranged from 5-19 hours during pair formation, 19-31 hours during incubation, and 5-8 hours during brooding (Table 2). This variability resulted from individual differences in phase length and the fixed nature of the recording schedule. Zoo camera footage was only used sparingly, since (1) internal cameras did not always capture all nests, (2) flipper bands were inconsistently visible, whereas in the footage recorded from outside the enclosure, band colours were spoken into the camera, and (3) since individual behaviour may depend on the presence and actions of other individuals in the enclosure, it was preferable to analyse footage in which all pairs were recorded simultaneously.

Two breeding pairs were excluded due to insufficient visibility on both the camera types, resulting in 13 analysed pairs. Pair 7 was included despite limited visibility: their missing observations were supplemented with footage from other days, during which the camera angle captured the central part of the enclosure where their nest was located. To ensure comparability with other pairs, this material was selected from similar times of days and from fasting or feeding days. Similar substitutions were made if 3-4 hours were missing in other pairs. Pair 4 failed to lay eggs but was included in the analysis of pair formation. Of the 12 pairs entering incubation, total footage per pair ranged between 28 and 41.5 hours (Table 2).

Table 2: Time per breeding phase and in total coded of northern rockhopper penguin pairs included in this study (n = 13 pairs).

Pair ID	Pair Formation (h)	Incubation (h)	Brooding (h)	Total Time Coded (h)
1	9.5	25	5	39.5
2	10.5	31	-	41.5
3	5	23	-	28
4	19	-	-	19
5	12.5	21.5	5	39
6	10.5	22	8	40.5
7	5	24	8	37
8	12.5	23.5	-	36
9	11.5	21.5	5	38
10	11	19	7.5	37.5
11	9	25	-	34
12	9	19	7.5	35.5
13	5.5	25.5	7.5	38.5

Despite attempts to match recording conditions (e.g. time of day, fasting vs. feeding day), supplementary footage may have introduced minor contextual biases that could not be fully controlled.

2.5 Behavioural Observations and Coding

2.5.1 General Coding Procedure

All video coding was performed using the software BORIS v8.27.1 (Behavioral Observation Research Interactive Software) (Friard & Gamba, 2016). Focal animal sampling was applied across all observation sessions. This method was chosen due to the relatively small sample size, which allowed for focal sampling to be applied, leading to a solid data set that allowed the focus on pair specific behaviour (Lehner, 1992).

Observations included the following metadata: observation date and time, focal subject identity, behaviour type, and start and end time of each behaviour.

2.5.2 Annotation Rules

Five-Second Rule

The five-second rule was implemented in the annotation of recorded behaviours. When a state behaviour was interrupted for less than five seconds, it was treated as part of the same bout. When the interruption lasted longer than five seconds, the re-uptake of that behaviour was considered a new bout.

Nest Proximity Rule

All behaviours of individuals were coded only when they were within three metres of the nest. If an individual left the surroundings of the nest, no behaviour was coded for that individual, even if it remained visible in the video. This was done because the focus was on the pair relationship, not interactions with other individuals in the enclosure. If both individuals were more than three metres from their nest, but still close to each other, no data were collected (“out of view”). If one individual was within three metres of the nest but the partner was not, only the behaviour of the nest present individual was coded.

Observer Bias Rule

All video material was coded by a single observer to ensure consistency in behavioural annotation. This approach minimised variation in subjective interpretation, particularly for variables that required judgement calls, such as proximity estimation. Since direct measurement inside the enclosure was not possible, estimations of the three-metre radius around the nest were based on standardised visual references. Firstly, distance was approximated by the body length of northern rockhopper penguins, with 4-5 body

lengths corresponding roughly to three metres. Additionally, the average distance between two adjacent nests was known to be approximately 1-1.5 metres, so the distance to a third nest served as a supplementary reference. This behavioural threshold was supported by practical observations during data collection: in many cases, individuals responded to disturbances within approximately three metres of their nest. However, auditory clues and long-distance responses (e.g. vocalisations from a partner across the enclosure) were not systematically accounted for in this study and thus excluded from behavioural annotation. The three-metre radius was therefore defined as a pragmatic and reproducible spatial threshold for pair-related behaviour in the context of visual observations only.

2.5.3 Important Behavioural Definitions

Behavioural data were coded according to a custom ethogram developed for this study (Table 3), distinguishing between state events (with measurable durations) and point events (recorded as occurrences). All behaviours were timestamped in BORIS with start and stop times (for state events) or marked by time of occurrence (for point events), allowing for precise quantification of interaction frequency and duration. This classification enabled a detailed analysis of both temporal patterns and discrete social behaviours.

Table 3: Behavioural Ethogram of Northern Rockhopper Penguins.

Behaviour	Type	Modifier	Operational Definition
Allopreening partner	State event	Recipient	Receiving preening from the partner.
Allopreening partner	State event	Active	Rubbing the head, bill or flipper on partner, or "mouthing" them.
Mutual preening	State event		Both individuals in a pair engage in preening the other.
Mutual display	Point event		A pair in proximity to each other simultaneously perform an ecstatic display. Penguins lean slightly forward, bill opened and emitting loud calls. May commence with a variety of soft vocalisations and head shaking, building in intensity and subsiding in a similar fashion. Often involves a body stretch in the beginning, commenced by a bowing of the head, were the trumpeting starts, and the penguin then stretching upwards with flippers extended vertically whilst calling.
Nest building	State event		Collecting nest material, carrying it to the nest site, arranging it. Movement with the head and material close to the nest site, or scraping with the foot

			counts as nest building only if the movement of nest material is seen. May involve the help from the partner; one collecting material and the other arranging it.
Incubation switch	State event		An incubating individual gets up and leaves the nest, and the partner takes over the incubation of the egg(s). The time from when one partner stops incubating to the other partner taking over is measured. Once the other partner is in the nest, incubation for this individual starts and the incubation switch is over.
Brooding switch	State event		A brooding individual gets up and leaves the nest, and the partner takes over the brooding of the hatchling. The time from when one partner stops brooding to the other partner taking over is measured. Once the other partner is in the nest, brooding for this individual starts and the brooding switch is over.
Proximity to partner	State event	Beak length	Penguin is at a distance to the partner up to beak length. The individuals may be touching.
Proximity to partner	State event	Beak length up to 3 metres	Penguin is in proximity to partner, from beak length up to 3 metres. They are not touching.
Proximity to partner	State event	More than 3 metres	Penguin is at a distance to partner pf at least 3 metres. They are not touching. The partner may not be in view.
Incubating egg(s)	State event		Egg(s) are positioned between the feet against the brood patch. Incubation may happen upright, or in lying position.
Brooding hatchling	State event		Hatchling is positioned between the feet against the brood patch. Brooding may happen upright, or in lying position.
Out of view	State event		Both individuals in a pair are not visible by the camera or are at least 3 metres away from the nest (see nest proximity rule chapter 2.5.2). The behaviour in relation to each other can not be determined.

Success Groups

Pairs were split into two reproductive success categories. A pair was defined as successful if it hatched at least one egg. Unsuccessful pairs were those that either did not lay any eggs or failed to hatch them.

Adoptees were defined as pairs that were initially unsuccessful but received a second chick from a successful pair and therefore had an introduced brooding phase.

These variables were linked to the behavioural dataset to examine associations between pair behaviour and reproductive outcomes.

Established Pairs

Established pairs were defined as those where the same two individuals had already been paired during the 2023 breeding season. This variable was used to test whether partner familiarity influenced their following years success. Their reproductive success in the former year was unknown.

Proximity Categories

Three proximity categories were coded: (1) from touching up to beak length, (2) from beak length up to three metres, and (3) more than three metres. The third category could include the individual being out of view, in the water, or elsewhere in the enclosure (Fig. 2).

Proximity in this context was a dyadic behaviour, meaning that both individuals in a pair were assigned the same proximity value at any given time. BORIS recorded the duration of each event in seconds for each individual. To obtain the true pair-level duration of time spent at each distance band, durations from both partners were summed and then divided by two (to correct for possible coding error). From the total observation time per pair, the share of time spent in each proximity category was calculated as a percentage.

Figure 2: Intra-pair proximity seen in pair 12 as a) proximity within 3 metres, and b) proximity within beak length. Female individual is on the left (colour band on left flipper) and the male (colour band on right flipper) is incubating the eggs in their nest.

2.5.4 Pair Coordination and Bonding

Pair Coordination

Three state behaviours were used to analyse coordination within pairs.

(1) Incubation coordination: measured as the variable “nest switch”, defined as the time interval between one partner leaving the nest and the other beginning incubation or brooding of the chick (Fig. 3).

(2) Partner proximity: measured as the average total duration of proximity within three metres.

Longer proximity bouts were proposed as attentive cooperation, enabling the partner to monitor the surroundings of the nest; this could be threats such as nest material theft or the partner being under duress. To compare success groups, the percentages of proximity up to beak length and up to three metres were added together, and that duration was then calculated per pair.

(3) Nest building cooperation: measured as the time spent building the nest (Fig. 3).

Nest building was analysed as an indicator of shared parental investment. For each sex, total time spent nest building was divided by total observation time per pair. The values were then compared between success groups.

Figure 3: Two coordination behaviours seen in pair 7 (a) and pair 12 (b). Both females have their colour band on the left flipper, and the male on the right flipper. a) Brooding switch: the male (right) leaves the nest and the female (left) moves to get into the nest. b) Nest building by the male: The female (left) incubates the eggs in the nest and the male (right) picks up nest material.

Pair Bonding

Three variables were considered indicative of pair bonding.

(1) Allopreening: measured by the individual using the state behaviour “active preening partner” and “mutual preening” (Fig. 4).

The combined investment of both individuals in a pair was calculated by adding active preening of both individuals in a pair together, divided by two. This resulted in the total active preening variable per pair.

(2) Partner proximity: measured as the average total duration of proximity within beak length distance.

To compare success groups, the percentage of proximity duration within beak length was calculated per pair. Whilst proximity up to beak length in combination with proximity up to three metres was also analysed in the context of pair coordination, it was included here as a component of bonding behaviour, as many bonding behaviours require close intra-pair proximity.

(3) Mutual display: the point state was measured as a rate per hour per pair (how often a pair displayed together in an hour) (Fig. 4).

