Timing of egg laying, moult, and annual changes in body weight in captive kororā (little penguins; *Eudyptula minor*) at the National Aquarium in Napier, New Zealand from 2013 to 2021

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Zoology at Massey University, Palmerston North

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Abstract

This study looked at the timing of egg laying, moult, and annual changes in body weight for captive little penguins housed at the National Aquarium, Napier. The aquarium has been keeping handwritten diaries they call “penguin diaries” for each year, and these diaries hold day-to-day information about the captive little penguins. Using the data from these diaries from 2013 to 2021, we could extract dates for when eggs were laid, who laid the egg, how many were laid, the occurrence of replacement clutches and the incubation period for the eggs. In the same way, we got information about when they started and ended their moult, which allowed us to calculate the duration of the moult. The aquarium provided the monthly body weights and daily feed intake data as an Excel file. Body weight was observed with the bird's annual cycle.

The timing of egg laying for the first clutch started in September, and the replacement clutch was observed in October for the second clutch and November for the third clutch. The timing of moult was observed from December to January and ranges from October to March for both male and female little penguins. An increase in body weight was seen as the bird starts to moult and would decrease as the bird undergoes their moult and would be the lowest when they come out of their moult. Feed intake would increase in December with the bird's moult coming up. Female birds showed more variation in their body weight during the breeding season than males. Therefore, the birds showed annual changes to their body weight as they progressed through their annual cycle. Their egg-laying and moult timing are similar to that of wild little penguins.

Acknowledgements

First, I would like to thank my parents for their constant support and encouragement regarding my future, from allowing me to study abroad and pursue my academic dreams. Their tremendous love and support for my abilities have inspired me always to push forward. The sacrifices they have made, big and small, have been a foundation for me to pursue my educational goals, and I will forever be grateful for their sacrifice and thankful that I was born their daughter.

I would also like to thank my older brother, who is the main reason I fell in love with the sciences. He was the person who would talk to me about the world we live in and the surrounding space. He once quoted Neil DeGrasse Tyson when I was in high school.

"We are stardust brought to life, then empowered by the universe to figure itself out – and we have only just begun" - Neil DeGrasse Tyson.

This quote has forever stuck with me and always plays in my mind whenever I have any self-doubt or feel stuck. As I grew up and got more involved with my bachelor's degree, he always listened to "interesting facts" I learned. He would always read my essays, and even with my Master, he would always be there to check up on me and constantly listen to my progress. His constant understanding and occasional humour motivated me through the highs and lows of my thesis journey.

Next, I am forever grateful to my supervisor, Prof John Cockrem; thank you for agreeing to meet with me when I was unsure how to proceed after my bachelor's and for inviting me into the world of the little penguins; you have provided me with exceptional supervision, consistent guidance, and mentorship. You have played an incredibly pivotal role in my research, and I will always be grateful and blessed for knowing such a supportive and knowledgeable mentor.

I am also very grateful for my fiancé, Tyrone; your unwavering support has been an anchor through this journey. Thank you for always believing in my ability even when I did not. Your emotional strength and willingness to lend a listening ear have helped me stay focused on this journey. I will always look at you as my inspiration, for I am truly fortunate to have found a life partner who not only celebrates my wins but also stands by me during my losses or, in better words, “life lessons”. Thank you once again for supporting me through my thesis journey and the journey we call life.

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Moreover, finally, I would like to thank the little penguins that are a part of this study. I have listed them by name in Section 2 subsection 2.3 of this study. Having read the diaries from 2013-2021, I feel connected with these birds, as reading about their daily activities made this work more personal.

Table of Contents

1. General Introduction

1.1 Kororā (little penguins; *Eudyptula minor*)

Little penguins (*Eudyptula minor*) [Māori: kororā] belong to the scientific order Sphenisciformes, to which all other species of penguins belong to (Department of Conservation, 2023; New Zealand Birds Online, 2023). There are 17 living species within the order Sphenisciformes, distributed across the Southern Hemisphere, from the Galapagos Islands to Antarctica (Marchant and Higgins, 1990b). Although penguins are the most evolved common aquatic bird, they still rely on going to land to perform essential behaviours associated with their annual cycle.

Within this order, kororā are regognised as a single species with two subspecies by the Ornithological society of New Zealand (Checklist Committee of the Ornithological Society of New Zealand, 2022). The two subspecies are the *Eudyptula minor*, which includes all birds found on the shores of New Zealand (Peucker *et al.*, 2009; Cargill *et al.*, 2020), and *Eudyptula novaehollandiae*, which includes the Australian population and some Otago birds (Braidwood *et al.*, 2011; New Zealand Birds Online, 2023). While the two subspecies are genetically different, their distribution and sympatry in southern New Zealand is poorly known (Grosser *et al.*, 2015; Grosser *et al.*, 2016). The plumage on their back is characteristically blue-grey with a blue-black head, white belly, and chin (Fig 1.1). They are also reported to be the smallest species of penguins, reaching a size and weight of just over 25 cm and 1 kg, respectively (Ropert-Coudert *et al.*, 2007; Borboroglu and Boersma, 2013; Department of Conservation, 2023). The IUCN Red List (2020) has classed them as "least concern", and the Department of Conservation (2023) in New Zealand classifies them as "at risk" and declining.

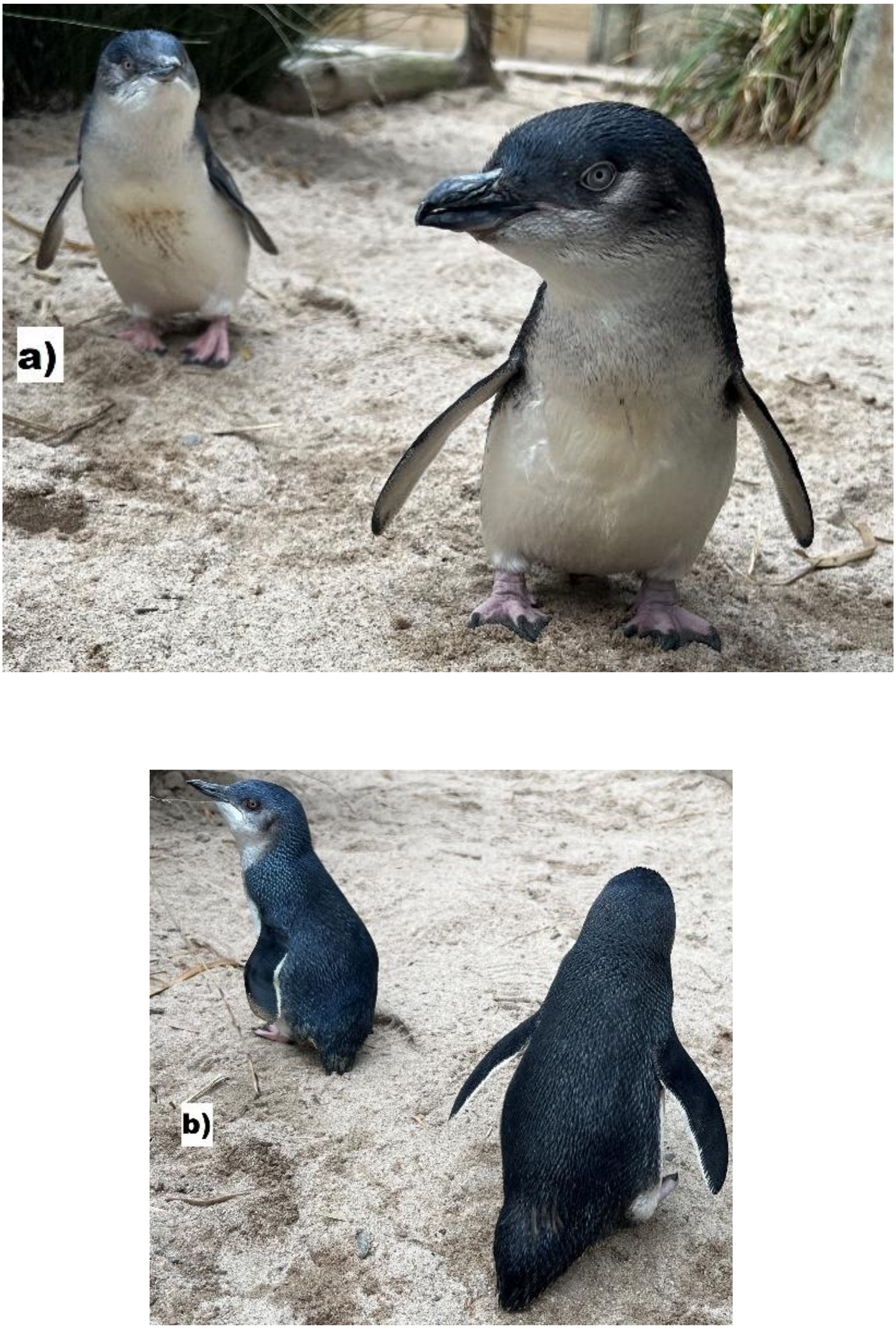

Fig 1.1. a) Dora (left) and Dave (right) showing their white belly and chin. b) Dora (left) and Dave (right) showing their blue-grey and blue-black head respectively. Photos by Ritika Ganesh (2021).

The annual cycle of these birds consists of a series of critical and distinct life events. It can be closely linked to food availability, habitat, and reproductive needs (Gales and Green, 1990), which are also subjected to human disturbances, climate conditions, and even ocean temperatures, which can affect prey availability (Agnew *et al.*, 2015; Berlincourt and Arnould, 2015). Little penguins in New Zealand spend prolonged periods at sea foraging during April through to May to help prepare for the breeding season (Forest & Bird, 2018). Penguins usually come ashore from June to begin their annual cycle. Egg laying usually occurs in the months from July to October and their incubation spells average 36 days (Kemp and Dann, 2001b). Once the chicks start to hatch, they enter into the guard stage of their cycle which is from September to December the parents make back-and-forth trips to the sea to bring back food and care for their chicks until they are fully grown at eight weeks. The parents then return to sea after the chicks are independent to start rebuilding their reserves for their moult. The moulting process takes around 2-3 weeks and usually starts for the little penguins between December and March. During this time, they are vulnerable to predators and must undergo a fasting period since they are unable to swim. Then, from April, the cycle repeats (Fig 1.2).

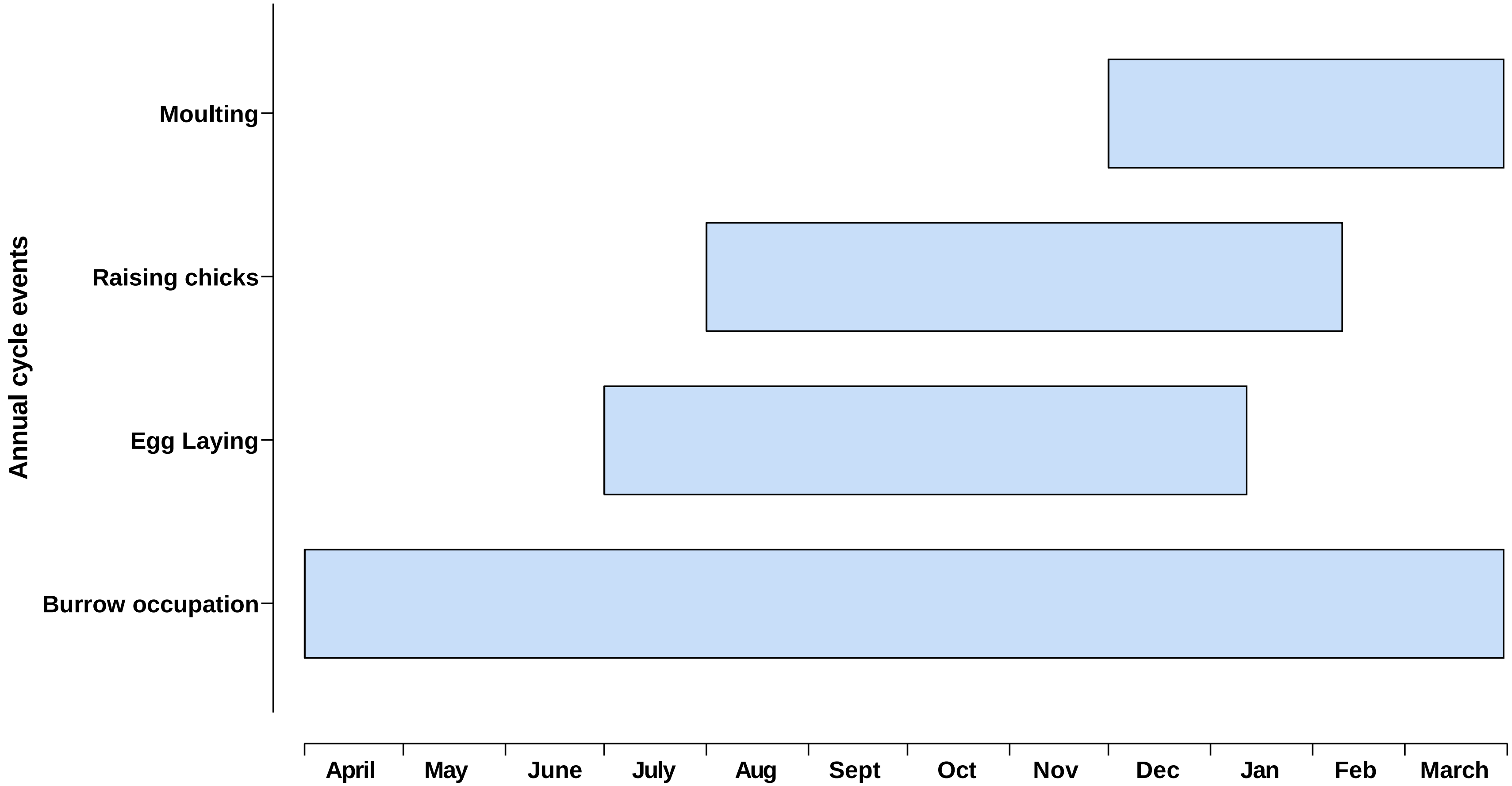

Fig 1.2 Annual Cycle of Little Penguin (kororā).

1.2 Body weight and condition index

Wild kororā are constantly alternating between fasting and feeding since they feed exclusively at sea but spend the majority of their annual cycle on land (Warham, 1958). The birds have to undergo a fast during periods where feeding is competing with other activities such as breeding and moult (Groscolas and Robin, 2001). To understand changes in body weight, observation studies have looked at foraging of little penguins around different times of the year. The majority of studies focus on foraging of Australian little penguin colonies, but the New Zealand colonies experience different prey conditions and therefore cannot be assumed to be similar to Australian studies. There are some foraging studies that have emerged for different New Zealand colonies (Agnew *et al.*, 2013; Chilvers, 2017; Elsom, 2022) in more recent years and by understanding their foraging behaviour, we are able to understand how their body weight changes depending on which process of their annual cycle they are at.