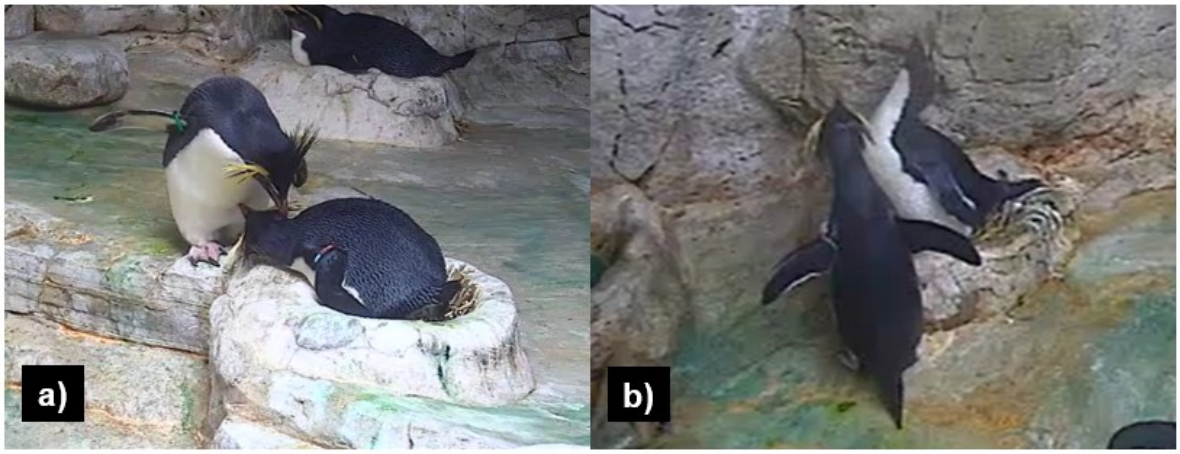

Figure 4: Two pair bonding behaviours seen in pair 7 (a) and pair 1 (b). The male from pair 7 has a colour band on the right, and the female on the left flipper. The male from pair 1 has the colour band on the left, and the female on the right flipper. a) Mutual preening: The female (right) is incubating the eggs in the nest, whilst both individuals preen each other. b) Mutual display: The male (left) and the female (right) display ecstatically; their body is stretched upwards, they are trumpeting and extending their flippers vertically.

2.5.5 Predictors of Success

All affiliative (pair bonding and coordination) behaviours were aggregated to the pair level. Total durations (seconds per hour) were calculated over the whole breeding season for nest building effort, proximity within beak length, proximity up to three metres, total active preening, and nest switch duration. For age, the pair mean age and absolute difference was calculated, the birthing status was split into three categories (zoo-born, mixed-born, and wild-born), and established pairing history were added as covariates.

2.6 Data Analysis

All statistical analyses were performed using R Statistical Software (v4.5.1; R Core Team, 2025) with the tidyverse for data manipulation, rstatix and car for assumption testing and univariate comparisons, lme4 and lmerTest for mixed-effects modelling (with emmeans for model-based estimates), logistf for penalised regression, and ggplot2 for data visualisation. Readxl and lubridate were used for data import and date handling.

For each continuous outcome variable (e.g. behavioural rates, proportions), the normality of residuals within each comparison group was assessed using the Shapiro-Wilk test. Homogeneity of variances was tested using Levene's test.

If both assumptions were met (Shapiro-Wilk $p > 0.05$ and Levene's $p > 0.05$), a two-sample Student's t-test was performed. If normality held but variances were unequal, a Welch's t-test was applied. If either assumption failed, a non-parametric Wilcoxon rank-sum test was used.

For paired sex-difference analyses of incubation and brooding investment (female % - male % within pairs), one-sample t-tests on the within-pair differences were used to test whether the mean difference deviated from zero when assumptions were met. Otherwise, Wilcoxon signed-rank tests were applied. Variance equality between success groups for paired differences was also assessed with Leven's test.

Effect sizes were reported for all tests: Cohen's *d* (with Welch's correction where applicable) for *t*-tests, the rank-biserial correlation (*r*) for Wilcoxon tests, and generalised eta squared (ges) for ANOVAs. For multiple univariate tests on related variables, *p*-values were adjusted using Holm's method.

Switch duration analyses during incubation and brooding were conducted using linear mixed-effects models (LMMs) with log-transformed switch durations (s) as the response variable, breeding success group as a fixed effect, and pair identity as a random intercept to account for repeated measurements within pairs. The log- transformation was applied to meet model assumptions and reduce skewness in the duration data.

Nest-building analyses included two approaches. Sex differences were assessed using an LMM including sex as a fixed effect and pair identity as a random intercept. Second, breeding phase differences were assessed using one-way ANOVA (reporting ges) or Kruskal-Wallis tests when assumptions were violated. When behaviours were absent for multiple pairs in a phase (e.g. only three pairs engaged in nest building

during brooding), results were interpreted with caution and complemented by descriptive summaries.

The relationship between each social or cooperative behaviour and reproductive success was assessed using separate Firth logistic regression models. This method was chosen to reduce small-sample bias and to avoid separation issues, given the limited dataset (n = 13 pairs) and rare events for some predictors. Each behavioural predictor was modelled separately to minimize overfitting and multicollinearity (e.g. proximity within beak length was highly correlated with allopreening rate, r = 0.68). Model outputs included the log-odds coefficient (β), penalised likelihood ratio test (p-value), and 95% profile penalised likelihood confidence intervals.

All behavioural rates were expressed per hour of usable observation time to account for variation in observation effort among pairs. Descriptive statistics are presented as means ± SD for normally distributed data or medians ± IQR for skewed data. All tests were two-tailed, with α = 0.05. Graphical summaries include boxplots with overlaid individual points and annotated significance levels where relevant.

3. Results

3.1 Breeding Stages and Sex Differences in Investment

Of the 13 northern rockhopper penguin pairs included in the analysis, five successfully hatched at least one egg, producing a total of eight chicks during the 2024 breeding season. Twelve pairs laid eggs, the one pair that did not was removed from the enclosure on the 21st of March 2024. During the brooding phase, no direct comparison with unsuccessful pairs was possible, as they had not hatched any eggs and therefore did not enter this phase. However, three unsuccessful pairs that raised an adopted chick were included and compared to successful pairs during brooding. A full list of pair IDs, pair formation, incubation and hatching dates, as well as success classification, is provided in the Appendix (Table 5).

3.1.1 Pair Formation Phase

The duration between initial visible pair formation and the onset of incubation due to egg laying, ranged from 7 to 28 days (median = 16.5, SD = 7). An unpaired two-sample t-test (equal variances assumed) found no significant difference in the mean pair formation length between successful (n = 5) and unsuccessful (n = 8) pairs ($t(11) = 0.79$, $p = 0.449$). Cohen's $d = 0.46$ indicated a small effect size (Fig. 5).

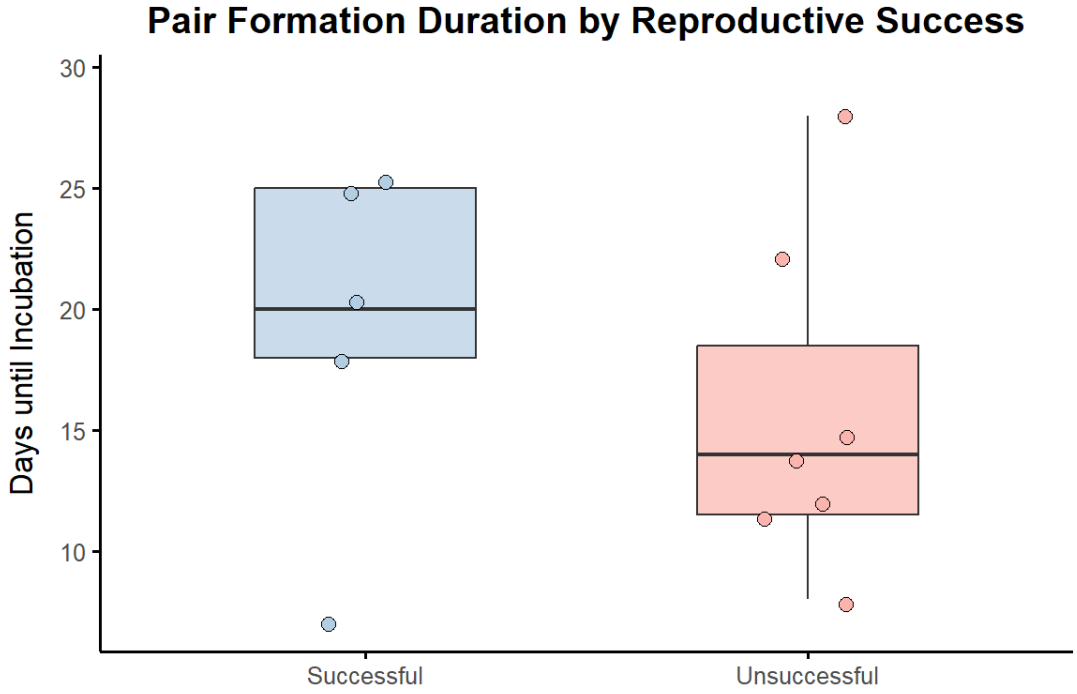

Figure 5: Pair formation duration in relation to reproductive success in northern rockhopper penguins. The number of days between observed pair formation and incubation onset is shown for successful (n = 5) and unsuccessful (n = 8) pairs. Although successful pairs tended to exhibit slightly longer pair formation periods, the difference was not statistically significant (t(11) = 79, p = 0.449, Cohen's d = 0.46).

3.1.2 Incubation Phase

The incubation time of the five successful pairs consisted of 37-40 days. The seven unsuccessful pairs did not hatch any eggs, and therefore their incubation phase ended either when they adopted a chick or were removed from the enclosure when the breeding season was over at the end of April 2024. This means that no statistical test can be done to compare the incubation time of successful and unsuccessful pairs.

A one-sample t-test on the paired differences (female – male percentage) revealed that the mean difference was significantly greater than zero ($t(10) = 3.66$, $p = 0.004$). Females invested a substantially higher proportion of time incubating (median = 71.6%) than males (median = 28.4%), with a large effect size (Cohen's $d = 1.06$) (Fig. 6).

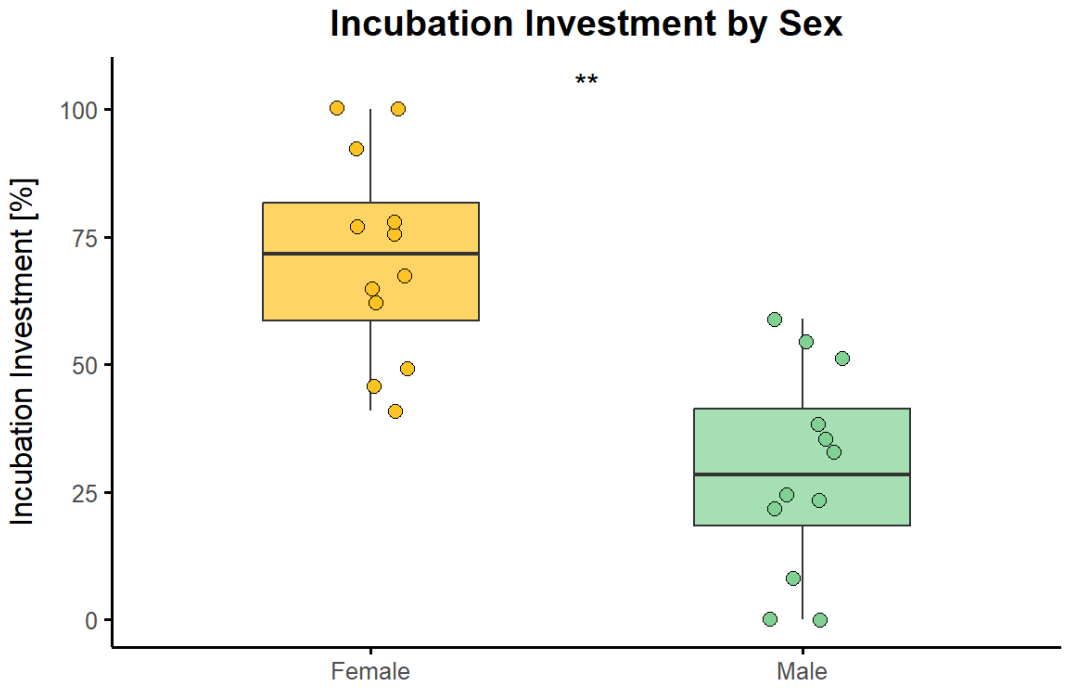

Figure 6: Incubation investment by sex of northern rockhopper penguins. Each point represents the percentage of total incubation time contributed by the female or male within each of the 12 pairs (percentages per pair sum to 100%). Females invested significantly more time incubating than males (paired t-test, t(11) = 3.66, p = 0.004, Cohen's d = 1.06).

The overall magnitude of sex difference in incubation investment did not differ significantly between successful ($n = 5$) and unsuccessful pairs ($n = 7$; Wilcoxon rank-sum test, $W = 20$, $p = 0.745$, $r = 0.117$). However, a Levene's test revealed significant variance heterogeneity ($F(1,10) = 7.28$, $p = 0.022$), meaning that successful pairs showed a relatively consistent female bias in incubation (median = 51.7%, SD = 12.0), whereas unsuccessful pairs displayed considerably more variation in sex differences (median = 23.9%, SD = 53.1) (Fig. 7).

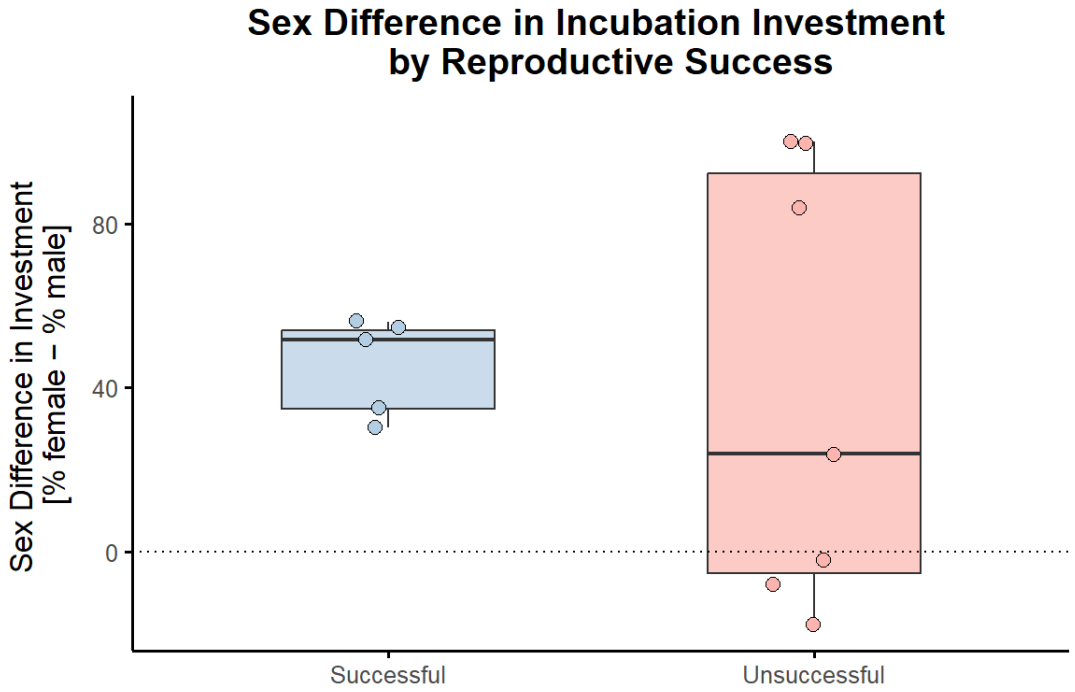

Figure 7: Difference in incubation investment between sexes across successful and unsuccessful pairs of northern rockhopper penguins. The y-axis shows the difference in percentage of incubation time between females and males per pair (% female minus the % male). Positive values indicate a higher relative incubation investment by the female, while values closer to zero reflect more equal sharing. Each data point represents one pair. No statistically significant difference was found between both groups in their investment of incubation (p = 0.745), although variation was higher among unsuccessful pairs (p = 0.022, Levene's test).

3.1.3 Brooding Phase

The zookeepers allowed each pair to raise their chick(s) for two weeks before transferring the young for monitoring into another section of the enclosure. Consequently, the brooding period was fixed at 14 days for all pairs. For adopting pairs, the brooding phase began once a chick from another pair was placed in their nest by the zookeepers.

Four pairs were excluded from the brooding analysis as they neither hatched nor adopted any chicks, leaving eight pairs for analysis (five successful, three adoptees). A one-sample *t*-test on the paired difference in brooding effort (female – male percentage) showed no significant sex bias (mean difference = -16.5%, 95% CI [-55.6, 22.7]; $t(7) = -0.99$, $p = 0.353$). Cohen's d = 0.35 (small) confirmed only a minimal effect. Thus, there is no evidence for a consistent sex asymmetry in brooding investment (Fig. 8).

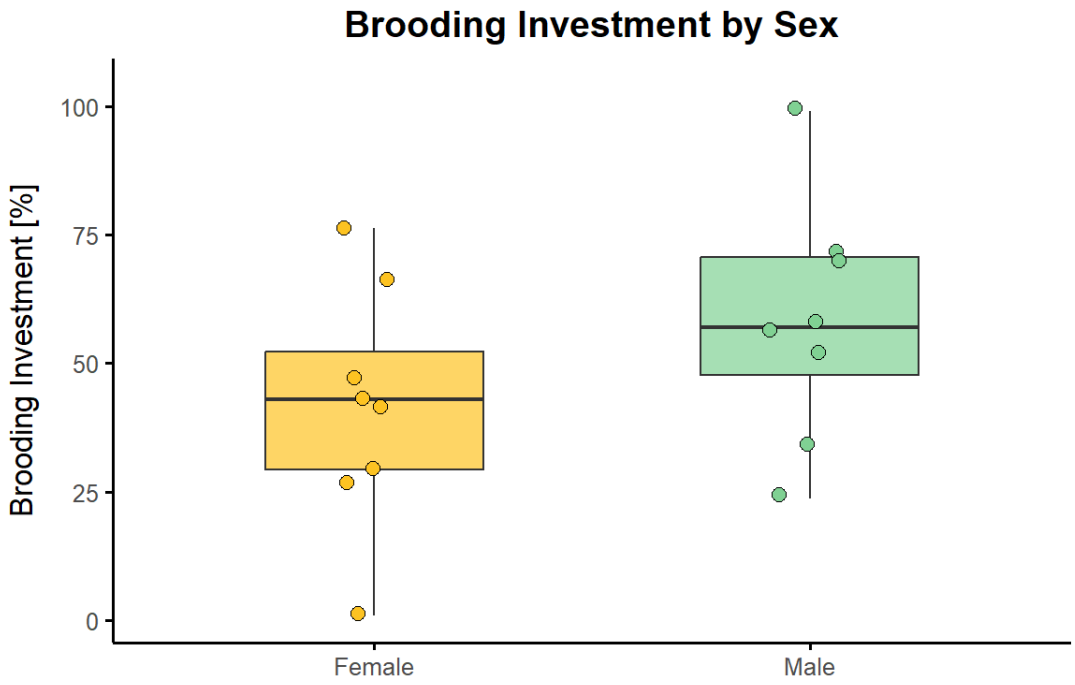

Figure 8: Brooding investment by sex of northern rockhopper penguins. Each point shows the percentage of total brooding time contributed by the female or male within each of the eight analysed pairs (percentages per pair sum to 100%). Females brooded slightly less than males, but this difference was not statistically significant (paired t-test on female-male differences: t(7) = -0.99, p = 0.353, Cohen's d = 0.35).

The overall magnitude of sex difference in brooding investment did not differ significantly between successful pairs (n = 5) and adoptees (n = 3; $t(6)$ = 0.29, p = 0.78, Cohen's d = 0.23). In both groups, the direction (female vs. male-biased) and magnitude of brooding-effort differences varied widely across the eight pairs (Fig. 9).

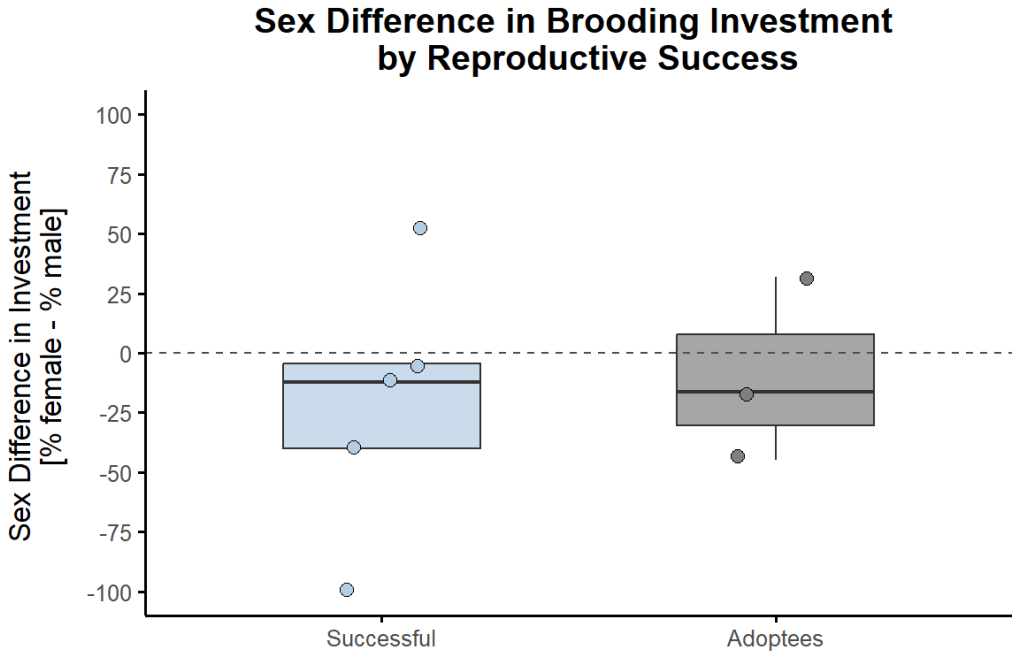

Figure 9: Difference in brooding investment between sexes across successful pairs and adoptees of northern rockhopper penguins. The y-axis shows the difference in percentage of brooding time between females and males per pair (% female minus the % male). Positive values indicate a higher relative brooding investment by the female, while negative values reflect a higher relative brooding investment by the male. No statistically significant difference was detected between groups (t(6) = 0.29, p = 0.78, Cohen's d = 0.23).