During the months after the birds finish their previous season moult, they are seen to forage for long periods to replenish their fat stores and energy reserves in preparation for the new breeding season. During the breeding and moulting season these birds must stay on land which lead to long-term fasting situations (Groscolas and Robin, 2001). The condition index which is a measure of the fat mass changes as the bird goes through its annual cycle (Stevenson and Woods, 2006). Birds from different colonies may align their breeding season (Groscolas and Robin, 2001) with the changes in prey availability to allow for a more successful breeding season (Berlincourt and Arnould, 2014). During the breeding season, female birds undergo an energetically demanding role in terms of egg-laying (Ramirez *et al.*, 2015). Once incubation has started both male and female birds will take turns foraging and staying with the egg. Birds are seen foraging <20 km from their colony, and breeding season varies from year to year. Birds will go through 1-day trips before incubation, but Collins *et al.* (1999), observed that birds spent longer time foraging during the incubation period. They also found that birds would have shorter foraging trips the younger their chicks were, and as their chicks were approaching their time to fledge the parents spent longer foraging (Buller, 1882; Collins *et al.*, 1999). This may be due to the increased energy demands associated with chick rearing (Buller, 1882). The birds body condition is very important during the breeding season, as it can affect egg laying (Robinson *et al.*, 2005).

Once the breeding season is over, they return to forage in preparation for their moult. This is a long-term fasting situation as little penguins do not forage during moult (Reilly and Cullen, 1983). They can fast for prolonged periods and by utilising the fat stores they build up after the breeding season (Green *et al.*, 2004). To prepare for the long-term fast birds will spend 3 weeks extensively feeding and double their body mass. Their condition index during moult is the highest due (Hocken, 2000) to this hyperphagia observed as the birds spend time at sea building up their nutrient stores and requires the birds to adjust their feeding behaviours to allow for such an intensive feeding period, especially if other colonies are present in nearby that could be seen as competitors (Thiebot *et al.*, 2014). Successful birds build enough fat to survive during and after the moulting period. The body condition of the birds is important for their survival and being in poorer body condition does pose difficulties during their moult (Cuming, 2009).

1.3 Breeding

After the end of the previous seasons moult, little penguins will spend time at sea foraging in preparation for the next breeding season. The commencement of the breeding season for little penguins depends on the breeding colony and can vary from place to place. Different factors are being investigated that play a role in determining the initiation of breeding in penguins, and the understanding of these cues, for example, photoperiod, may be an essential step in understanding when the birds decide to breed (Cockrem, 1995a). Buller (1882) had birds that started breeding from late June to September. Knight and Rogers (2004) showed that the colony at Philip Island laid eggs from September to October, but there were some years when the birds were seen laying eggs as early as May and would go through till late December. Reilly and Cullen (1981), found that the birds at Summerland on Phillip Island whose data has been collected for 11 years started breeding around August to September but had cases where birds started breeding as early as May or June.

Green *et al.* (2008) noted that one of the factors that could be influencing the timing of egg laying and the breeding of these birds is the availability of food. Food availability depends on prey abundance, which can be directly affected by sea surface temperatures (SST) and changes in chlorophyll-a (Agnew *et al.*, 2015; Colombelli-Négrel *et al.*, 2022). Potts *et al.* (2013) noted that a warmer SST leads to reduced spawning rates and higher egg mortality rates of fish species. Banks *et al.* (2002) saw that in 2012-2013, due to lower prey abundance, some birds spent much more time foraging, resulting in lower breeding success.

Little penguins are normally seen laying two egg clutches and normally have 2-3 days between the laying of each egg (Kemp and Dann, 2001a). Sometimes, if a bird has laid early there maybe chances of replacement clutches being laid towards later months in the breeding season or after chicks have fledged (Agnew *et al.*, 2014). There are also cases of replacement clutches being laid in case the first clutch didn’t survive (Dann *et al.*, 1995). According to a study by Herald (2018), they analysed six years of data from five different penguin breeding colonies, to determine the annual breeding success during El Nino/La Nina perturbations. Their study found that there were fewer double breeders among the birds that started breeding late which may be due to the higher sea temperature around the South of New Zealand. A penguin's body condition can also affect the timing of egg laying (Robinson *et al.*, 2005). The study found that the penguin colony on Phillip Island had synchronous egg-laying in 2000 but asynchronous egg-laying in 2001, and the researchers believe that body condition may have influenced the breeding of little penguins. While the body conditions of the penguins were good in 2000, they were lower in 2001 than in the previous year. Banks *et al.* (2002) also found that penguins tend to lay eggs earlier if changes in sea surface temperatures and Chlorophyll-a occur earlier (Carroll *et al.*, 2016b). This variation in sea surface temperature has been recorded to influence the timing of breeding and egg-laying in little penguins (Crockett, 1977; Falshaw, 2007).

1.4 Moult

Moult in little penguins is the renewing of their entire plumage. In most cases, moult is seen to occur from January to March (Croxall, 1982; Reilly and Cullen, 1983; Department of Conservation, 2023). The feathers of penguins are unique and hence their moulting style is also unique and not seen in other birds. Unlike other birds whose feather are in tracks, some species of penguin feathers are densely packed in 30-40 feather per square centimetres (Dawson *et al.*, 1999). Little penguins are avid swimmers, and their feather quality and coverage is important for their survival. Their feathers provide them with thermal insulation but are also impenetrable to water and wind (Dawson *et al.*, 1999). Moult occurs annually and is usually after the birds have completed their breeding season (Reilly and Cullen, 1983).

Due to the nature of penguin moult where there is a high metabolic demand to replace their feathers, Gales *et al.* (1988a), found that there was an approximate decrease in body weight by 50 g per day during this moulting period. Successful birds build enough fat to survive during and after moulting period which is approximately around 14-17 days and it counts for 8.4% of their annual energy budget (Kowalczyk *et al.*, 2014; Kowalczyk *et al.*, 2015). There have been some instances that show that birds that had a successful breeding attempt or those that didn’t breed moult earlier than those whose breeding attempts were unsuccessful (Gales *et al.*, 1988b). The new feathers that grow are a reflection of the birds successful fat stores and isotopic studies (Cherel *et al.*, 2000; Jaeger *et al.*, 2009; Bushman, 2016) have used this new plumage to understand the signature of prey consumed during the start of moult. Once moult has started the nutrients that are utilised from the fat and protein store to help with the keratin production (Cherel *et al.*, 2005). Sometimes the birds may have started their moult later due to not building enough reserves. The range when it comes to timing of moult depend on the location of the colony (Reilly and Cullen, 1983) but also many other factors such as food availability during and after the breeding season or pre -moult season (Stahel and Gales, 1987; Collins *et al.*, 1999).

1.5 Kororā in captivity

This study looks at little penguins in captivity to see if they experience a similar annual cycle since they are not in the same setting as in the wild. In the wild, little penguins are found all along the coast of New Zealand, Stewart and Chatham Islands (Department of Conservation, 2023). There are also breeding colonies around Western Australia (Dann, 1988) to the Far East, such as South Solitary Island and New South Wales, including the Bass Strait and Tasmania (Dann, 1992). They have abundant space and freedom to forage, build nests and form colonies that sometimes range from 100 to over 1000 birds. Today's largest breeding penguin colony is on Phillip Island in Australia, with 40,000 breeding pairs on the Summerland Peninsula (Penguin Foundation, 2024).

Breeding management is not the focus of the National Aqaurium; but captive rearing does occur in other zoos. The Melbourne zoo in Australia recorded variable chick survival rates from 2010 – 2016 (Geraldene, 2020) and initiated a trial in 2016 as a form of breeding management. They looked at specific circumstances where chicks could be fostered under surrogate parents and found that the timing of fostering chicks is crucial to have a more successful outcome. In the wild, little penguins have been observed to leave their chicks in their nest unattended around 2 weeks of age (Chiaradia and Kerry, 1999) but the study done by Melbourne zoo observed at least one parent in the nest right up to and including 5 weeks of age (Geraldene, 2020). The study provided insight in the application of fostering chicks as a breeding management (Dunn, 1986; Geraldene, 2020). There are other breeding management practices such as hand rearing (Klusener *et al.*, 2018) that is utilised across the world for a variety of other captive species but it does require more time and resources (Kuehler and Good, 1990; AZA Penguin Taxon Advisory Group, 2014). There is not enough research published to help provide insight to the implications captivity has on the rearing of little penguins in captivity.

There are also a few rare cases where they are found outside of Australia and New Zealand in captivity. Japan has 42 fairy penguins spread around four zoos (Takeuchi, 2022). They also have been trying to breed genetically-diverse birds by intra-park breeding. They can also be found in one zoo in the UK (Belfast Telegraph, 2018) and a variety of zoos in the US (Bronx Zoo, 2015; Gros, 2022). Being in captivity, they do not have to travel distances to forage for food, and the penguins in this study are fed three times a day on a fixed number of fish per day, depending on where they are in their cycle. Wild little penguins forage various species as food, from small fish and squid to crustaceans (Cullen *et al.*, 1992; Geurts, 2006). The birds in this study are only fed *Sprattus sprattus* and are provided with a multivitamin inserted into the gill of the first fish they eat. In terms of social structure, little penguins form large colonies in the wild, leading to complex social structures, but in captivity, the number of individuals in the colony is significantly less. The birds in this study have still formed their colony and established breeding pairs.

Another factor the wild birds deal with is predators. Their predator list ranges from the fur seal, stoats to domestic cats and dogs (Cannell *et al.*, 2016). In some cases, they may be found in coastal towns and are at risk from being hit by a car or boat (Harrigan, 1992). The wild birds also have other causes such as disease (Jansen van Rensburg, 2010) and environmental challenges such as storms that play into their survival. But the birds at the aquarium have no worries about predators as they are protected in their enclosure. They may experience disturbance from visitors (Sherwen *et al.*, 2015; Chiew *et al.*, 2019), but the aquarium has policies and procedures in place to keep their birds as stress free as possible. For the birds in captivity, any signs of distress from getting into a fight with other birds, not eating, or disease such as bumblefoot is immediately checked out by a veterinarian and monitored by the penguin keepers. Exposure to a variety of stimuli and environmental factors allows the birds in the wild to be more active and aware but, the birds in captivity have to have enrichments added to the enclosure to provide stimulation. The birds in this study have a beach with lots of foliage but also jets around the pool to stimulate flowing water. In some cases, the penguin keeper might throw a fish into the pool during feeding time for those birds that like to feed in the water.

Human interaction with wild little penguins is limited but there is research going on with penguin colonies, but it mostly involves, checking nest boxes, microchipping, weighing and in some cases drawing blood etc (Colombelli-Negrel, 2019; Cargill *et al.*, 2020) and occurs at certain times of the year for example, during the breeding or moulting season. This is done to keep interaction with humans to a minimum. But the captive birds are exposed to the penguin keepers at least three times a day, there are divers that come to clean the pool, and the aquarium also hosts encounters, so the birds are exposed to a variety of people. Interactions with humans can cause these birds to become less wary of us.

In terms of the annual cycle, we have some understanding about how the birds in the wild progress through it. They need to spend a considerable amount of time at sea foraging, especially during their moult (Reilly and Cullen, 1983). For at least two weeks, little penguins are unable to forage for food as they grow a new plumage of feathers. Their foraging in the wild depends on various factors such as sea surface temperatures (Carroll *et al.*, 2016a), chlorophyll-a (Mickelson *et al.*, 1991), and prey availability (Agnew *et al.*, 2015; Berlincourt and Arnould, 2015). The penguins used in the study are captive and are currently located at the National Aquarium in Napier. The reason for their arrival at the aquarium is explained in section 2.3. These birds have access to a pool and pre-established nest boxes of their choice. The study is aimed at understanding how the birds in captivity with all these differences with their wild counterparts experience their annual cycle.

1.6 Outline of thesis

This study was conducted to investigate whether little penguins in captivity experience the same or similar annual cycle as their wild counterparts. The National Aquarium has been collecting daily information about these birds and by using that information we were able to determine their timing of egg laying, and their timing of moult. The birds were weighed monthly and the number of fish each bird ate making up their food intake were both recorded and stored on an Excel file which was also sent over by the aquarium. In terms of condition index, birds that were present at the aquarium will only be included as we do not have the flipper length of those that passed in prior years. Using both the information of body weight and food intake we can understand the changes these birds undergo with respect to their annual cycle.

Section 3 talks about the annual changes of the birds' body weight, condition indices and food intake from 2013 to 2021 and will look to answer whether birds in captivity experience annual changes in their body weight and condition index and how food intake changes depending on their annual cycle?

Section 4 discuss the timing of breeding such as when does the timing of egg laying occur in captive little penguins? And what is the average incubation period for captive birds whose eggs have hatched?

Section 5 discusses the timing of moult and looks at answering question such as when does moult begin in captive little penguins? What is the duration of moult in captive little penguins? How does body weight change during moult? does egg laying affect timing of moult? And does age affect the duration of moult?

2. The National Aquarium of New Zealand

2.1 Introduction

The National Aquarium is a public aquarium that is located on Marine Parade in Napier, New Zealand and holds a variety of animal exhibits. Before it was established as the aquarium it is today, it started out as a fish tank measuring 3 ft deep by 18 in high and 18 in wide (Fowler, 2018) in the store of retailer Les Mills in 1954 (The National Aquarium of New Zealand, 2024). The popularity of this fish tank led to a public aquarium being built in the basement of Napier's War Memorial in 1957. The National Aquarium of today was moved to its present location in 1976, and in 2002, the aquarium went through a renovation that included the Oceanarium exhibit, which was then opened to the public as The National Aquarium of New Zealand. The aquarium is home to the little penguins, kiwi and tuatara. With the penguins, they offer a close encounter opportunity to the public, so the penguins get exposed to different people besides just the penguin keepers, and they also feed the penguins three times a day at set times.

2.2 Penguin Cove: little penguin habitat at the National Aquarium

2.2.1 Enclosure and husbandry

The National Aquarium in Napier houses a colony of little penguins in an outdoor enclosure called the Penguin Cove. The enclosure boasts a large pool at the centre that holds 36,000 litres of water and is 1.5 m deep. It is surrounded by a sandy beach and a rock wall. It includes permanent and movable homes catering to the penguins' requirements. The surrounding environment is adorned with logs, rocks, and flax bushes to provide a natural habitat for the penguins. The penguins are also exposed to the natural day/night cycle as the top of their enclosure is open and only covered with a mesh (Fig 2.1.c).

Multiple jets in the pool keep the water moving and create an illusion of flowing water. The little penguins enjoy swimming against the current. Also, there is an underwater viewing area for the public to watch these birds as they swim (refer to Fig. 2.1 b). The birds are fed three times a day at 9:30 am, 1:30 pm and 3:30 pm and usually must come to the feed station (Fig. 2.1 d). The penguins are fed a diet of sprattus, and during the first feed of the day, a multivitamin tablet is placed in the fish's gills. This is the first fish fed to each bird to ensure adequate nutrients. The birds are also hand-fed (Fig 2.2), but keepers may throw fish into the water for the birds that prefer to eat in the water, such as Timmy since he suffered a spinal injury. During the breeding season, the keepers will put out nesting material consisting of long grass or leaves to allow the birds to build their nests (Fig 2.3).