3.2 Coordination Behaviours by Success

3.2.1 Nest Switching

Nest switch counts recorded varied widely among pairs. A total of 122 nest switches was recorded during incubation, ranging from 2 to 44 counts per pair (median = 8, SD = 11.3). Notably, pair 11 accounted for 44 of the total switches during incubation: in that pair, the female repeatedly vacated the nest without the male taking over, resulting in a very high and long number of switch events. During brooding, the total number of switches recorded was 22 times. During this phase, the switch counts per pair varied from 0-6 times (median = 2, SD = 1.95), indicating considerably less variability in this phase.

Incubation Switch

A linear mixed-effects model on the log-transformed switch duration (in seconds) during incubation with pair identity as a random intercept showed no significant effect of reproductive success (log-scale estimate $\beta = -0.717 \pm 0.590$, $t(14.4) = -1.22$, $p = 0.244$). Back-transformed, unsuccessful pairs ($n = 7$) had a geometric mean switch duration of 21.9 s (95% CI 9.18 - 52.3s), versus 10.7s (95% CI 3.72 - 30.2s) in successful pairs (n

= 5) (Fig. 10). Although successful pairs tended to switch their incubation duties roughly twice as fast, this difference did not reach statistical significance. The random-effect variance for pair identification (σ^2 = 0.85) remained substantial, indicating considerable between-pair heterogeneity in switch behaviour beyond the success effect - such as seen with pair 11 or pair 3. The mixed-effects model down-weighted this intra-pair clustering via its random intercept, and the log-transformed analysis remained therefore non-significant.

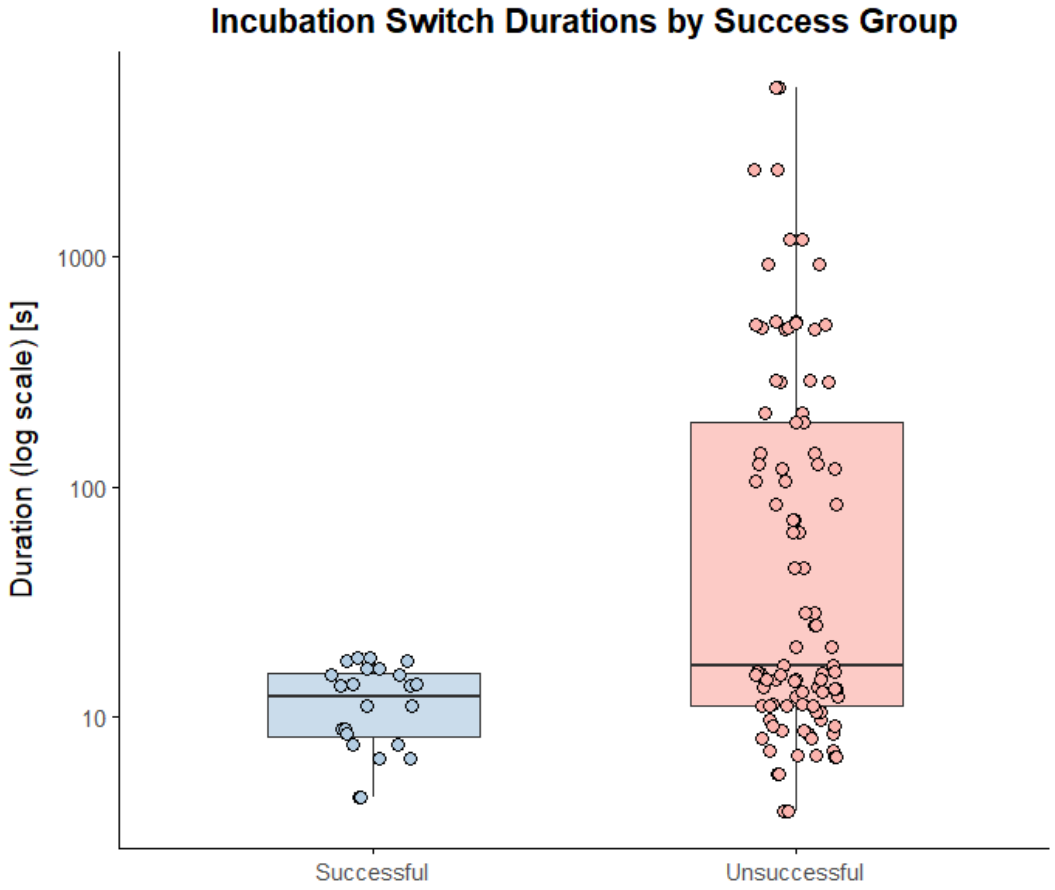

Figure 10: Incubation switch durations of northern rockhopper penguins by success group. Boxplots showing the individual incubation switch durations (s, log10 scale) recorded during incubation for successful (24 incubation switches) and unsuccessful (98 incubation switches) pairs. No statistically significant difference was detected between groups (p = 0.78).

Brooding Switch

A linear mixed-effects model on the log-transformed switch durations (seconds) during brooding with pair identity as a random intercept revealed no significant effect of success (β = -0.209 ± 0.269 log-s, t(7.7) = -0.78, p = 0.46). Back-transformed, adoptees exhibited a geometric-mean switch duration of 9.34s (95% CI 5.46-16.0s) compared to 5.7s (95% CI 4.75 - 12.1 s) in successful pairs. The low number of events per pair, and

the small sample size of brooding pairs (n = 8) limits power to detect anything but large effects, but the point estimates suggest broadly similar switch-speed patterns in both success groups (Fig. 11).

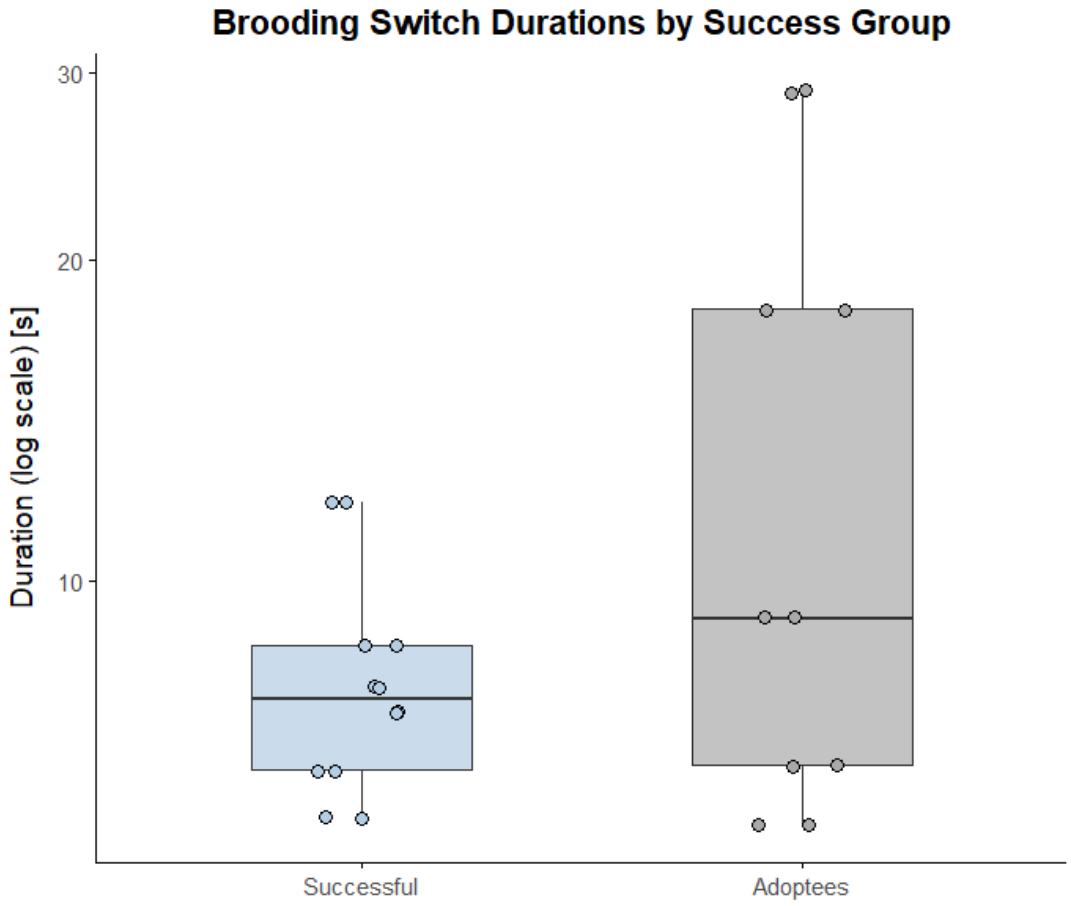

Figure 11: Brooding switch durations of northern rockhopper penguins by success group. Boxplots showing the individual brooding switch durations (s, log10 scale) recorded during brooding for successful (12 brooding switches) and unsuccessful (10 brooding switches) pairs. No statistically significant difference was detected between groups (p = 0.46).

3.2.2 Proximity within 3 Metres

Proximity was assessed both in terms of coordination (spatial presence within three metres) and bonding (physical closeness up to beak length), as these capture distinct affiliative processes.

Successful pairs (n = 5) allocated a larger share of their total proximity time to distances within three metres (M = 87.1%, SD = 3.0%) than unsuccessful pairs (n = 8; M = 71.4%, SD = 10.2%), but the difference was not statistically significant (t(7.24) = -2.10, p = 0.073), with a large effect size (d = 1.05) (Fig. 12).

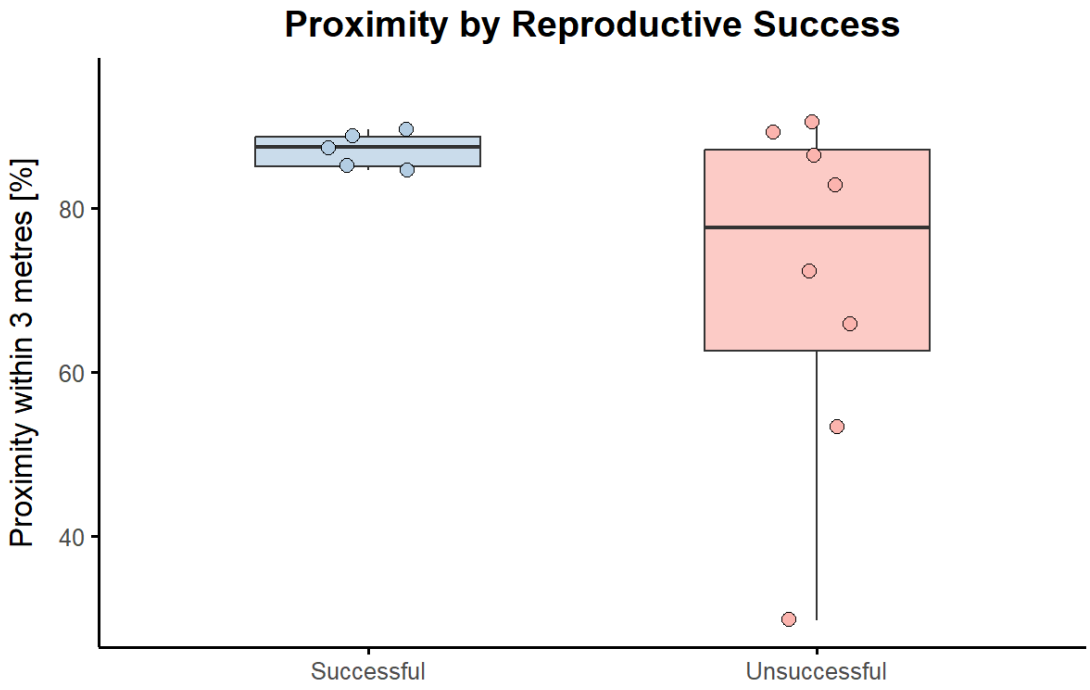

Figure 12: Boxplot showing the percentage of observation time that breeding pairs spent in proximity within 3 metres of each other, grouped by reproductive success. The left box represents successful pairs, the right box the unsuccessful ones. Successful and unsuccessful pairs did not differ significantly in their mean proximity percentage spent within 3 metres of each other (p = 0.073).

3.2.3 Nest Building

Nest Building Investment during Breeding Phases

Nest building rate per hour differed significantly between breeding phases (one-way ANOVA: $F(2, 25) = 10.10$, $p = 0.0006$, ges = 0.447). Post-hoc comparisons with Bonferroni correction indicated that pairs invested significantly more in nest building during incubation ($n = 13$) compared to pair formation ($n = 12$; $p = 0.0007$), whilst differences between brooding ($n = 8$) and the other phases were not statistically significant after correction (all $p \geq 0.059$). Although mean nest building rates during brooding were comparable to those in incubation, this was based on only three pairs exhibiting any building activity in this phase, in contrast to all pairs in the other phases.

Sex Difference in Nest Building Investment

A linear mixed-effects model predicting nest-building rate (seconds of nest building per hour of usable footage) from sex (with pair identity as a random intercept) revealed a highly significant sex difference (LMM: $\beta = 40.8 \pm 15.96$ s/h, $t = 2.56$, $p = 0.013$). On average, females build nests at 23.1 ± 11.4 seconds per hour, whereas males build at 63.9 ± 15.6 seconds per hour (i.e. 23.1 + 40.8). Although the random-effect variance for pair identification collapsed to zero, indicating little between pair-variability beyond

sex difference, the fixed-effect of sex was robust, showing that males consistently invest substantially more time in nest building than their female partners across breeding phases.

During pair formation, females (*n* = 13) contributed 26.8% of the dyadic nest building time, males 73.2% (*n* = 13). During incubation, females (*n* = 12) contributed 31.9% and males 68.1% (*n* = 12). During brooding, only three pairs were observed building the nest, with 22.9% being females (*n* = 3) and 77.1% males (*n* = 3) building the nest (Fig. 13).

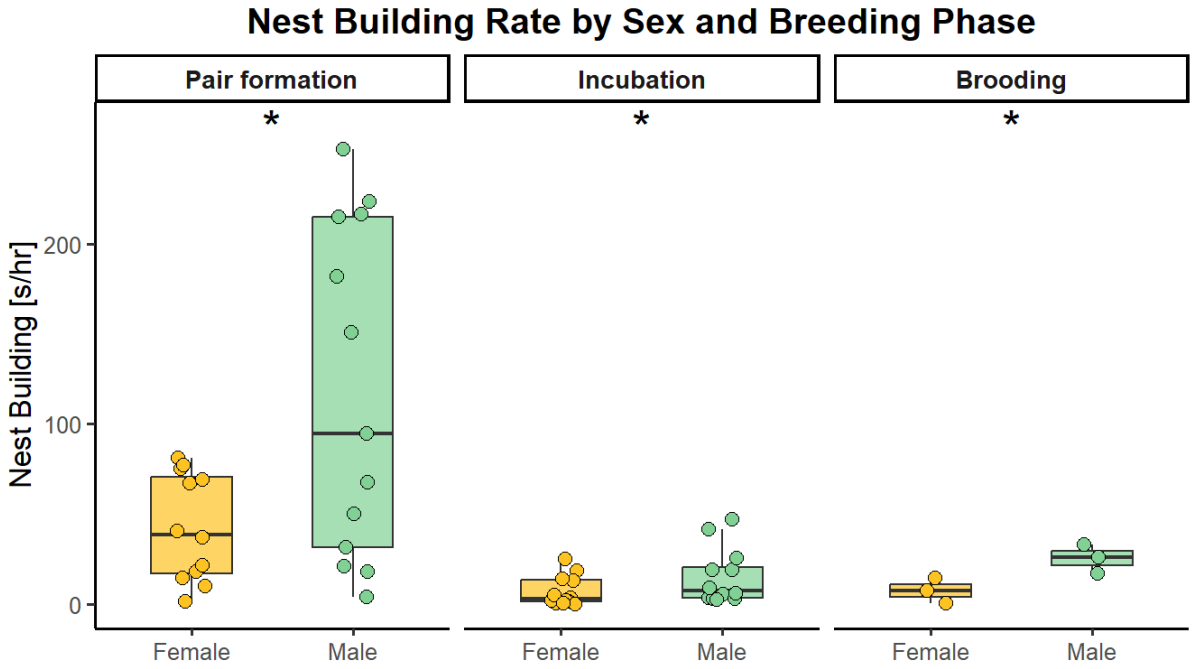

Figure 13: Boxplots of nest building rate by sex and breeding phase of northern rockhopper penguins. Boxplots show the distribution of nest building investment, expressed as seconds of nest building per hour, for female (yellow) and male (green) northern rockhopper penguins during three breeding phases: pair formation, incubation and brooding. All panels share a common y-axis to highlight the large drop in nest-building after the formation phase. A single asterisk above each panel indicates a significant main effect of sex in a linear mixed-effects model (β = 40.8 ± 15.96 s/h, t = 2.56, p = 0.013), with males building significantly more than females in all phases.

Nest Building Investment per Success Group

Season wide nest building effort did not differ between successful (*n* = 5) and unsuccessful (*n* = 8) pairs (*W* = 17, *p* = 0.714). A Shapiro-Wilk test indicated that rates in the unsuccessful group were not normally distributed (*W* = 0.639, *p* < 0.001), whereas those in the successful group were approximately normal (*W* = 0.973, *p* = 0.894). On average, unsuccessful pairs build nests at 19.5 ± 15.2 s/h, compared to 23.3 ± 18.7 s/h for successful pairs, indicating that total nest building investment across the season was not significantly different between success groups.

3.3 Bonding Behaviours by Success

3.3.1 Active Preening

There was no significant difference in total active preening during pair formation between successful (n = 5) and unsuccessful (n = 8) pairs ($t(11) = 0.572$, $p = 0.579$), with a small effect size (Cohen's $d = 0.041$). Total active preening did not differ during incubation ($t(10) = -1.32$, $p = 0.215$). The effect size was moderate (Cohen's $d = 0.305$). The same as during the other two breeding phases, during brooding, successful pairs (n = 5) and adoptees (n = 3) showed comparable active preening ($t(6) = -0.223$, $p = 0.831$), with a small effect size (Cohen's $d = 0.053$).

3.3.2 Proximity up to Beak Length

Successful pairs (n = 5) spent a significantly greater proportion of their proximity time during the total breeding season within beak length (M = 78.4%, SD = 1.71%) than unsuccessful pairs (n = 8; M = 59.7%, SD = 21.6%; $t(7.14) = -2.43$, $p = 0.045$, d = 1.22) (Fig. 14).

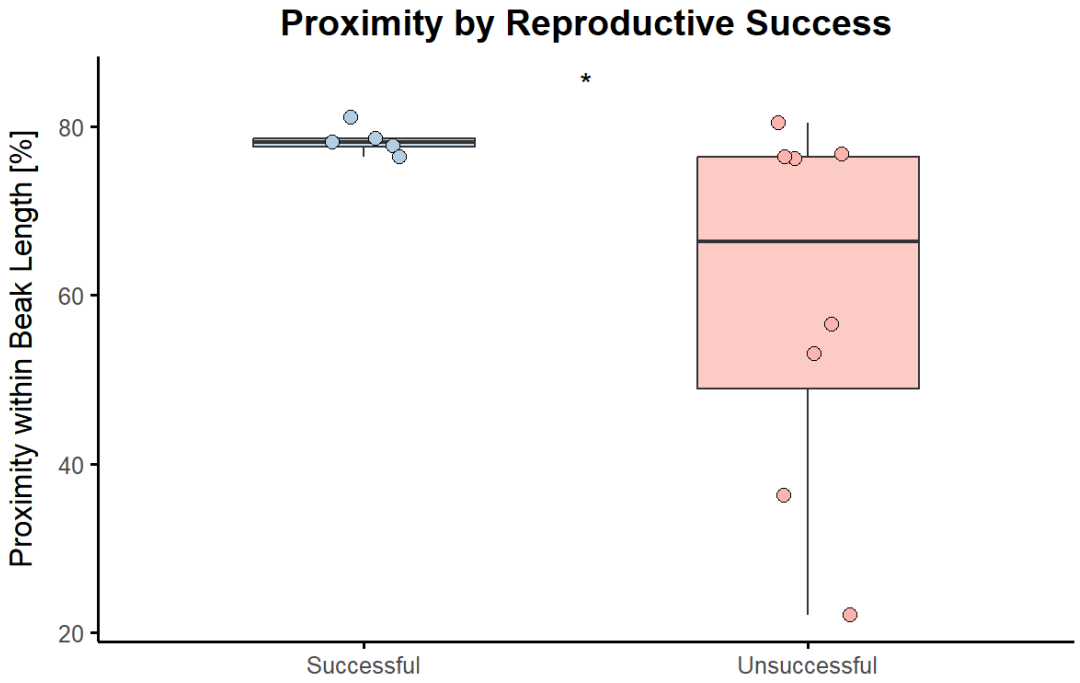

Figure 14: Boxplot showing the percentage of observation time that breeding pairs spent in proximity up to beak length, grouped by reproductive success. The left box represents successful pairs, the right box the unsuccessful ones. Successful and unsuccessful pairs differed significantly in their mean proximity percentage spent up to beak length (p = 0.045).