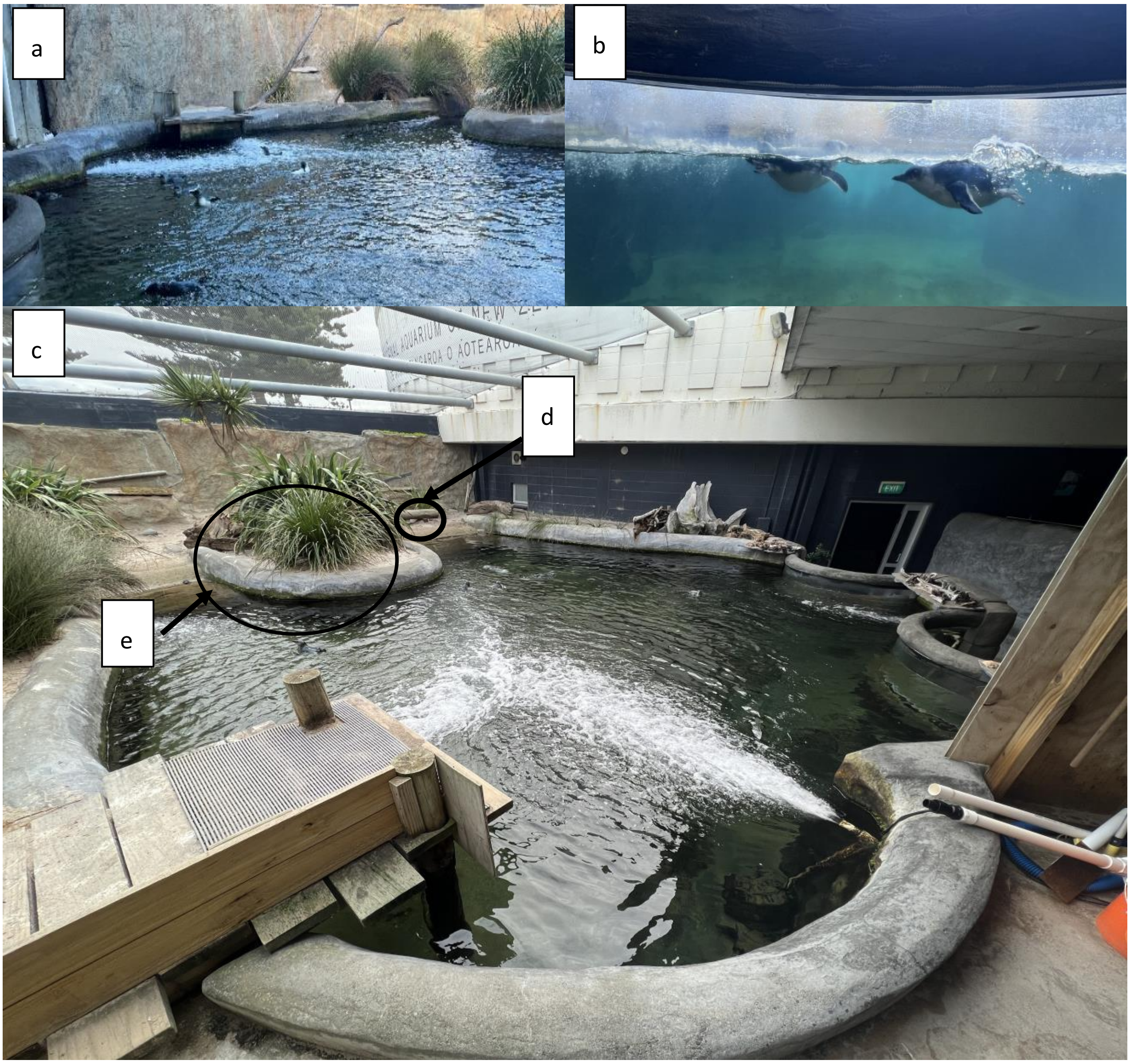

Fig 2.1. Images of the penguin enclosure at the National Aquarium, Napier. a) shows the pool with some of the penguins swimming and the jets. b) shows the underwater viewing window for guests to see the penguins swimming. c) shows the overall enclosure with the open roof cover with mesh and the stone back walls. d) The feeding station. e) The island. Images taken by Ritika Ganesh (2022)

Fig 2.2. Images of the feeding station and the birds being hand-fed by Prof John Cockrem at the National Aquarium of New Zealand. Images taken by Ritika Ganesh (2022).

Fig 2.3. Dave and Kaewa sitting on the nest they made with the nesting material provided. Image taken by Ritika Ganesh (2022).

2.2.2 Nestboxes

At the aquarium, you can find two kinds of nest boxes. One is the permanent box (Fig 2.4 a) that is fixed to the aquarium wall, and the other is the "holiday homes" - portable boxes (Fig 2.4 c) for birds that haven't settled in the permanent burrows yet. These boxes are particularly useful for less dominant birds as they can be moved to a safe place where they will be more comfortable. There are eight boxes in the burrow, known as the "permanent boxes", each with a top lid that can be opened. However, the eighth box is currently closed due to water leakage causing dampness. Martin and Burny have made their home in the burrow located under the flax bush (Fig 2.4 d) on the Island (Fig 2.1.e). Since this spot is frequented by other birds passing by to get to the feeding station, a log has been placed in front of the burrow to prevent Martin from chasing after them.

Fig 2.4. The permanent boxes. a) shows the permanent nest box entrance and the lid closed. b) shows the permanent nest box with the lid open and a little penguin inside. c) shows a movable burrow. d) Burrow on the island under a flax bush, which is occupied by Martin and Burny.

2.3 The penguins in the study

2.3.1 Females

Table 2.1. Female kororā in this study. Reasons for being at the aquarium and their breeding history from 2013 to 2021.

Name	Time at Aquarium	Reason for coming to the aquarium	Mates	Number of clutches	Eggs laid	Eggs hatched
Betty	2008 – current	Malnourished chick	Mo	7	14	5
Burny	2018 – current	Burns on the sole of her feet	Martin	3	8	0
Dora	2014 – 2023	Abandoned chick	Dave (2021)	1	2	0
Draco	2007 – current	Head injury from being hit by a car when crossing the road	Gordon (2013), Pocket (2013), Pacino (2015-2017), Mr.Mac unknown - current	7	12	0
Elmo	2003 – 2020	Amputated flipper from a bad injury	-	5	10	0
Flip	2014 – current	Amputated left flipper due to entanglement	Captain	8	16	7
Gonzo	2004 – 2018	Entanglement with a fishing line; flipper amputated	Jack	6*	11	0
Jack	1999 – 2018	Nerve damage down the right side and partially blind	Gonzo	7*	12	0
Lulu	2012 - 2020	Thin and weak chick on a beach in Thames	Mr.Mac	14	28	9

Name	Time at Aquarium	Reason for coming to the aquarium	Mates	Number of clutches	Eggs laid	Eggs hatched
Marina	2021-current	Found on Marine Parade	-	0	0	0
Miley	2014 – 2017	Emaciated new-fledged chick. Moved to the Antarctic Centre, Christchurch, in 2017.	Flip (2015)	0	0	0
Pepper	2015 – current	Captive born chick of Lulu and Mr.Mac	Tux	8	16	2
Pippa	2015 - 2017	Captive born chick of Lulu and Mr Mac. Moved to the Antarctic Centre in 2017.	-	0	0	0

*Gonzo and Jack were both females and a couple. They laid infertile eggs in their burrow, and the clutches noted here are the ones that keepers have written in the diary as belonging to either bird.

Fig 2.3. The female penguins in this study, from left to right, are 1-4, Betty, Burny, Dora, Draco, 5-8, Elmo, Flip, Gonzo, Lulu, 9, and Pepper. (Jack, Miley and Pippa don’t have any photographs of them). Images retrieved from The National Aquarium, Napier.

2.3.2 Males

Table 2.2. Male kororā in this study and reasons for being at the aquarium from 2013-2021.

Name	Time at Aquarium	Reason for coming to the aquarium	Mates
Captain	2015 - current	Loss of his right eye from an altercation with another seabird	Flip
Dave	2018 – current	Captive was born to parents Flip and Captain	Kaewa
Gordon	Unknown - 2015	Unknown	Draco
Mo	2012 – current	Abandoned chick	Betty
Mr.Mac	2009 – current	Abandoned chick	Lulu (till 2018), currently Draco
Pacino	2014 – 2017	Victim of a predator attack – permanent injury to one eye and flipper	Draco
Pocket	Unknown - 2014	Unknown	Draco
Timmy	2016 – current	Spinal injury due to boat strike	
Tux	2016 – 2021	Abandoned chick	Pepper

Fig 2.4. The male penguins in this study, from left to right, are 1-3 Tux, Captain, Timmy, 4-6, Dave, Mr.Mac, and Mo (Gordon and Pacino don't have any photographs of them). Images retrieved from The National Aquarium, Napier.

3. Body weight and food intake

3.1 Introduction

Body weight in little penguins' changes depending on a variety of factors such as seasonal changes, food availability, individual birds' health, breeding cycles etc., In the wild, little penguins undergo an annual cycle that causes their body weight to fluctuate throughout the year. Studies around little penguins' food intake is important when trying to understand their ecology. Little penguins are sensitive to changes in the availability of prey and are important species in understanding the surrounding ecosystem as they are consumers of small pelagic fish and understanding their diets proves useful when understanding their role within the marine ecosystem.

Condition index is used as a measure and is the ration of body mass by flipper length. This morphometric estimate of body condition is widely used to determine fat mass of the bird (Labocha and Hayes, 2011). Body condition scores are also used by captive management as a way to monitor the overall health and well-being of their birds. Condition of a birds changes as it goes through its annual cycle and can also be an indicator for the timing of the breeding season (Robinson *et al.*, 2005). Robinson *et al.* (2005), found that body condition increased prior to laying eggs both in 2000 and 2001 and continued to increase till incubating only in 2000. It was suggested that the reason the same increase wasn't seen in 2001 was due to factors such as lower food supply. When the bird is able to build up enough reserves or have a higher body condition it benefits them during breeding season from egg laying to chick rearing. Once chick rearing begins there is still a chance to fail even if the parent has a high body condition due to the energy demands required to feed themselves and their chicks (Chiaradia, 1999).

Seabirds, such as little penguins, depend on the sea for their food to help them with their annual cycle. Changes resulting from anthropomorphic factors, such as overfishing, can result in a decline in bird species, especially those that depend on a particular species of prey. However, little penguins have developed to be generalist-feeders that have a more diverse diet (Cavallo *et al.*, 2020) as they live in more coastal areas (Gales and Pemberton, 1990). Little penguins foraging range varies but are normally seen foraging within 20 km of their colony and they feed on small pelagic fish and various cephalopod species (Cullen *et al.*, 1992; Collins *et al.*, 1999; Chiaradia *et al.*, 2003; Chiaradia *et al.*, 2010).

Before the breeding season, the birds are seen foraging to prepare from April to May (Berlincourt and Arnould, 2015). After laying their eggs, both male and female birds take turns incubating and foraging at sea (Agnew *et al.*, 2015; Berlincourt and Arnould, 2015). This leads to them experiencing a short-term fast and forage cycle. This results in fluctuations in their body weight (Croxall, 1982). After the breeding season, the birds begin to prepare for their moult. In the wild, these birds spend 60% of their time at sea (Bethge *et al.*, 1997; Berlincourt and Arnould, 2014) and can travel over 70 km to forage (Carroll *et al.*, 2016a; Elsom, 2022). Little penguins are visual hunters and spend most of their daylight foraging.

There are studies that look at the feeding behaviour of little penguins (Schulz, 1987; Carroll et al., 2014), but it is difficult to know how much the bird has eaten without capturing it and then either studying its faeces or stomach flushing (Cullen *et al.*, 1992). However, working with data from a captivity setting, we not only have individual feed intake data, which will help to see how they increase or keep their feed intake depending on the stage of their annual cycle. We know exactly what fish species they eat, how much and when, and their monthly body weight. This will give us a starting point for understanding how the little penguins use their nutrient stores to enable them to progress through their annual cycle.

There are no current studies that look at captive kororā changes in body weight, condition indices and food intake in New Zealand. This is an important part of researching this species as captivity provides us with a novel environment to study these birds. Due to the various factors that play into the outcomes of the birds' annual changes in body weight in the wild. This study looks to understand whether captive birds experience a similar annual variation. The captive penguins in this study do not have to swim long distances for food; they also have a maximum number of fish allowed to be eaten per day. However, there are some exceptions; for example, during the breeding season, female penguins are allowed to eat as much as they want to cover the energetic costs of breeding. Both male and female birds have increased their maximum number of fish during moult. We can see that these captive birds are monitored, and the keepers use this information to support their birds. Since we have monthly body weight data and daily individual food intake data this study looks to see how they increase or decrease their body weight and their food intake depending on the stage of their annual cycle. Using the formula to calculate condition indices for the birds whose flipper measurements are available we can also see how body condition was affected from 2013 to 2021.

3.2 Methods

3.2.1 Body weight

The Aquarium provided us with an Excel sheet that had all the bird's monthly body weights from the years 2013-2021. Regular monthly health checks are generally done inside the enclosure and the portable scale they use the measure them is a Bilancia Minneapolis. In cases where the bird is required to be taken out of the enclosure into a holding room for administration of medicine, they are weighed using GSC scales no:60209. The diaries provided also had the exact dates for when body weight was taken and noted if any bird was missed and why. All the body weight measurements were given to us in kg and were converted to g.

3.2.2 Condition index

Condition index was also calculated for the birds that were present in 2023 as their flipper length, witdth and height measurements were taken and CI was calculated using the formula; condition index = body mass/flipper length^3 (Cockrem *et al.*, 2017).

3.2.3 Food intake

The birds are fed three times a day. They are fed sprats (*Sprattus sprattus)* which are caught in the North Sea and weigh between 7-10g. The first fish they eat always includes a multivitamin tablet. The aquarium provided us with each bird's daily feed intake, including the number of fish eaten at each feed, as well as the minimum number of fish the bird needs to eat in a day to maintain itself. The birds have a maximum number of fish they are allowed to eat each day, which changes from week to week. This number was not recorded in the diaries or an Excel spreadsheet but written on a whiteboard where the penguin keepers could keep track of and change it when necessary. During the breeding season, the female birds are allowed to eat as much fish as they want to help with the energy requirement associated with growing their sexual organs as well as the formation and laying of the eggs. The male birds still have a maximum restriction during this time. As moult approaches, the bird's maximum number of fish increases per day, but sometimes, if the bird seems "very hungry" or is still at the feeding station seeking out food, the keepers will make an informed decision to provide the bird with more fish to help them reach the weight that is established by the aquarium before they start their moult. Once they start their moult, the birds are not fed and are allowed to experience the same state of starvation as their wild counterparts.

3.2.3 Data analysis

Data were analysed using Prism (GraphPad Software, LA Jolla, CA). Mixed effects analysis were used to anayse body weight, Condition indices and food intake between birds and months and also to see if there was a relationship between bird and month for both males and females. To analyze food intake we converted the daily data into monthly data for birds to correspond with the monthly data for body weight and CI. Welch's t-test were used to compare sex differences for mean body weight. An unpaired t-test was used to compare sex differences for mean condition indices and food intake. This study used Sidak's multiple comparison when comparing differences between the months for body weight, condition indices and food intake. Data are presented as individual values or as mean $\pm$ S.E.

3.3 Results

3.3.1 Body weight

3.3.1.1 Female body weight

Fig. 3.1 shows monthly body weights of female penguins from 2013 to 2021. Body weights varied from 593 to 1516 g, with the great majority of body weights between 700 and 1100 g. The highest body weights of individual birds almost always occurred in December or January. An exception was late 2020 and early 2021 where one bird had a high body weight in December and one in February, four other birds did not have high body weight measurements around the time of moult, and mean body weights were low in January. Body weights were measured monthly, so there could have been increases then decreases in body weight, associated with moult, in between the body weight measurement dates.

Mean body weights were usually highest in December or January (Fig. 3.2). From 2016 onwards, the pattern of mean body weights during the year was a decrease from a peak in January to a minimum in March, then a gradual increase through the year (Fig. 3.2). Differences between years may have arisen due to the limitations of the monthly body weight measurements.