3.3.3 Mutual Display

Overall, mutual displays were relatively rare. Across the whole breeding season, successful pairs (n = 5) collectively produced 28 mutual display events (0.189 ± 0.080 events/h), whereas unsuccessful pairs (n = 8) produced 52 events (0.209 ± 0.128 events/h). These very low rates reinforce that mutual display is an infrequent behaviour in both groups. There is a non-significant difference in per-pair rates ($t(10) = 0.309$, $p = 0.764$, $d = 0.18$).

3.4 Behavioural and Demographic Predictors of Reproductive Success

Behavioural and demographic predictors of hatching success were evaluated for the 13 northern rockhopper penguin pairs. Ten separate Firth logistic regression models were fitted, six testing affiliative behaviours associated with cooperation and bonding, and four demographic components as a predictor of breeding success.

Among the tested predictors, both proximity within beak length and proximity between beak length up to three metres showed significant associations with reproduction success. The time pairs spent in proximity within beak-length was positively associated with success ($\beta = 0.0014$, 95% *CI* [0.00016, 0.00455], likelihood ratio $\chi^2 = 6.17$, $p = 0.013$). Similarly, time spent between beak-length and three metres to each other was also positively associated with success ($\beta = 0.0013$, 95% *CI* [0.00013, 0.00991], likelihood ratio $\chi^2 = 5.83$, $p = 0.016$). Other predictors of affiliative behaviours showed no significant effects. This included allopreening rate ($\beta = 0.0024$, $p = 0.620$), average nest switching duration ($\beta = -0.0014$, $p = 0.598$) and mutual display ($\beta = -0.165$, $p = 0.924$).

A penalised logistic regression of the association between birth status and success showed a significant association with success at the model level (Likelihood ratio test: $\chi^2(2) = 6.61$, $p = 0.037$). Mixed-origin pairs (one individual zoo born and the other wild born) had reduced odds of success ($\beta = -3.41$, 95% *CI* [-8.54, -0.52], $p = 0.018$), whereas wild-born pairs did not differ significantly from zoo-born pairs ($\beta = -0.34$, $p = 0.81$). No other demographic variables, including the mean age of the pair ($\beta = -0.064$, $p = 0.488$), age difference between male and female ($\beta = -0.026$, $p = 0.719$) and established status ($\beta = 1.099$, $p = 0.326$), were significant predictors of reproductive success (Table 4).

Table 4: Results of separate Firth logistic regression models testing behavioural and demographic predictors of hatching success in northern rockhopper penguins. β coefficients are on the log-odds scale; positive values indicate higher odds of success with increasing predictor values.

Predictor	β	95% CI	p-value	Sig.
Proximity ≤ beak length	0.0014	[0.00016, 0.00455]	0.013	*
Proximity beak length - 3 m	0.0013	[0.00013, 0.00991]	0.016	*
Allopreening rate	0.0024	[-0.0079, 0.0127]	0.62	n.s.
Nest switching duration	-0.0014	[-0.0065, 0.0037]	0.598	n.s.
Mutual display	-0.165	[-3.89, 3.30]	0.924	n.s.
Birth status: mixed-origin vs zoo-born	-3.41	[-8.54, -0.52]	0.018	*
Birth status: wild-born vs zoo-born	-0.34	[-2.88, 2.20]	0.81	n.s.
Mean pair age	-0.064	[-0.24, 0.12]	0.488	n.s.
Age difference	-0.026	[-0.17, 0.12]	0.719	n.s.
Established status	1.099	[-1.07, 3.27]	0.326	n.s.

4. Discussion

The aim of this study was to investigate the relationship between affiliative behaviours, linked to pair bonding and coordination, and reproductive success in northern rockhopper penguins (*Eudyptes moseleyi*) housed at the Vienna Zoo during the 2024 breeding season. The results indicate that several affiliative and cooperative behaviours differ between successful and unsuccessful pairs, supporting the assumption that behavioural coordination and pair bonding are important predictors of reproductive success.

The findings further suggest that reproductive success is not driven by any single behaviour or demographic factor, but is more likely shaped by a complex interplay of multiple behavioural and social dynamics within the pair. It appears to result from a combination of behavioural coordination, physical proximity, and birth status. Although not all expected relationships were statistically significant, the observed trends highlight the complexity of breeding cooperation and social interactions among penguin pairs. As the sample size was quite small, some trends might have been overseen in this population.

4.1. Breeding Phases

4.1.1 Pair Formation

No significant difference in pair formation length was found between successful and unsuccessful northern rockhopper penguin pairs at the Vienna Zoo, which aligns with findings from previous research. In other rockhopper penguin species, the egg-laying period in the wild is brief, with the yolk production spanning approximately 14-17 days, and is considered a rigid laying behaviour (Crossin & Williams, 2016). Consequently, little difference is observed in egg size, hatching success, or chick survival at the end of the brood and crèche periods between pairs that lay early or late in the breeding season (Poisbleau et al., 2008). The results here show a wide range of variation of courting and mating duration, with some pairs having a very short pair formation. As some pairs laid their eggs after 7 days, some pairs may have already formed before moving into Sections A and B, with courting behaviour beginning prior to data collection. Therefore, the duration of the pair formation phase cannot be considered a differentiating factor between success groups.

4.1.2 Incubation

For the five successful penguin pairs at the Vienna Zoo, incubation length ranged from 37 to 40 days, which is comparable to reported lengths in other rockhopper penguin species. Some studies have reported shorter incubation periods of 32-33 days

(Poisbleau et al., 2008), 32-34 days (Pütz et al., 2001) or 36 days (Lera et al., 2023). Variation may be partly due to differences in how incubation length is defined. In this study, the incubation period was measured from the laying of the A-egg, whereas Poisbleau et al. (2008) considered the beginning of incubation on the lay-day of the B-egg. Another factor may be species differences, as most available data derive from southern rather than northern rockhopper penguins.

Environmental conditions can influence incubation duration as well. For example, Emmerson et al. (2011) found that the phenological stages (arrival, egg-laying, and incubation length) of Adélie penguins (*Pygoscelis adeliae*) responded to different environmental triggers, and Black et al. (2018) observed longer incubation durations in gentoo (*Pygoscelis papua*) and chinstrap penguin colonies (*Pygoscelis antartica*) located further south, likely due to colder temperatures slowing embryonic development. In captivity, enclosure conditions such as temperature may similarly affect incubation length in northern rockhopper penguins, potentially contributing to the longer incubation period observed in this study.

4.2 Parental Investment

In wild rockhopper penguins, the parental roles during the breeding period are well defined, with both adults investing time and effort during the egg laying phase until the chicks fledge(Booth & McQuaid, 2013; C. L. Hull et al., 2004; Lera et al., 2023; Ludynia et al., 2013; Pütz et al., 2018). It has been reported that for the first week after laying, both adults remain at the nest, with the female typically taking the first solo incubation shift until the male returns to relieve her, allowing her to feed (Pütz et al., 2018). Females generally invest more time in incubation, whilst males spend more time guarding chicks during the guard stage, often remaining at the nest for almost the entire period (Lera et al., 2023; Ludynia et al., 2013).

Although food-related pressures are reduced in captivity due to regular feeding, the division of parental care was broadly similar to that in wild populations. Females spent significantly more time incubating the eggs and there was a trend of males brooding the chicks slightly more than females, even if this difference remained insignificant. During the brooding phase, the division of investment differed widely among all pairs, regardless of reproductive success or adoption status. Reduced environmental and predator pressures in captivity may lead to less guarding behaviour by males, thereby decreasing sex-depending differences and resulting in more equal brooding duties. Another factor may be that females in captivity do not deplete their body reserves as much during incubation compared to wild counterparts (Kemper et al., 2007) due to a constant high-quality food source, reducing the necessity for males to take over during

brooding. Research has shown that sex-differing investment is adaptive in penguins when environmental stressors are changing, further supporting these results (Saraux et al., 2011).

In Adélie penguins (*Pygoscelis adeliae*), the sex of the offspring has been found to influence the duration of foraging trips during the early guard stage, where females rearing a female chick performed longer foraging trips than females rearing a male chick and males rearing either a male or a female chick (Beaulieu et al., 2009). This may relate to females adapting their parental investment, leaving the males to either invest more or less in the guarding stage. This might also explain the highly differing sex-specific percentages in brooding investment between the eight pairs. However, in the population of northern rockhopper penguins at the Vienna Zoo, the sex of the chicks was unknown, and therefore the possible effect of differing brooding investment by offspring sex could not be assessed.

These results demonstrate novel insights into pair investment during brooding, where there is no significant sex difference observed, with differing results to other species of penguins or rockhopper penguins in the wild. When comparing sex-specific investment between successful and unsuccessful pairs, no significant differences were found in any breeding phase. These findings suggest that sex-specific effort alone may not determine reproductive outcome, and that multiple breeding strategies can lead to success. Coordinative and bonding behaviours are likely to play a more influential role.

4.3 Coordination and Pair Bonding Behaviours

4.3.1 Proximity

Intra-pair proximity is often greater in strongly bonded pairs (Bales et al., 2021; Galante & Margulis, 2022). In this study, successful pairs spent significantly more time within beak-length proximity compared to unsuccessful pairs, suggesting that close physical distance is an important indicator of reproductive success. Among unsuccessful pairs, the standard deviation in beak-length proximity was markedly higher (21.6% vs. 1.71%) in successful pairs, indicating substantial behavioural variability, whereas successful pairs displayed more consistent proximity patterns.

Proximity within a three-metre radius did not differ significantly between success groups, although a large effect size suggested a trend towards greater time spent within this range among successful pairs. Such proximity may reflect both pair bonding and cooperative behaviours, such as remaining near the nest to assist a partner or protect offspring (Foster, 1985). Notably, both beak-length proximity and proximity from beak length up to three metres were the only behaviours that significantly predicted

hatching success in the Firth logistic regression model, further supporting intra-pair proximity as a key marker of coordination and bond strength.

Previous studies have shown that intra-pair proximity is negatively correlated with the duration of pair bonding, with long-term pairs maintaining the closest distances (Galante & Margulis, 2022). As exact pairing histories were not available for this population, further research could investigate whether the proximity patterns observed here are linked to bond duration.

4.3.2 Nest Building

Nest-building investment in this population peaked during the pair formation phase of the breeding season. In rockhopper penguins, nest material serves primarily to insulate eggs, making it most relevant before and during incubation (Ancel et al., 2013; Frere et al., 1992; Morandini et al., 2021; Moreno et al., 1995). Although mean investment appeared higher during brooding than incubation, this pattern was driven by only three pairs (two adoptees and one successful pair) that showed unusually high nest building activity after hatching. In contrast, during incubation all pairs contributed to nest maintenance. Once chicks hatch, their down feathers provide thermal protection, generally reducing the need for additional nest insulation during brooding (Hilton et al., 2004). During pair formation, nests are constructed in preparation for egg laying, whereas in incubation, continued material gathering likely helps maintain insulation and structural stability.

Across the entire breeding season, males invested significantly more time in gathering nesting material than females. This pattern is consistent with the division of labour observed in other penguin species, where males collect materials and deliver them to the incubating female, who then arranges them (F. M. Hunter & Davis, 1998; Moreno et al., 1995). The greater male investment in nest building at Vienna Zoo likely reflects the fact that females spend more time incubating, giving males time to perform this role. Parental strategies in penguins vary by species, sex, and environmental conditions (Beaulieu et al., 2009; Morrison et al., 2016), meaning that the male bias observed here may not be directly comparable to wild populations. Female biased provisioning may enhance reproductive success for females, whilst male involvement is often context-dependent (Saraux et al., 2011).

Despite these sex differences, overall nest building investment - whether by males, females or both parents combined - did not differ between successful and unsuccessful pairs, and it did not predict reproductive success in the Firth regression model. This suggests that, within this captive setting, nest building effort alone is not a reliable

predictor of reproductive outcome. Whilst nest site quality is critical for egg survival in the wild, the mechanisms involved in the quality are multifaceted.

Nest site characteristics are strongly associated with breeding success in several penguin species, including magellanic penguins (*Spheniscus Magellanicus*), Adélie penguins (*Pygoscelis adeliae*), and African penguins (*Spheniscus demersus*). Research shows that nest position within the colony (Frere et al., 1992; Morandini et al., 2021), nest material theft (Morandini et al., 2021; Moreno et al., 1995), nest cover (which protects against predators and extreme temperatures; (Stokes & Boersma, 1998)) and nest size (Morandini et al., 2021) all influence success in wild populations. In captivity, however, some of these pressures are reduced. Access to a steady supply of nesting material by zoo staff, stable enclosure temperatures, and the absence of predation likely reduce the ecological pressures typically influencing nest site quality. As such, while nest-site location and structure may still play roles in success, pair variation in nest building effort or sex-specific investment does not appear to be predictive in this context.

Male effort in nest building may instead reflect pair-specific behavioural strategies. In species like the little penguin, investment patterns remain stable across years within pairs, but differ between pairs, suggesting consistent but individualised strategies (Saraux et al., 2011). This highlights the value of studying reproductive investment over multiple seasons, as single year observations may miss consistent pair-specific behaviours that do not directly predict reproductive success.

Parallel research on the same population of northern rockhopper penguins at the Vienna Zoo further highlights the complexity of nest-related behaviours. Praefke (2025) found that successful pairs engaged more frequently in nest material theft during early breeding stages, suggesting that competitive interactions over materials, and the stress or dominance dynamics they entail, may also influence breeding outcomes.

4.3.3 Allopreening

Across all breeding phases, preening rates did not differ significantly between successful and unsuccessful pairs, and effect sizes were generally small, except for a moderate effect during incubation. In the Firth logistic regression model, preening rate was not a significant predictor of reproductive success. These findings indicate that, in this population, allopreening alone does not directly explain variation in hatching outcome.

Nevertheless, allopreening in birds is widely recognised as a means of maintaining social bonds with key partners, showing some similarities to allogrooming in primates. It can play important roles in courtship and pair maintenance (Spoon et al., 2006),

promoting cooperation in parental care (Kenny et al., 2017) or the re-establishing familiarity between mates after separation (Kushlan, 2011). However, preening is also proposed to function as a coping mechanism following stress or conflict (Henson et al., 2012). Allopreening therefore likely serves a dual function: reinforcing pair bonds and mitigating social tension. Comparative evidence shows it is more prevalent in species exhibiting high parental cooperation and long-term pair stability (Kenny et al., 2017). In common guillemots, partner allopreening was associated with long-term fitness, while allopreening between neighbours correlated with lower aggression and enhanced short-term reproductive outcomes (Lewis et al., 2007). Moreover, post-conflict allopreening appears to act as a reconciliatory behaviour in birds, further supporting its stress-relief role (Radford, 2008).

In the high-pressure context of breeding, preening may thus be performed not only as an affiliative display, but also as a stress-relief behaviour. If allopreening serves as a bonding and stress-coping behaviour, variation in stress levels between pairs could obscure consistent differences between success groups, explaining the lack of significant effects here. Stress responses are particularly relevant during the breeding season, as they can influence coordination, parental effort, and ultimately reproductive outcome (Angelier & Chastel, 2009).

Evidence from the same Vienna Zoo population supports this interpretation: Praefke (2025) examined “soothing behaviours” - including allopreening within 5 minutes after an agonistic encounter - as a proxy for stress. These behaviours were more frequent during pair formation, decreased during incubation, and rose again in brooding. The only significant difference between successful and unsuccessful pairs occurred during pair formation, with successful pairs engaging in more soothing behaviour. This pattern aligns with the present finding that successful pairs allopreened slightly more early in the season. In many seabird species, high allopreening rates during early breeding are associated with long-term pair stability (Kenny et al., 2017), so its occurrence here may reflect a combination of bond strengthening and stress-coping functions. However, because both processes typically peak at the start of breeding, disentangling their relative influence in this context remains challenging.

4.3.4 Mutual Displays

Mutual displays were rare in this population, with only 80 observations recorded over the entire season, with all pairs showing a rate per hour of under 1.1 and no mutual displays recorded for pair 4. Mutual displays did not differ between breeding phases or success groups. This low number, combined with their context-specific nature, may explain the absence of a detectable relationship between mutual display rates and

reproductive outcome. Similarly, the logistic regression model found no evidence that mutual displays predicted success, suggesting they are not a primary behavioural marker of pair bond strength in this setting.

Mutual displays involve coordinated vocal and visual elements distinct from solo ecstatic displays, which are typically used for territory defence and mate advertisement by males (Favaro et al., 2014; Jouventin, 1982). Their vocal function have been studied in many penguin species, found to act as individual recognition (Aubin et al., 2000; Jouventin & Aubin, 2002). Further, mutual displays have been linked to pair bonding: in African penguins (*Spheniscus demersus*), mutual display calls were found to be more acoustically similar between partners than between non-partners, consistent with a role in reinforcing bonds (Baciadonna et al., 2022). They are also thought to facilitate partner recognition upon reunion after foraging trips at sea (Favaro et al., 2021; Jouventin, 1982).

The function of mutual displays in *Eudyptes* species remains less well studied, and their role in northern rockhopper penguins is not fully understood. If they serve similar purposes as to those in other penguin taxa, their occurrence in the wild may be closely tied to foraging trips and reunion events. In captivity, however, the absence of extended foraging periods and altered incubation/brooding schedules could reduce the need or change their function. This may help explain the low number of events recorded here. Given that mutual displays are typically linked to specific social contexts rather than ongoing courtship, low numbers of mutual displays, especially later in the breeding season, are to be expected. Consistent with this, few events and no association were found with reproductive success in this captive *Eudyptes moseleyi* population.

4.3.5 Nest Switching

Neither the incubation switch nor the brooding switch duration was significantly different between success groups. Although cooperation between a pair is crucial for reproductive success (Baldan & Ouyang, 2020; Griffith, 2019), the present results suggest that the latency to take over incubation or brooding (the time between individual leaving the nest and the partner beginning to incubate or brood) does not reliably indicate cooperation level in this population.

Incubation Switching

During incubation, successful pairs switched incubation duties roughly twice as quickly as unsuccessful pairs, but this difference was driven largely by two unsuccessful pairs (pair 3 and 11) that exhibited irregular breeding behaviour. In both cases, the females incubated alone and, when leaving the nest to feed, were not relieved by their partners,

even when the males were present nearby. This complete absence of nest switching cooperation likely contributed to their breeding failure.

In *Eudyptes* penguins, incubation and brooding reliefs are generally well-coordinated with foraging trips, with partners alternating duties to maintain near-continuous nest attendance (C. L. Hull et al., 2004; Pütz et al., 2018). In royal penguins (*E. schlegeli*), relief timing has been shown to vary with foraging trip duration and food availability (C. Hull et al., 1997), while in southern rockhopper penguins (*E. chrysocome*), parental roles during nest attendance and provisioning are relative balanced but sensitive to environmental conditions (Raya Rey et al., 2007). This suggests that failure to coordinate nest switches, as observed in pairs 3 and 11 here, may reflect a breakdown in this fundamental breeding rhythm.

In wild seabirds, coordinated incubation reliefs reduce egg exposure, maintain optimal incubation temperatures, and conserve energy, all of which are linked to higher hatching success (Lislevand et al., 2004; Sládeček et al., 2019). Delays or failure to relieve the partner can result in egg desertion and embryo mortality, particularly when the other bird is absent on extended foraging trips (Davis, 1982). Consequently, in the wild, the duration of incubation bouts may be more indicative of breeding success than the brief hand-over latency measured here.

Brooding Switching

Brooding switch durations were also similar between successful pairs and adoptee pairs, with no outliers. Adoptees were unsuccessful pairs that were selected by zookeepers to raise an additional chick because they were known to be cooperative partners. Anecdotal reports from the keepers noted, for example, that the male in pair 6 could no longer mount the female due to age-related limitations, preventing natural breeding, despite previous success. Although classified as unsuccessful in this study, this pair, and likely the other adoptees, displayed cooperative and strong pair bonding behaviours. In Adélie penguins (*Pygoscelis adeliae*), failure to relieve the partner during the early chick rearing stage (6-8 days old) was linked to chick starvation (Davis, 1982), reinforcing the importance of coordination during this period - although in this study, switch duration itself did not differ between groups.

The absence of significant differences or extreme outliers in brooding switch durations between successful pairs and adoptees suggests that reproduction in these pairs was unsuccessful for reasons other than cooperation, indicating that the zookeepers selection of adopting pairs was well judged.

Nest Switching as an Indicator of Coordination

Overall, these findings provide first insights into nest coordination in northern rockhopper penguins, suggesting that nest switch duration alone is not a reliable indicator of coordination or breeding success in captivity. Instead, the complete absence of takeover behaviour, as observed in certain unsuccessful pairs, may be a more meaningful marker of poor coordination. The lack of general difference between success groups indicates that some unsuccessful pairs cooperated effectively but failed for other reasons.

In the wild, *Eudyptes* species often exhibit highly synchronised incubation and brooding schedules, with switch timing adapted to optimise foraging efficiency and chick survival (C. Hull et al., 1997; Raya Rey et al., 2007). These patterns are shaped by the need to balance energy reserves during extended foraging at sea, and disruption of this coordination can negatively affect breeding outcome. In captivity, where there is no need for long foraging trips and predation or weather pressures do not exist, the urgency of immediate relief is reduced. In the wild, takeover cues are likely triggered by the partner's return from foraging, whilst in captivity, the precise cues concerning handover remain unclear. The duration of nest switching may carry less ecological relevance.

Future research should examine the communication cues that initiate nest relief in captive penguins, as this may clarify how coordination operates outside the ecological context of the wild.