There were differences between birds in the occurrences of peaks in body weight (see Figs. 3.3 to 3.6). The monthly measurements mean that observed high body weights did not necessarily indicate high body weights associated with moult. Betty had high body weights in January. Burny had high weights in February and December 2019 and in August and December 2020. Dora had a high weight in November 2016 and in February from 2017 to 2021. Draco had high weights in January 2014, 2015, and 2020. Elmo had a high weight in January 2015, November 2016 and October 2017. Flip had high weights in January 2015 2017, 2018, and 2019, and also had weight increases in September

2019 and November 2021. Gonzo had high weights in January 2013 and January 2015, and in February 2016, 2017, and 2018. Jack had high weights in February 2014, 2015, and 2016. Lulu had high body weights in January 2013, 2014, 2015, and 2016, and also in December 2016, December 2019, and February 2018. Miley had high weights in October 2014 and October 2015, and in November 2016. Pepper had high weights in January 2017, 2018 and 2019, and September of 2020. Pippa had a high weight increase in February 2017.

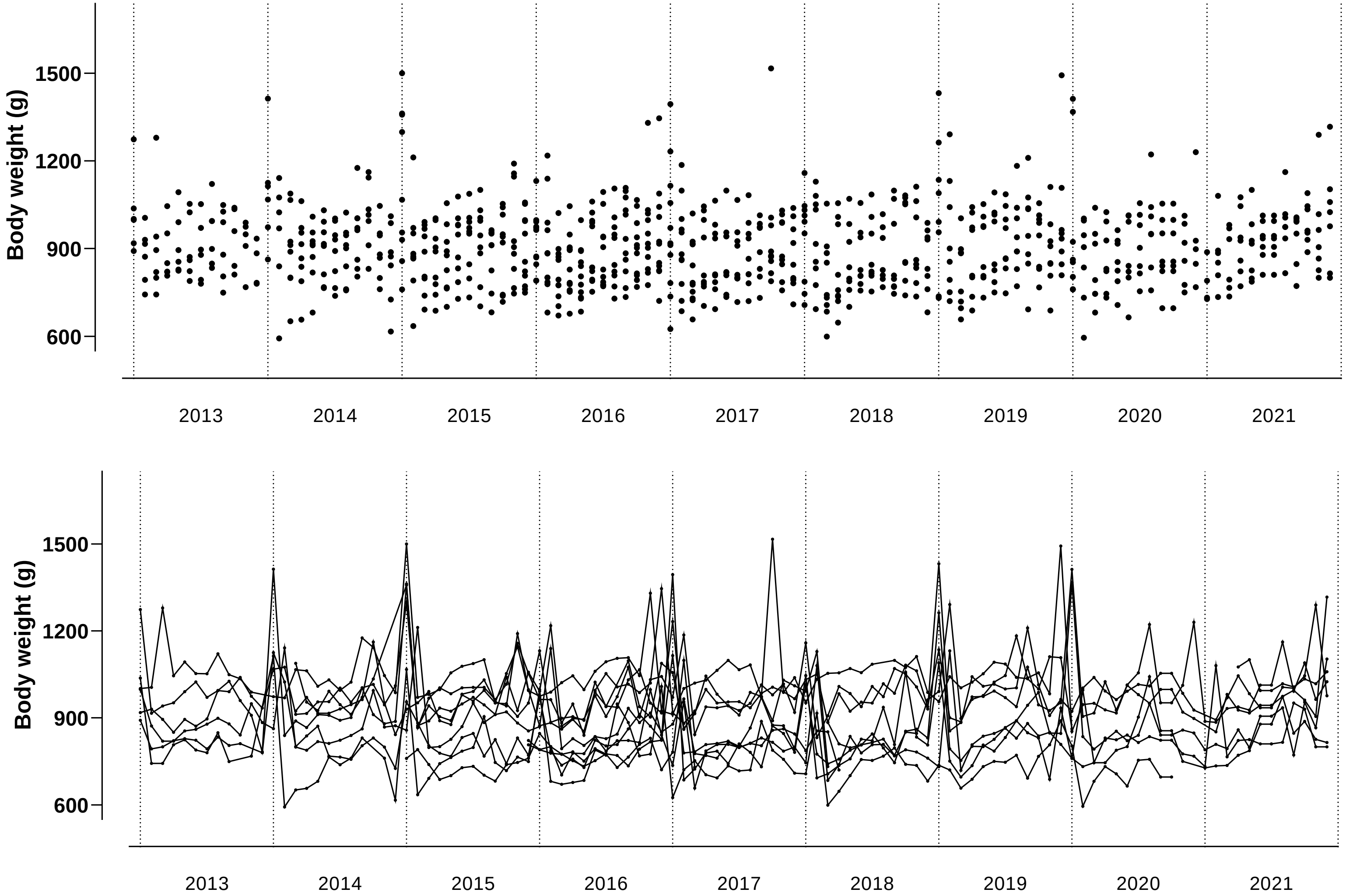

Fig. 3.1. Body weights of female kororā. Data are monthly measurements. The numbers of female kororā each year were n = 6 (2013), n = 7 (2021), n = 8 (2014, 2019 and 2020), n = 10 (2018), and n = 11 (2015, 2016 and 2017).

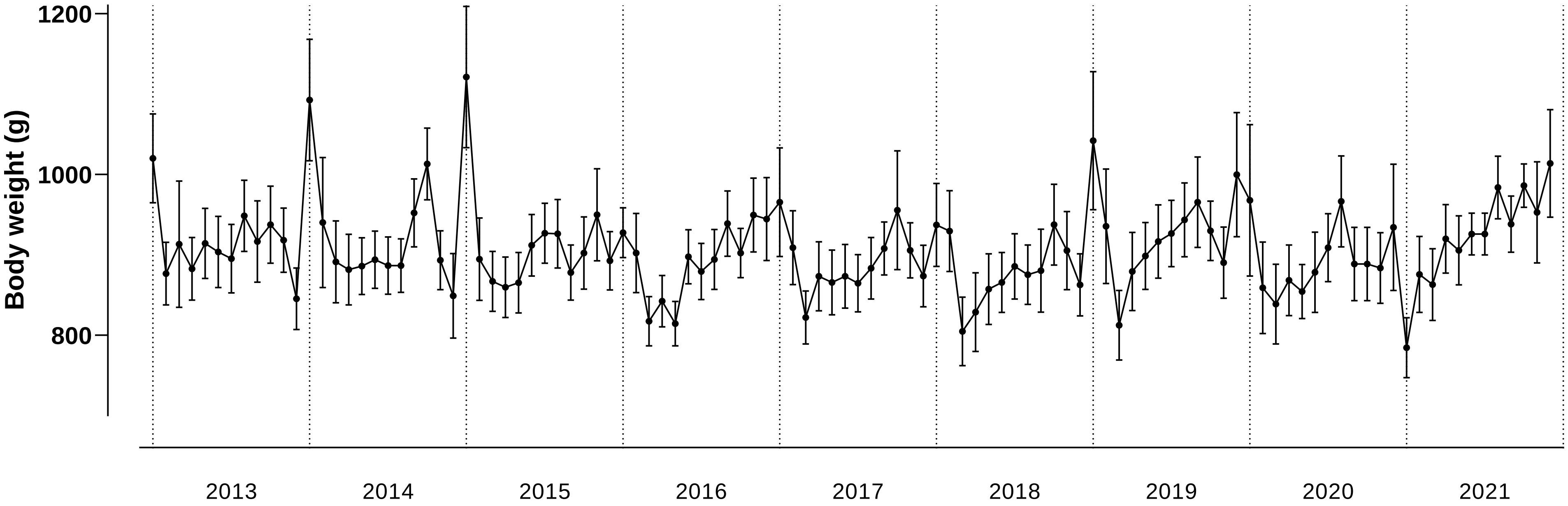

Fig 3.2. Mean + SE body weight of all female kororā. Data are monthly measurements.

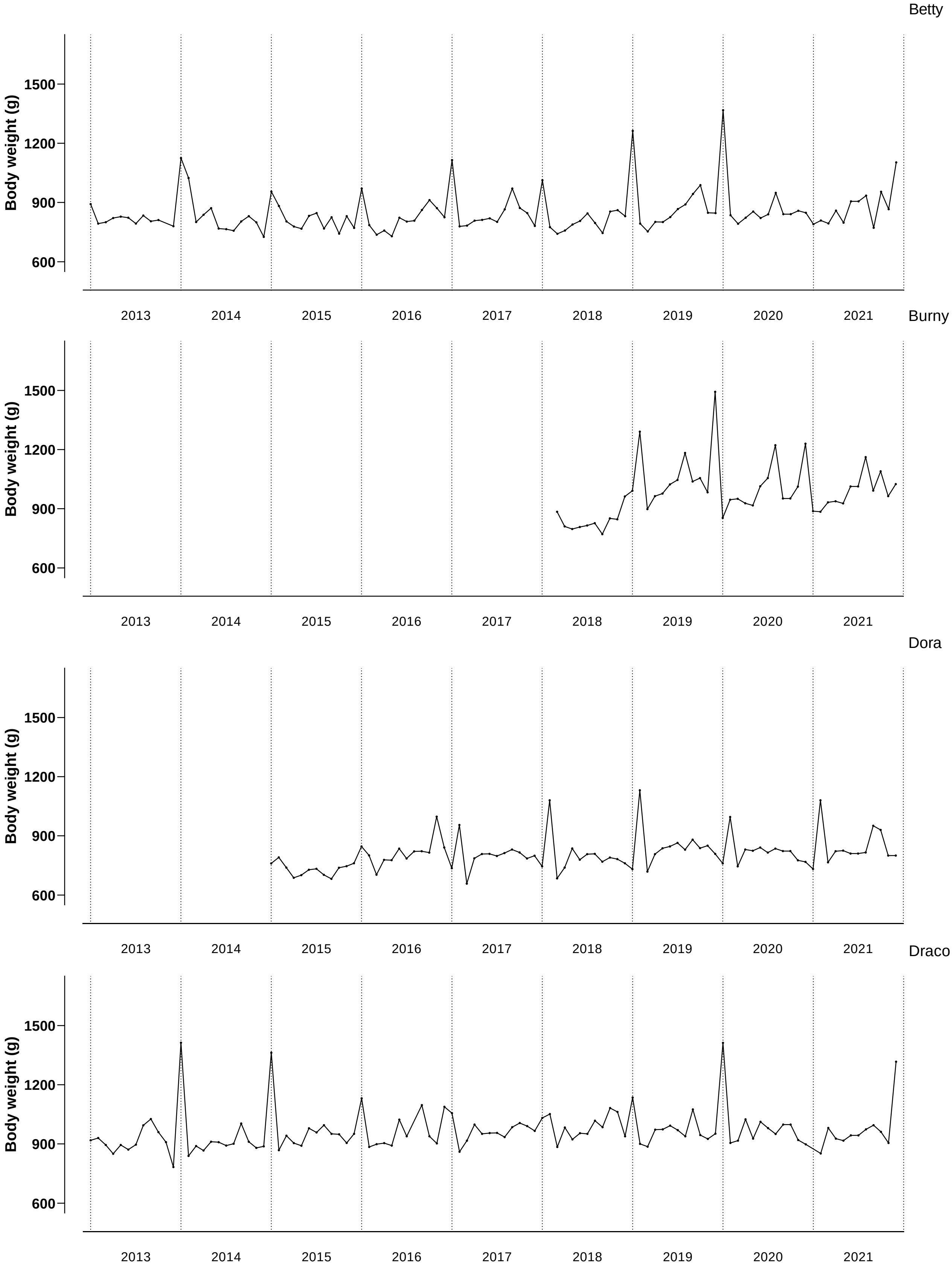

Fig. 3.3. Individual body weights of Betty, Burny, Dora and Draco from 2013 to 2021. Data are monthly measurements.

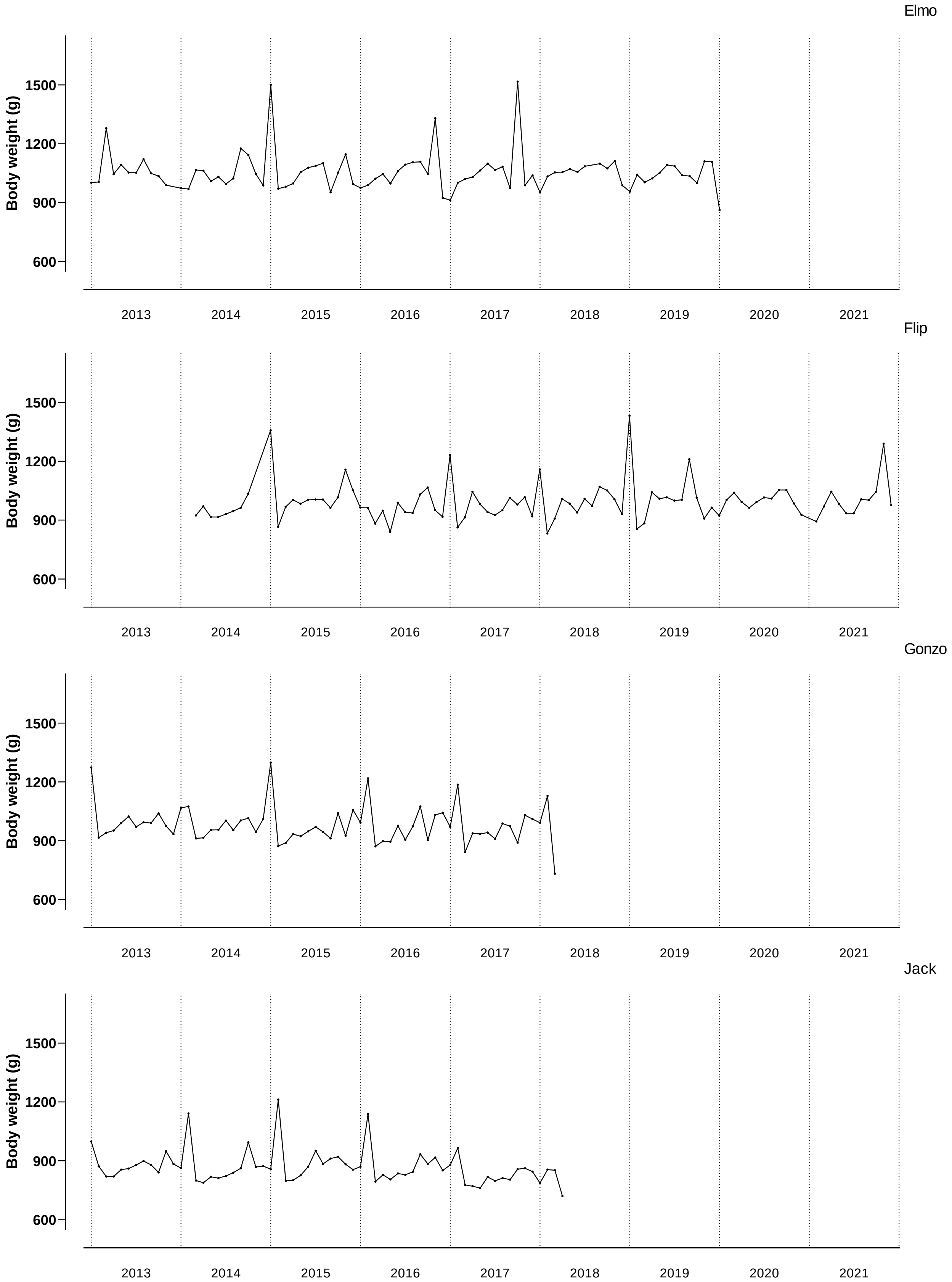

Fig. 3.4. Individual body weights of Elmo, Flip, Gonzo and Jack from 2013 to 2021. Data are monthly measurements.

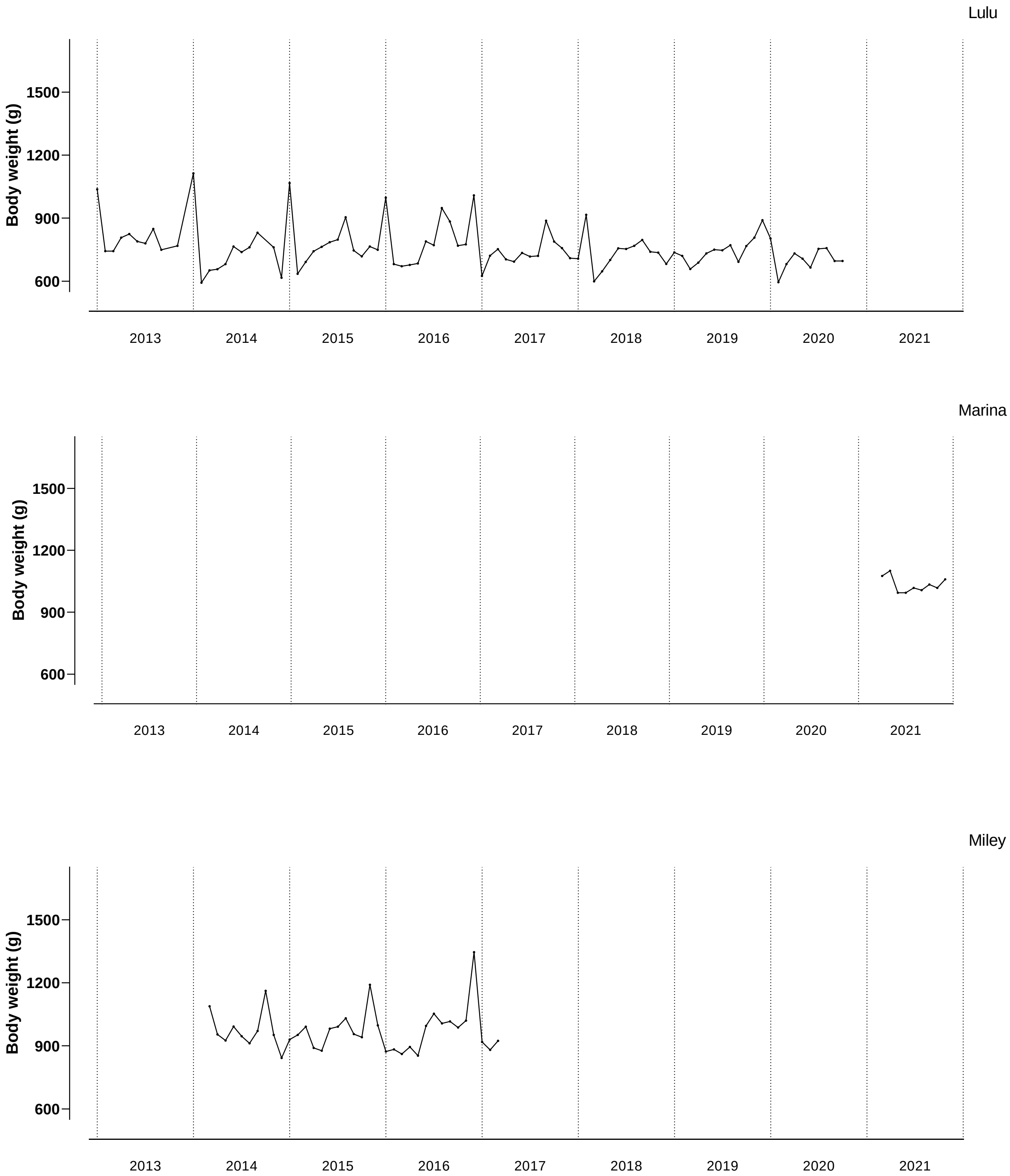

Fig. 3.5. Individual body weights of Lulu, Marina and Miley from 2013 to 2021. Data are monthly measurements.

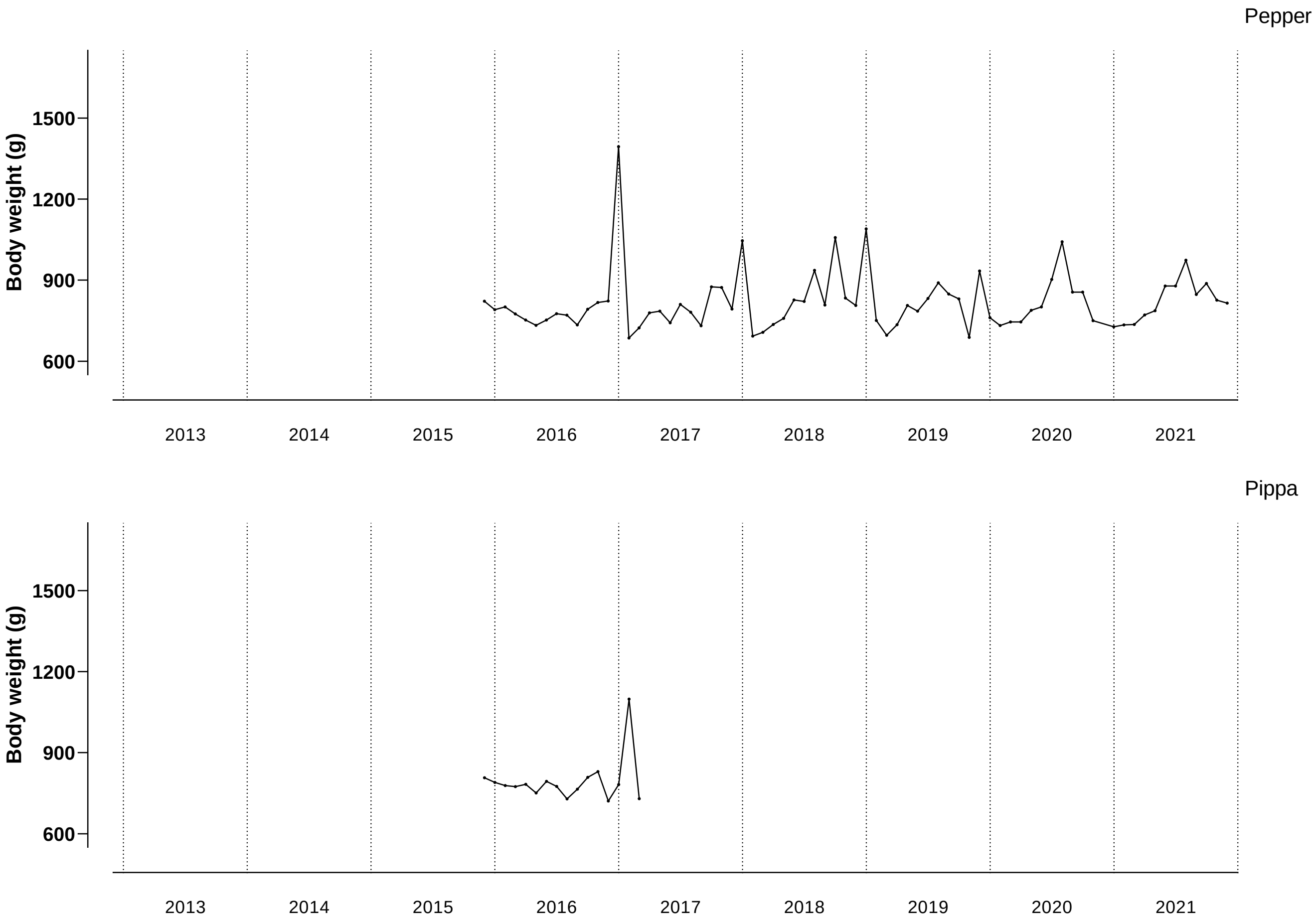

Fig. 3.6. Individual body weights of Pepper and Pippa from 2013 to 2021. Data are monthly measurements.

Mean body weights of individual females ranged from 759.0 ± 10.4 to 1056.3 ± 11.1 g (see Table 3.1). The mean body weight of the lightest bird (Lulu) was 73% of the mean body weight of the heaviest bird (Elmo).

Table 3.1. Body weights (g) of female kororā. Birds were weighed monthly.

Name	Mean	S.E.	*n*	Min.	Max.	Number of years
Lulu	759.0	10.4	89	593	1113	8
Pippa	794.8	21.6	16	721	1098	3
Dora	808.2	9.2	84	658	1131	7
Pepper	817.6	13.0	72	686	1394	7
Betty	848.6	9.8	105	729	1368	9
Jack	866.9	11.0	62	720	1212	6
Draco	967.1	10.1	104	783	1413	9
Miley	976.9	17.0	35	853	1346	4
Gonzo	980.8	12.3	61	732	1299	6
Burny	982.0	20.7	46	771	1493	4
Flip	993.8	10.2	91	832	1432	8
Marina	1033.4	12.5	9	994	1101	1
Elmo	1056.3	11.1	81	862	1516	8

There was an annual cycle of body weight in female penguins (see Fig. 3.7). Mean body weights were lowest in March, increased from April to August, remained relatively constant from August to December, rose to a peak in January, then declined in February and March. Mean, standard error, minimum and maximum body weights each month are shown in Appendix Table 7.1.

Mean body weights of female birds differed between birds (mixed effects model; $F_{11,67} = 47.00$, *p<0.0001*) and between months ($F_{4.421,254.8} = 10.56$, *p<0.0001*), with a significant interaction between bird and month ($F_{121,634} = 2.808$, *p<0.0001*). Sidak's multiple comparison tests showed that mean body weights differed between May and June (*p<0.0001*), July and August (*p=0.0168*) and February and March (*p=0.0152*), and did not differ between other successive pairs of other months.

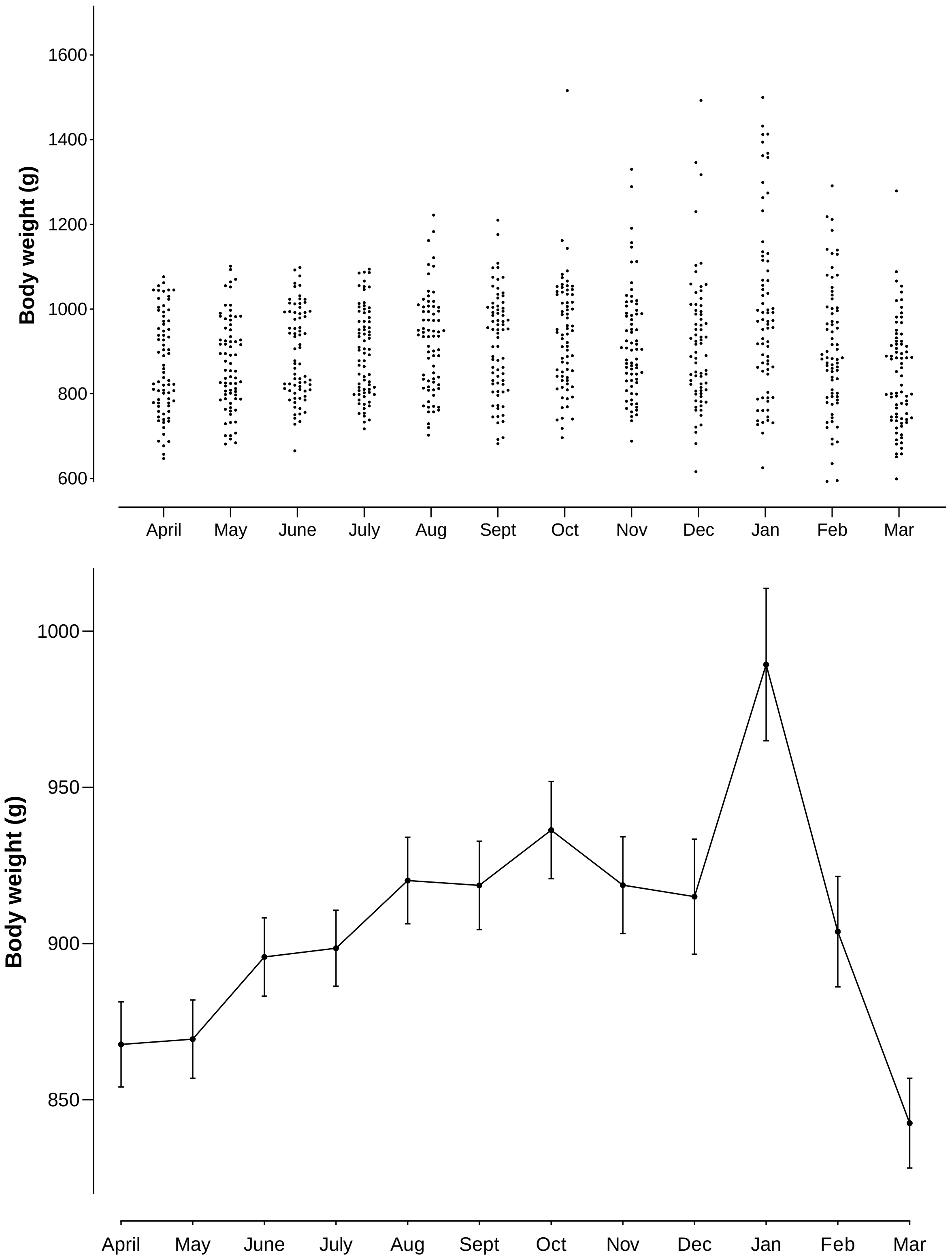

Fig 3.7. Individual and mean (± S.E) monthly body weights of female kororā at the National Aquarium. Data are from 2013 to 2021. There were from 6 to 11 female kororā at the Aquarium each year.

3.3.1.2 Male body weight

Fig. 3.8 shows monthly body weights of male penguins from 2013 to 2021. Body weights varied from 850 to 1700 g, with the great majority of body weights between 900 and 1200 g. The highest body weights of individual birds almost always occurred in December or January. An exception was late 2020 and early 2021 One bird had a high body weight in Novmeber and one in December, three other birds did not have high body weight measurements around the time of moult, and mean body weights were low in January. Similar to the females, body weights were measured monthly, so there could have been increases then decreases in body weight, associated with moult, in between the body weight measurement dates.

Mean body weights were usually highest in December or January (Fig. 3.9). Males showed a pattern of mean body weights peaking in January and then to a minimum in March, then a gradual increase through the year (Fig. 3.9). From 2017 onwards, the pattern of mean body weights during the year was a decrease from a peak in December to a minimum in January or March, then a gradual increase through the year (Fig. 3.9). Differences between years may have arisen due to the limitations of the monthly body weight measurements.

There were differences between birds in the occurrences of peaks in body weight (see Figs. 3.10 to 3.12). The monthly measurements mean that observed high body weights did not necessarily indicate high body weights associated with moult. Captain had high weights during the months of January 2017 and 2018 and also in December of 2018 and 2019. Dave had high weight in January of 2020. Gordon had high weight in November of 2014. Martin had high weights in December 2019 and 2020. Mo had high weight in January of 2015, 2016, and 2018 and then his weight didn't fluctuate as much from 2019 to 2021. Mr Mac had high weight from January 2013 to 2017, and then his weight didn't fluctuate from 2018 to 2021. Pacino doesn't show very significant increases in weight, but there was a high weight in March 2014 and December 2016. Pocket had high weight in January 2013 and 2014. Timmy doesn’t show any big increase or decrease in weight and stays in the range of 950 to 1400g. Tux only shows on high increase in weight during December 2019.