4.4 Demographic Background

4.4.1 Birth Status

In the Firth logistic regression model, pair birth status significantly predicted the reproductive success of northern rockhopper penguins. Whilst pairs consisting of two zoo-born individuals or two wild-born individuals did not differ in their likelihood of success, pairs of mixed birth status showed significantly lower odds of success. Of the five successful pairs, three were both zoo-born and two were both wild-born. On the contrary, the uncoordinated pairs 3 and 11, which did not share incubation duties and had extreme nest switching durations, were both mixed heritage.

One possible explanation is that mixed-origin pairs may differ in their breeding strategies or communication styles, reducing coordination and the ability to form a strong pair bond. Although direct evidence in penguins is scarce, research in captive breeding programmes indicates that differences in origin can influence cooperation and breeding success (Farquharson et al., 2018). The reproductive success of wild-born animals

in captivity is influenced by stress adaptation, social integration, and species-specific behaviours (Willoughby & Christie, 2017). They often face challenges adapting to artificial environments, which can negatively impact breeding (Farquharson et al., 2018), and they may also have difficulties with the lack of proper mate choice in captive populations (Borecki et al., 2024).

Captive-born individuals, on the other hand, tend to be better adapted to artificial environments and social interactions in captivity, showing lower stress responses and more consistent breeding success (Farquharson et al., 2018).

In African penguins (*Spheniscus demersus*), Borecki et al. (2024) found that wild-born individuals may initially display slightly higher reproductive performance if they retain strong parental behaviours, but this advantage often diminishes within one or two generations in captivity. These dynamics suggest that not only birth origin, but also the length of time in captivity, could be relevant. For example, the male in pair 11 was rescued from starvation in Australia only a few years ago and may not be fully adapted to captive life.

The precise mechanisms underlying reduced success in mixed-origin pairs remain unclear in this northern rockhopper penguin population and merit further study. Understanding the influence of birth origin is important for breeding programme management and should be considered in pairing decisions.

4.4.2 Established Status, Age Difference and Mean Age of Pairs

All other demographic variables tested in this population showed no significant predictive power in any model, meaning there was no evidence supporting the hypothesis that established pairs had an increased likelihood of reproductive success. Neither mean age nor age difference between partners predicted reproductive outcome.

Research on penguin breeding success has demonstrated a relationship between the quality and duration of pair bonds, with several species showing that long-term partnerships can enhance reproductive performance (Borecki et al., 2024; Nisbet & Dann, 2009). However, the development of a strong pair bond typically occurs over multiple seasons rather than within a two-year period. Therefore, using only the previous breeding year as a measure of an established relationship may limit the ability to detect its influence on reproductive success. The absence of breeding records for earlier years, and uncertainty regarding the exact duration of each pair bond, further constrains the analysis of reproductive success in relation to pair establishment.

Similarly, no effect of mean age or age difference was observed. In long-lived seabirds, reproductive performance often peaks at middle age, when individuals have both fully

physical capacity and extensive breeding experience, and may decline in older age classes (Nisbet & Dann, 2009; Williams, 1984). If most birds in this study fell within an intermediate age range, a lack of variation could mask such patterns. Furthermore, in species with biparental care, large age differences between partners may affect coordination if one partner is inexperienced, but this effect is not always detectable in small samples (Bried & Jouventin, 2002). In captivity, factors such as consistent food availability, absence of predation risk, and veterinary support may mitigate age-related declines in condition.

Taken together, the absence of demographic effects in this study may reflect both biological factors and methodological constraints (small sample size, incomplete breeding histories). Long-term monitoring with more detailed life-history data would help clarify whether demographic traits influence reproductive success in northern rockhopper penguins. In the context of the present findings, where behavioural coordination rather than demographic traits appeared more closely linked to breeding outcomes, this suggests that partner compatibility and cooperative behaviour may be stronger determinants of success than age or establishment status in this captive population.

4.5 Methodological Considerations and Limitations

These findings revealed correlations between pair stability, cooperative behaviours, and reproductive success, but did not identify clear single behavioural variables that reliably explain pair bond strength or reproductive success. This suggests that reproductive success likely results from a combination of multiple behavioural and contextual factors. Furthermore, other inter-relational aspects, such as agonistic interactions or stress responses within the colony may also influence breeding outcomes. These aspects have been addressed in a complementary study conducted on the same colony during the 2024 breeding season in another Master Thesis (Raefke R.T., 2025).

One major methodological limitation was the incomplete camera coverage of the enclosure. Not all behaviours could be recorded with equal visibility, leading to the necessity of supplementing the dataset with video footage from the zoo's internal cameras. Whilst efforts were made to minimise bias by matching filming conditions (e.g. time of day, fasting versus feeding days), variations in visitors presence, sun exposure, or camera angles may have introduced subtle inconsistencies in the observed behaviours.

Another constraint was the lack of precise measurements within the enclosure. The three-metre radius around nests, used to categorise proximity behaviours, was estimated visually by a single coder to avoid disturbing the breeding colony (instead of

measuring inside). While this consistent coding reduced inter-observer variability, it does not eliminate the possibility of systematic distance estimation errors. Future studies could address this by measuring distances during the non-breeding season to establish clear spatial reference points, thereby improving the accuracy of proximity-related behavioural data.

The sample size also limited statistical power. With only 13 pairs, dividing individuals into successful and unsuccessful groups reduced within-group variation and may have obscured subtle behavioural effects. Additionally, pair success in a single breeding season may not reflect long-term pair quality, as some strong pairs may fail in one season due to age-related factors or other transient conditions. Multi-year studies would therefore be valuable to confirm these findings and better capture the dynamics of pair bonding and cooperation over time.

Lastly, this study was restricted to visual observation and did not include other potentially relevant cues, such as vocalisations or physiological indicators (e.g. stress hormones), which could provide further insights into the mechanisms underlying pair coordination and bonding.

These limitations are common in behavioural studies of social species in ex-situ environments and should be considered when interpreting the relationship between cooperative and bonding behaviours and reproductive success. Future research could address these constraints through enhanced camera coverage, spatial measurements of the enclosure, and the continuation of long-term monitoring of this penguin colony across multiple breeding seasons.

5. Conclusion

This study investigated how pair bonding and coordination behaviours relate to reproductive success in northern rockhopper penguins (*Eudyptes moseleyi*) at the Vienna Zoo. The results demonstrate that while multiple behaviours contribute to pair dynamics, proximity within beak length and proximity from beak length up to three metres emerged as a consistent predictor of hatching success. This finding highlights the importance of close spatial association between partners as a marker of pair bonding and cooperative investment in reproduction. Other behaviours, such as nest building, allopreening, and nest switch durations, showed no clear direct link to success, although they may still play an indirect or complementary role in maintaining pair stability. The birth status of the pairs emerged as a significant predictor of success, suggesting that perhaps pairs of differing birth origin might have different breeding strategies or do not effectively communicate during the breeding season, leading to failed coordination. This further supports the idea that pair coordination is important in reproductive success outcomes.

Importantly, these results suggest that reproductive success should not only be evaluated by comparing successful and unsuccessful groups but also by examining specific pairs. Some pairs showed clear signs of disorganised or uncoordinated behaviour, indicating that pair-level assessments may be crucial for targeted management decisions.

These findings also have implications for the chick adoption practice implemented at Vienna Zoo. No differences in brooding behaviour were observed between successful pairs and adoptees, suggesting that placing chicks with well-coordinated pairs is an effective and appropriate management measure. However, adoption carries poptential risks. Introducing a chick to poorly coordinated or inexperienced pairs may increase stress or reduce care quality, and repeated adoptions could reinforce pair bonds in otherwise unsuitable partnerships by creating a false impression of reproductive success. While this may provide a short-term management benefit for maintaining ex-situ populations, the long-term effects on pair stability and overall breeding outcomes remain uncertain. This underlines the value of behavioural monitoring to ensure that adoption is applied selectively, and should be done under careful consideration and close supervision.

This study gives first insights into the intra-relational behaviours of northern rockhopper penguins in captivity, and their sex-specific investment during the breeding season. Sex-specific differences in incubation investment and male-dominated nest building investment were observed but did not translate into reproductive success differences,

suggesting that demographic and individual factors alone can not explain breeding outcomes. Similarly, pair history and age showed no significant effects, underlining that behavioural coordination, rather than static demographic characteristics and sex-based role division, is central to reproductive success in this species.

Here, it is emphasised that reproductive success in northern rockhopper penguins is a multi-factorial process, shaped by the interaction of affiliative behaviours, coordination, and pair-specific dynamics across the breeding season. They also demonstrate the value of fine-scale behavioural monitoring in ex situ populations, offering potential practical implications for captive management, such as using proximity as a non-invasive behavioural indicator of pair compatibility.

Further research should expand on these findings by incorporating multi-year data, if possible larger sample sizes, and additional behavioural variables, including inter-pair interactions and auditory communication, to further clarify the mechanisms that underpin successful reproduction in this species. By integrating such behavioural insights into conservation management, zoos may be better equipped to support sustainable breeding outcomes in endangered penguin populations.

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Ending note

I used AI-assisted tools (ChatGPT by OpenAI) for language refinement, structural feedback and coding support in R. All research decisions, behavioural video coding and statistical analyses, as well as interpretations and conclusions, are entirely my own.

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Appendix

The table below lists all northern rockhopper penguin pairs included in this study, along with their success status (successful = at least one egg hatched, unsuccessful = no eggs laid or no eggs hatched), their pair formation, incubation and hatching date. Pair ID correspond to those used in the behavioural dataset.

Table 5: List of all northern rockhopper penguin pairs included in the study (n = 13), showing pair ID, pair formation date (onset of courting), incubation date (date first egg was laid), hatching date (date first egg hatched or date unsuccessful pairs adopted second chick from successful pair), and success classification. Pairs classified as unsuccessful during incubation analyses were considered adoptees during brooding analyses if they received a chick.

Pair ID	Pair Formation Date	Incubation Date	Hatching / Adoption Date	Success Group
1	15.02.2024	01.03.2024	21.04.2024	Unsuccessful (Adoptee)
2	15.02.2024	27.02.2024		Unsuccessful
3	28.02.2024	10.03.2024		Unsuccessful
4	15.02.2024			Unsuccessful
5	15.02.2024	11.03.2024	19.04.2024	Successful
6	15.02.2024	29.02.2024	31.03.2024	Unsuccessful (Adoptee)
7	15.02.2024	22.02.2024	31.03.2024	Successful
8	15.02.2024	14.03.2024		Unsuccessful
9	15.02.2024	11.03.2024	20.04.2024	Successful
10	15.02.2024	06.03.2024	13.04.2024	Successful
11	19.02.2024	12.03.2024		Unsuccessful
12	15.02.2024	04.03.2024	10.04.2024	Successful
13	16.02.2024	24.02.2024	14.04.2024	Unsuccessful (Adoptee)