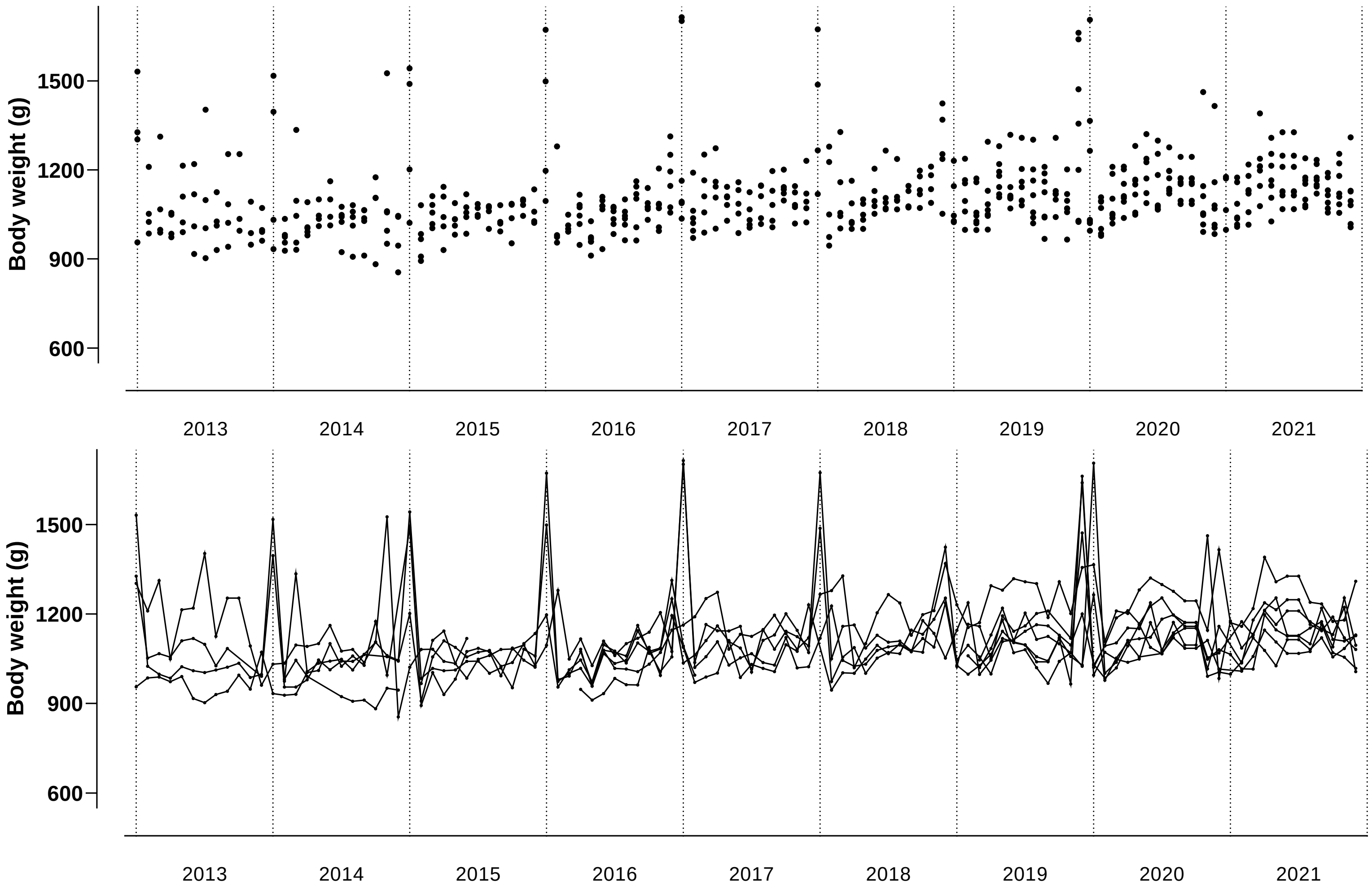

Fig 3.8. Body weights of male kororā. Data are monthly measurements. The number of male kororā each year ranged from n = 4 (2013), n = 5 (2014, 2016, 2018), n = 6 (2016 & 2017), and n = 7 (2019, 2020, & 2021) birds.

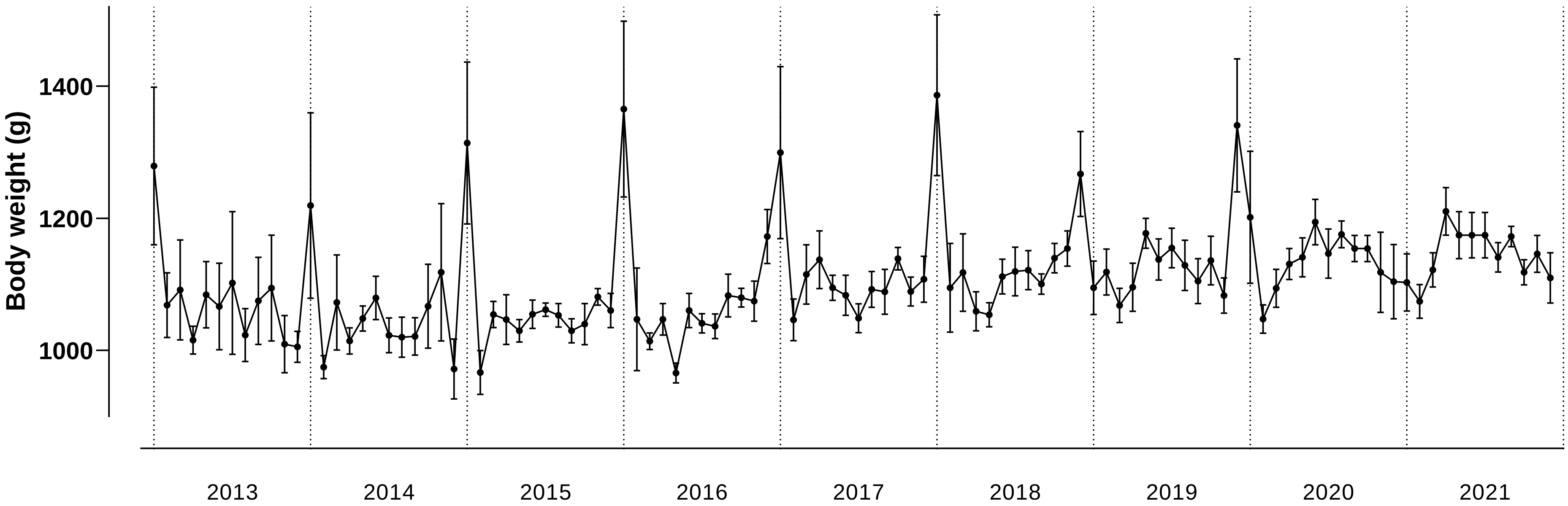

Fig 3.9. Body weight means ± SE of all male kororā. Data are monthly measurements.

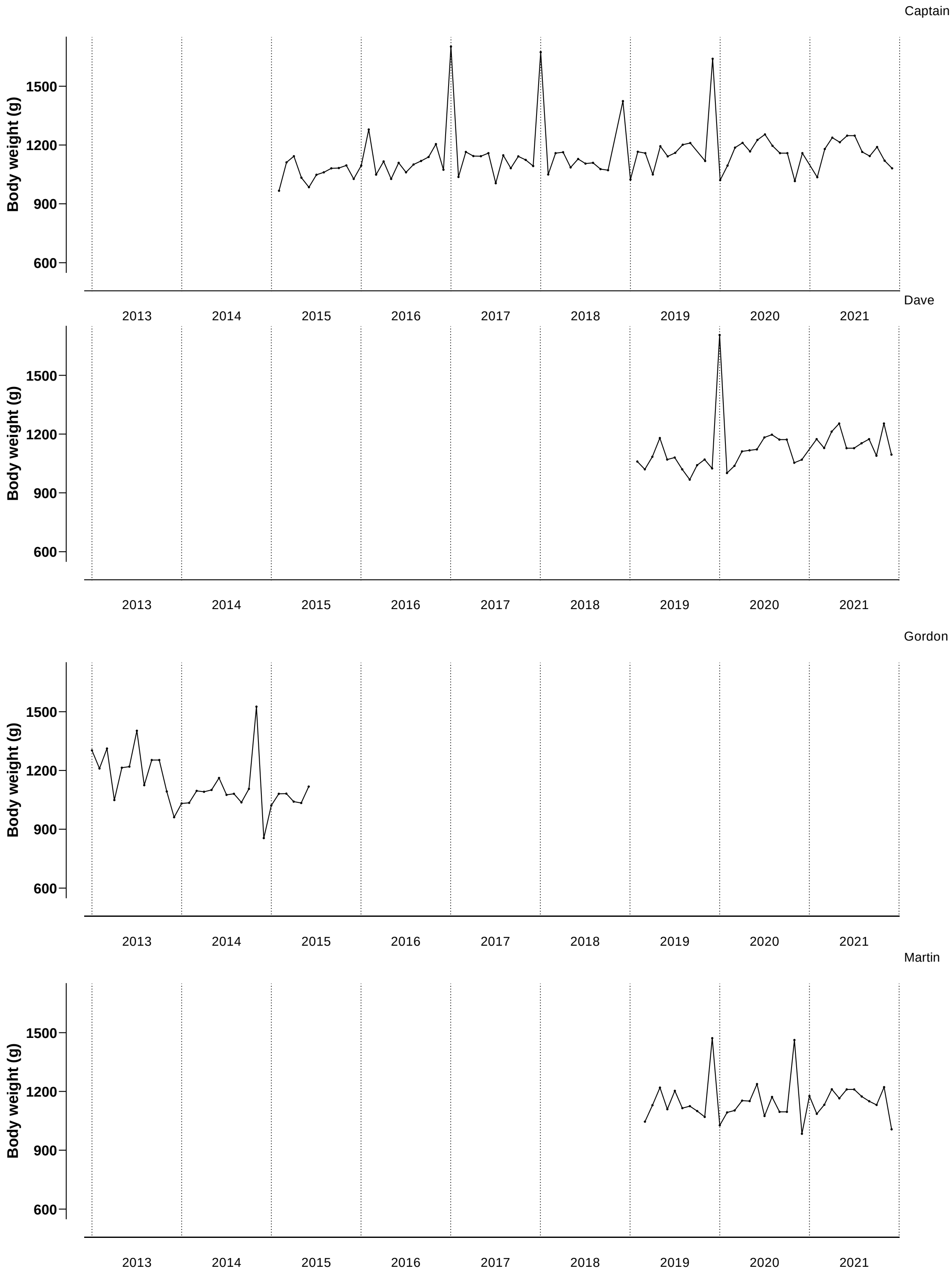

Fig 3.10. Individual body weights of Captain, Dave, Gordon and Martin from 2013 to 2021. Data are monthly measurements.

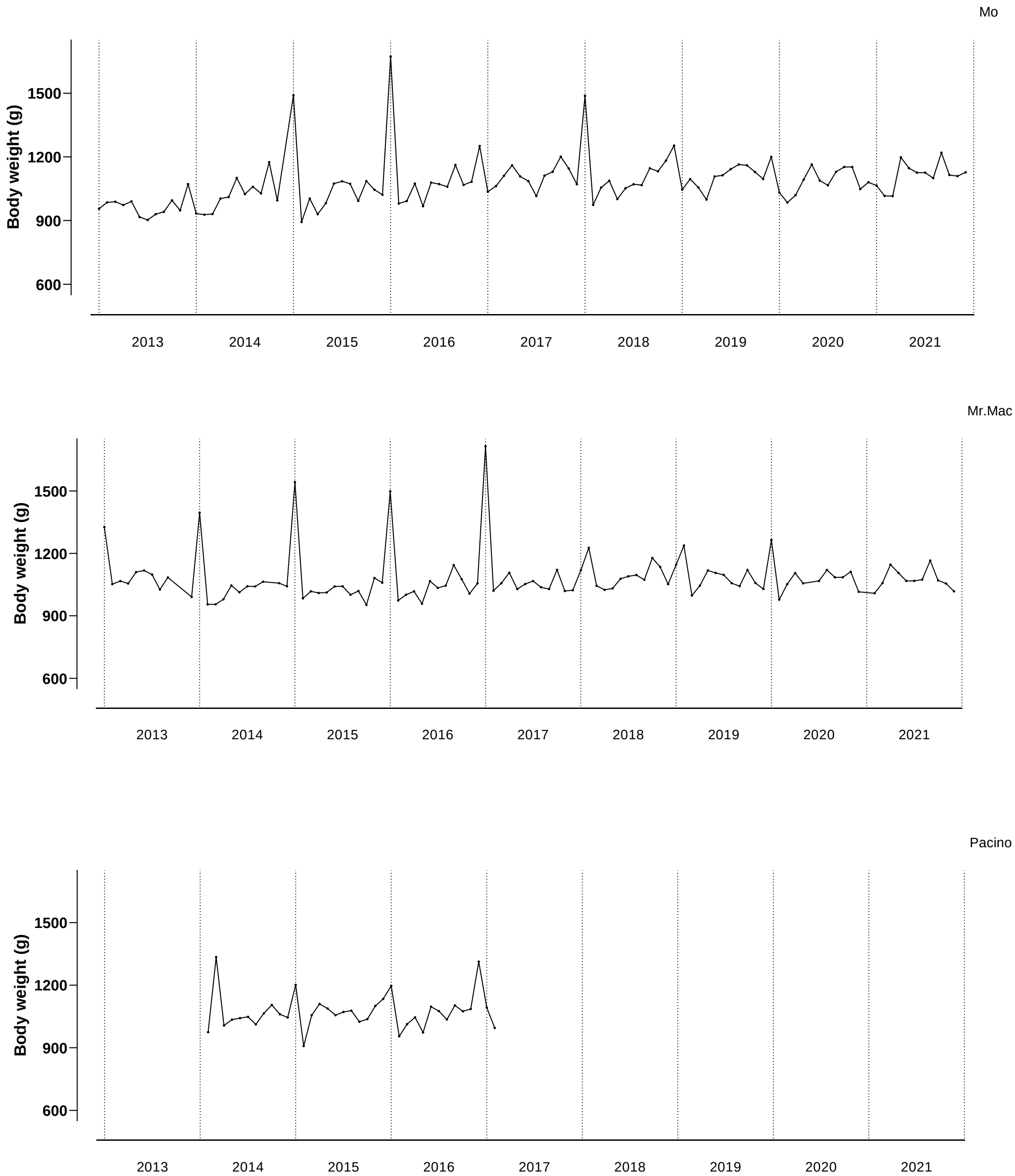

Fig 3.11. Individual body weights of Mo, Mr.Mac and Pacino from 2013 to 2021. Data are monthly measurements.

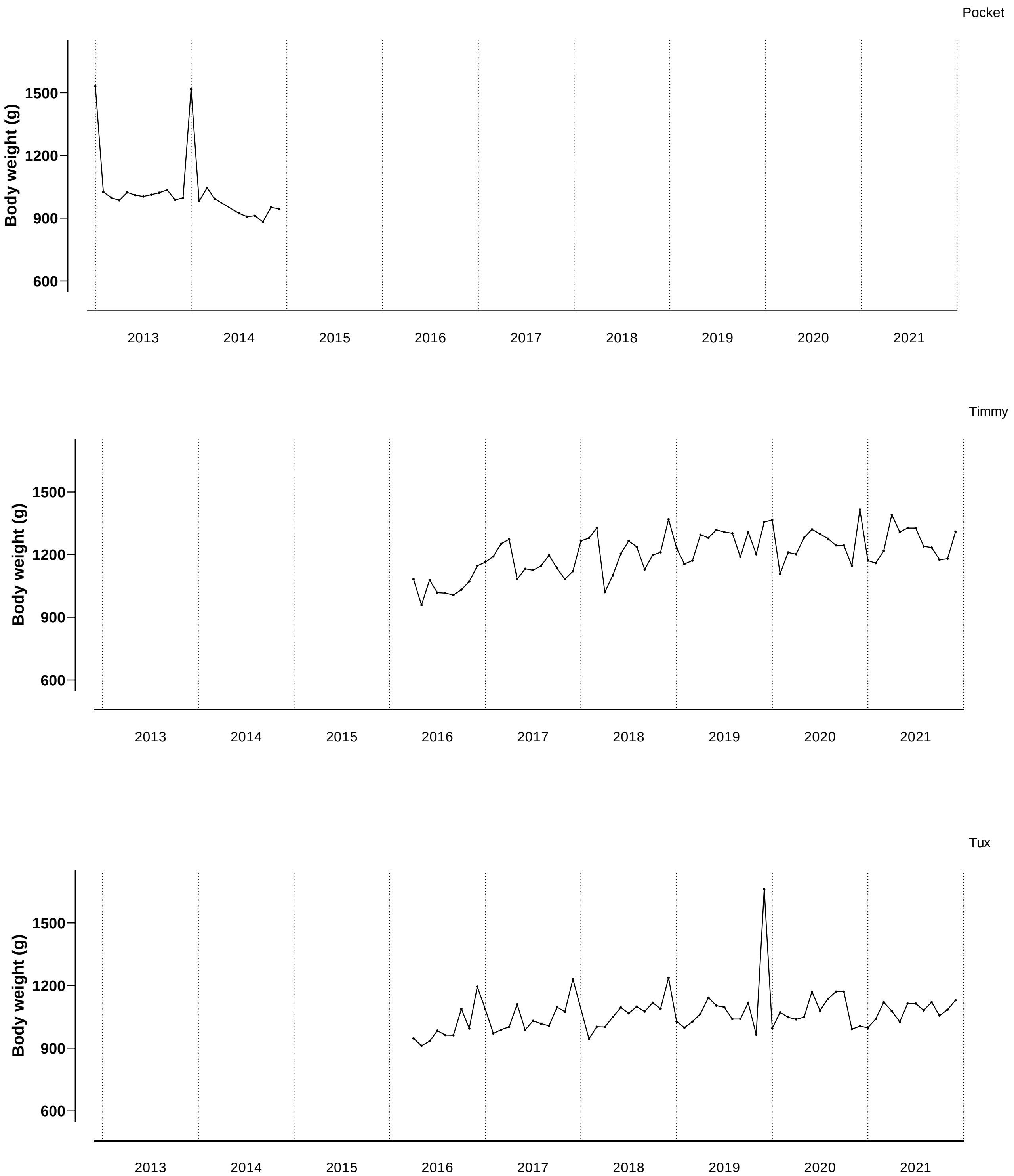

Fig 3.12. Individual body weights of Pocket, Timmy and Tux from 2013 to 2021. Data are monthly measurements.

Mean body weights of individual males ranged from 1039.3 ± 38.4 to 1205.4 ± 12.4 g (see Table 3.2). The mean body weight of the lightest bird (Pocket) was 86% of the mean body weight of the heaviest bird (Timmy).

Table 3.2. Body weights of male kororā. Birds were weighed monthly.

Name	Mean (g)	S.E. (g)	*n*	Min.	Max.	Number of years
Pocket	1039.3	38.4	20	882	1531	2
Tux	1066.7	12.3	68	911	1662	6
Pacino	1072.8	14.6	35	908	1335	4
Mo	1078.8	11.0	106	893	1672	9
Mr Mac	1082.3	11.4	101	953	1714	9
Dave	1128.0	21.3	34	968	1706	3
Gordon	1128.3	19.8	28	961	1403	3
Captain	1147.3	14.4	80	967	1702	7
Martin	1150.4	17.5	34	984	1472	3
Timmy	1205.4	12.4	69	958	1415	6

There was an annual cycle of body weight in male penguins (see Fig. 3.13). Mean body weights were lowest in Febuary, remained relatively constant from April to November, rose to a peak in January, then declined in February and March. Mean, standard error, minimum and maximum body weights each month are shown in Appendix Table 7.2.

Mean body weights of male birds differed between birds (mixed effects model; $F_{9,42}=5.308$, $p<0.0001$) and between months ($F_{3.433,129.5}=8.549$, $p<0.0001$), with a significant interaction between bird and month ($F_{99,415}$=2.229, *p<0.0001*). Sidak's multiple comparison tests showed that mean body weights differed between January to Febuary (*p=0.0006*), and did not differ between other successive pairs of other months.

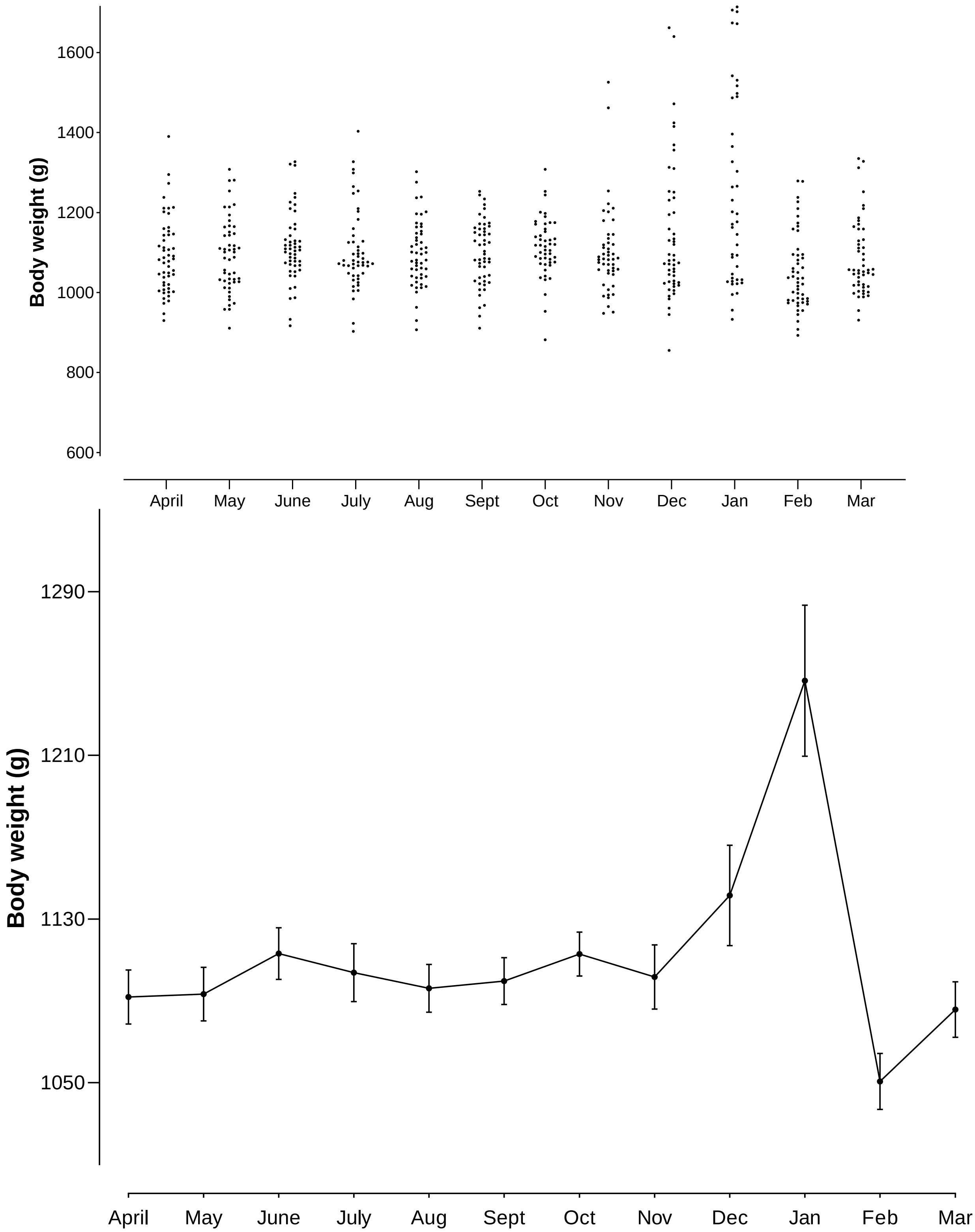

Fig 3.13 Individual and mean (± S.E) monthly body weights of male kororā at the National Aquarium. Data are from 2013 to 2021. There were from 4 to 7 male kororā at the Aquarium each year.

3.3.1.3 Female and male comparison

Male kororā were heavier than female kororā and this is consistent with them being bigger birds than the females. Both sexes show similar patterns of weight increase and decrease (Fig 3.14). The female birds show a lot more changes in body weight from May to October than the males. The males seem to be relatively constant. The mean body weight of males was greater than the mean body weight of females (table 3.3; Welch's corrected t-test t_{1329}=29.36, $p<0.0001$)

Table 3.3 Mean and S.E. of body weight (g) for female and male birds from 2013 to 2021.

	Mean	S.E.	*n*	Minimum	Maximum
Female	906.4	4.7	854	593	1516
Male	1110.0	5.1	577	882	1714

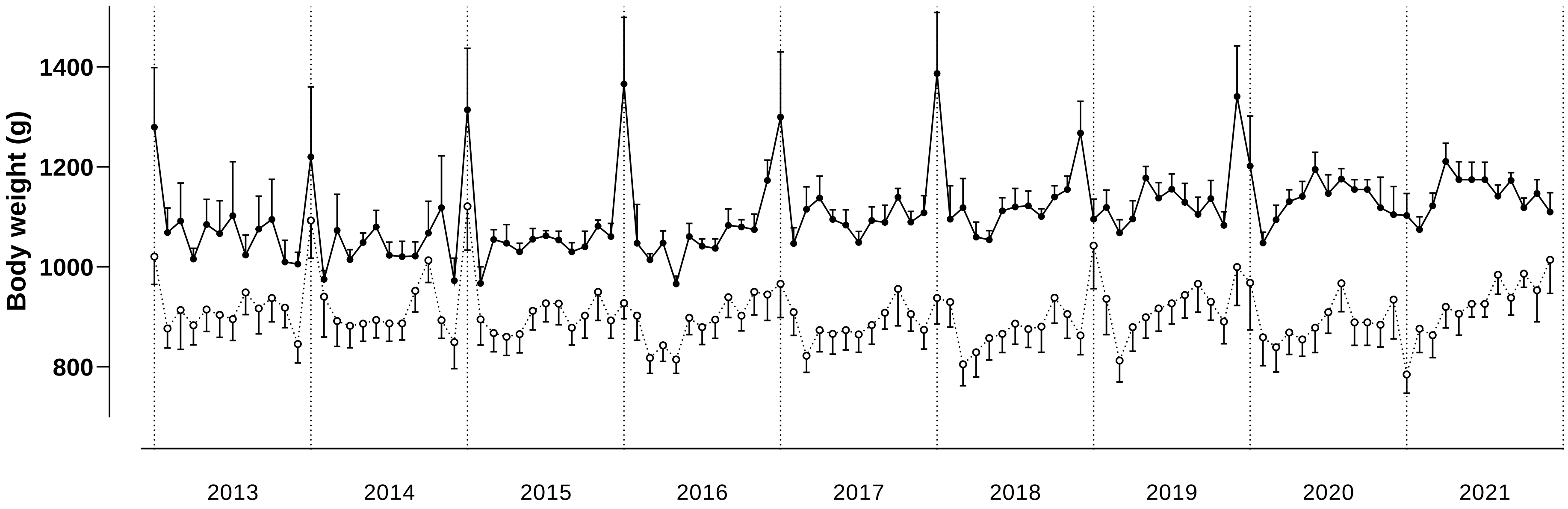

Fig 3.14 Monthly body weights mean (± SE) of all male (— ● —) and female (--- ○---) birds from 2013 to 2021 (National Aquarium, Napier).

3.3.2 Condition index

3.3.2.1 Females

Mean condition index (CI) of female birds ranged from 77.7 + 0.9 to 92.6 $\pm$ 1.9 (see Table 3.4). Mean condition index (CI) of female birds differed between birds (mixed effects model; $F_{5,37} = 17.79$, $p<0.0001$) and between months ($F_{3.497,124.6} = 11.31$, $p<0.0001$), with a significant interaction between bird and month ($F_{55,392} = 3.537$, $p<0.0001$). Sidak's multiple comparison tests showed that mean CI differed between July and August ($p<0.0179$), and did not differ between other successive pairs of other months. This significance can be traced back to Burny being the heaviest compared to the other birds during August of 2019, and 2020. Both those years in August, Burny was laying eggs when her body weight was taken.

Table 3.4. Condition indices mean and SE of females kororā[1].

	Mean	S.E.	*n*	Min.	Max.
Dora	77.7	0.9	84	63.3	108.8
Pepper	78.6	1.2	72	66.0	134.0
Betty	79.3	0.9	105	68.1	127.9
Draco	79.3	0.8	104	64.2	115.8
Flip	86.5	0.9	90	72.3	124.5
Marina	88.3	1.1	9	85.0	94.1
Burny	92.6	1.9	46	72.7	140.8

[1]Not all female birds were alive or present at the aquarium when flipper lengths were measured in 2023.

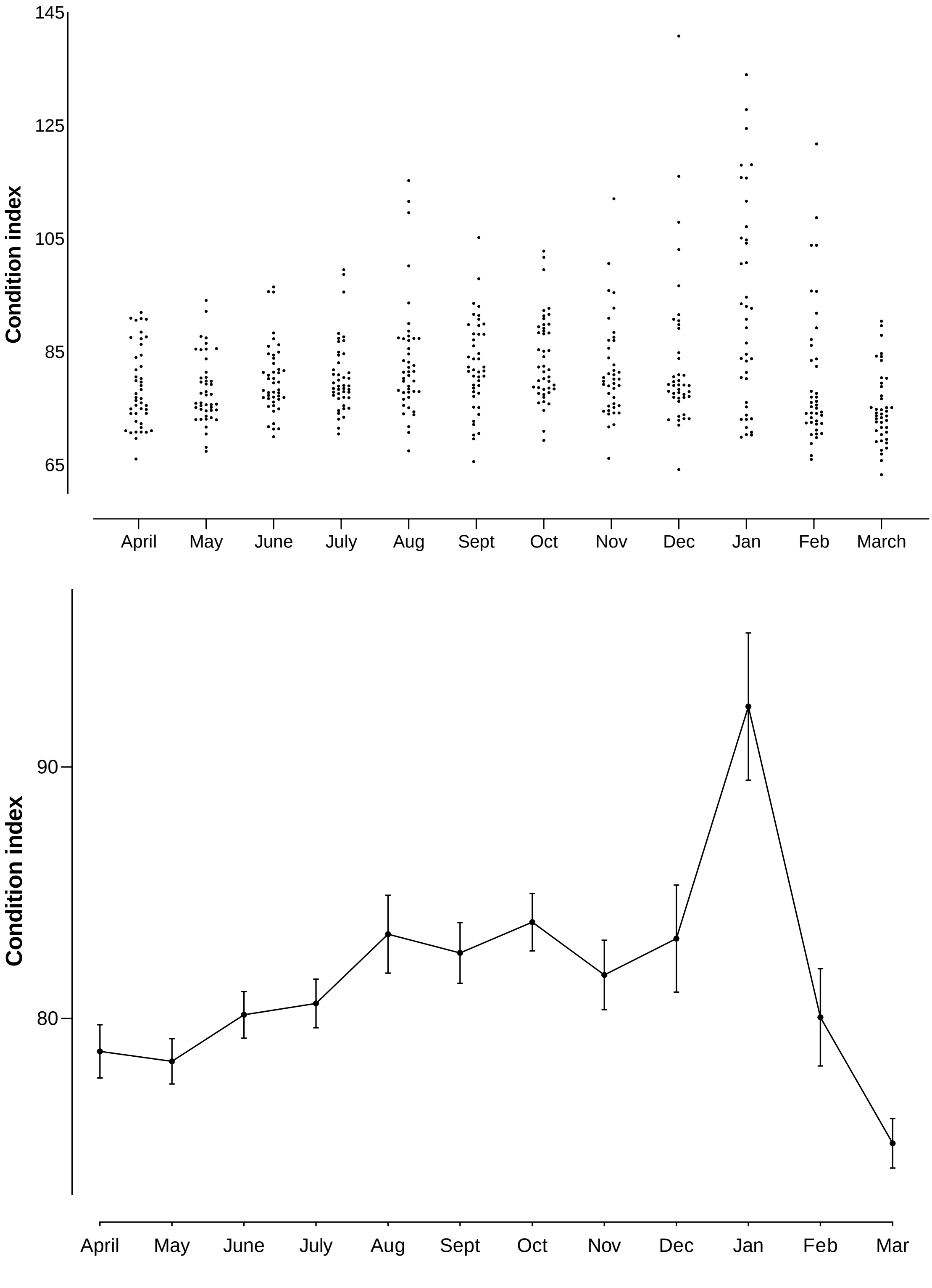

Fig 3.15 Individual and mean (± S.E) condition indices of female kororā at the National Aquarium. Data are from 2013 to 2021 and only 2 to 7 female birds were used as other female birds flipper lengths were not obtained.

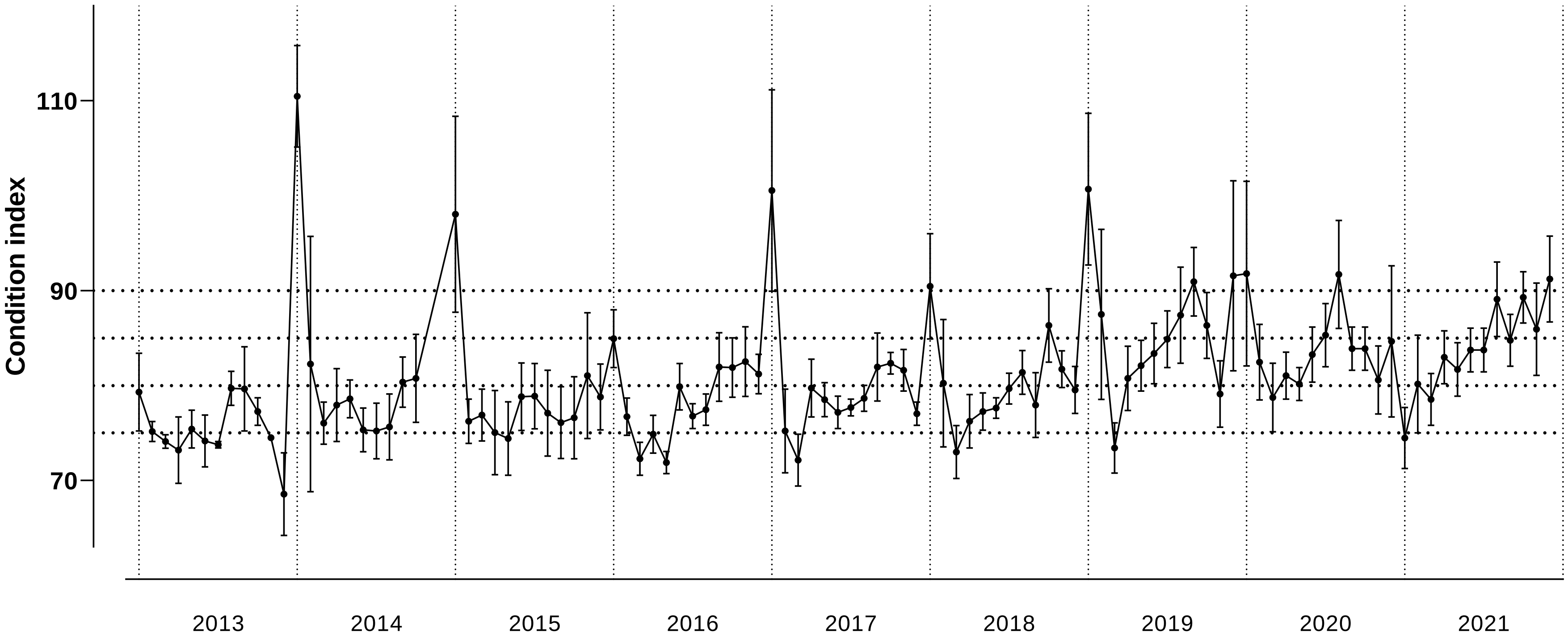

Fig 3.16. Condition indices of all female kororā where the number of birds per year ranged from 2 to 7. Not all female birds were alive or present at the aquarium when the flipper length collection was taken in 2023 at the National Aquarium.

There is an increase in CI from 2016 onwards which may be due to newer birds being added into the colony, since flipper lengths were collected in 2023 and some birds in the study passed away so we do not have a completed representation of condition indices for all female birds. Looking at the birds (Betty, Dora, Draco and Flip) whose flipper lengths we do have an were present at the aquarium from 2015 to 2021 we still see that increase in CI over the years from 2016 (Fig 3.17) similar to the CI of all the female birds (Fig 3.16). Mean condition index (CI) of female birds differed between birds (mixed effects model; $F_{3,44}=5.060$, $p=0.0043$) and between year ($F_{3.902,169.1}=3.992$, p-0.0044), with a significant interaction between bird and years ($F_{18,260}=1.672$, $p=0.0444$). Sidak's multiple comparison tests showed that mean CI differed between 2018 and 2019 ($p=0.0174$), and did not differ between other successive pairs of other years.

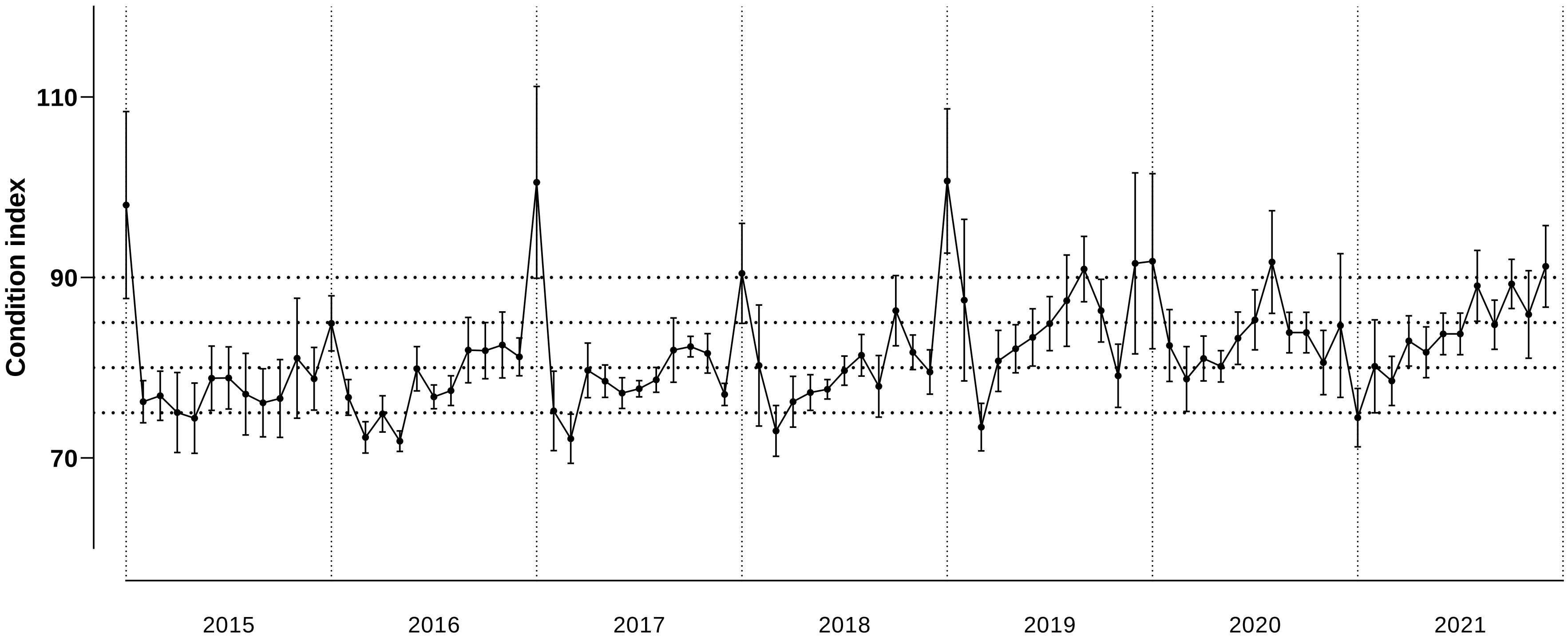

Fig 3.17. Condition indices of the female kororā present from 2015 to 2021 (n = 4). Not all female birds were alive or present at the aquarium when the flipper length collection was taken in 2023 at the National Aquarium.

3.2.2.2. Males

Mean condition index (CI) of male birds ranged from 84.4 ± 1.0 to 98.0 ± 1.0 (see Table 3.5). Mean condition index (CI) of male birds differed between birds (mixed effects model; $F_{6,36}$ =11.62, *p<0.0001*) and between months ($F_{2.980,100.8}$=7.569, *p=0.0001*), with a significant interaction between bird and month ($F_{66,372}$ =1.940, *p<0.0001*). Sidak's multiple comparison tests showed that mean CI differed between January and Febuary (*p=0.0020*), and did not differ between other successive pairs of other months. This significance can be traced back to the birds timing of moult. From the year 2016 to 2021 we see that mean CI for the birds through the years is increasing. This increase may by due to newer birds being introduced into the colony.

Table 3.5. Condition indices mean and SE of males kororā[1].

	Mean	S.E.	*n*	Min.	Max.
Tux	84.0	1.0	68	71.7	130.9
Mo	88.4	0.9	106	73.2	137
Mr Mac	89.5	0.9	101	78.8	141.7
Dave	94.0	1.8	34	80.7	142.2
Captain	95.6	1.2	80	80.6	141.8
Martin	96.7	1.5	34	82.7	123.7
Timmy	98.0	1.0	69	77.9	115

[1]Not all male birds were alive or present at the aquarium when the flipper length collection was taken in 2023.

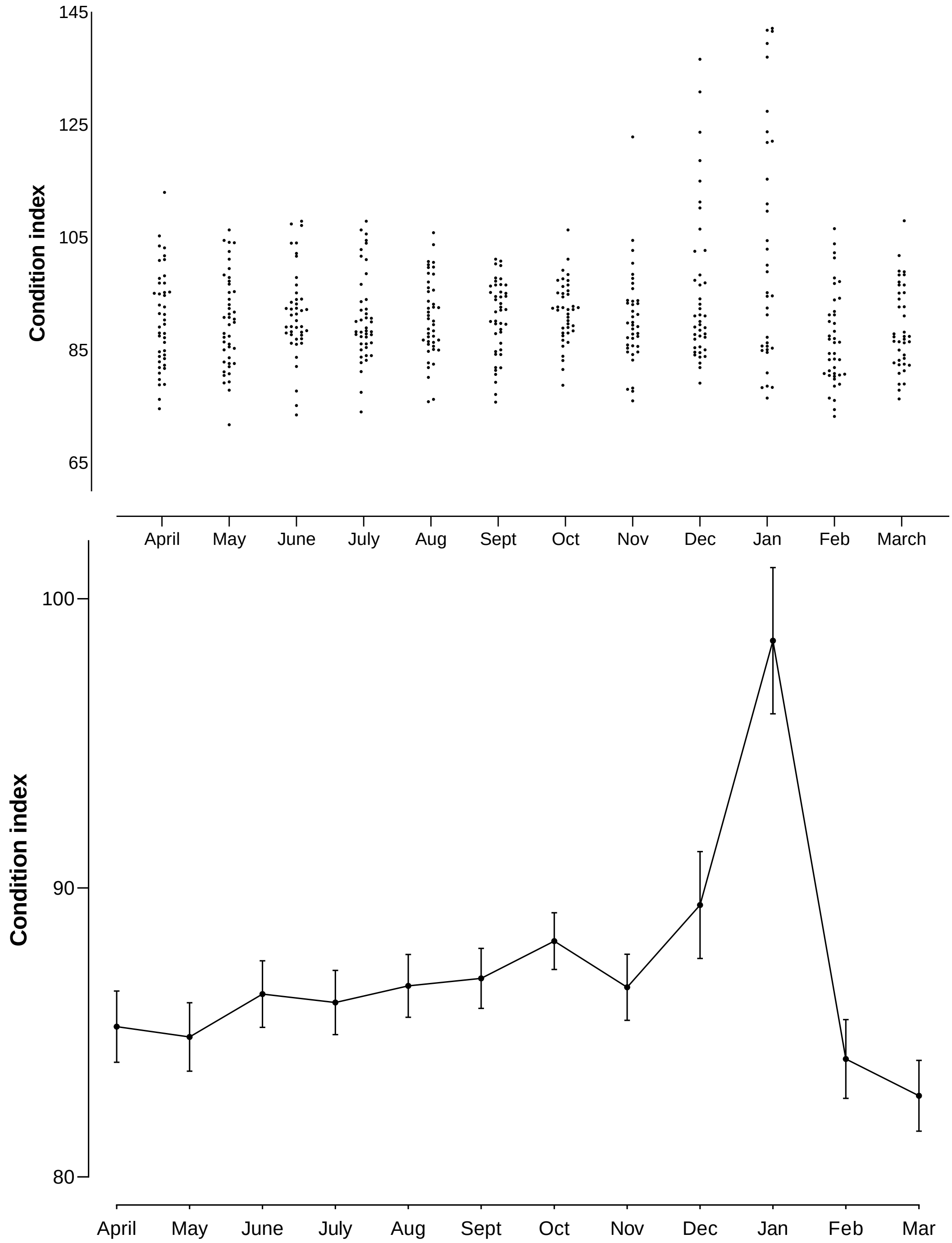

Fig 3.18 Individual and mean (+ S.E) condition indices of male kororā at the National Aquarium. Data are from 2013 to 2021 and only 2 to 7 male bird's data were used as other male birdFs flipper lengths were not obtained

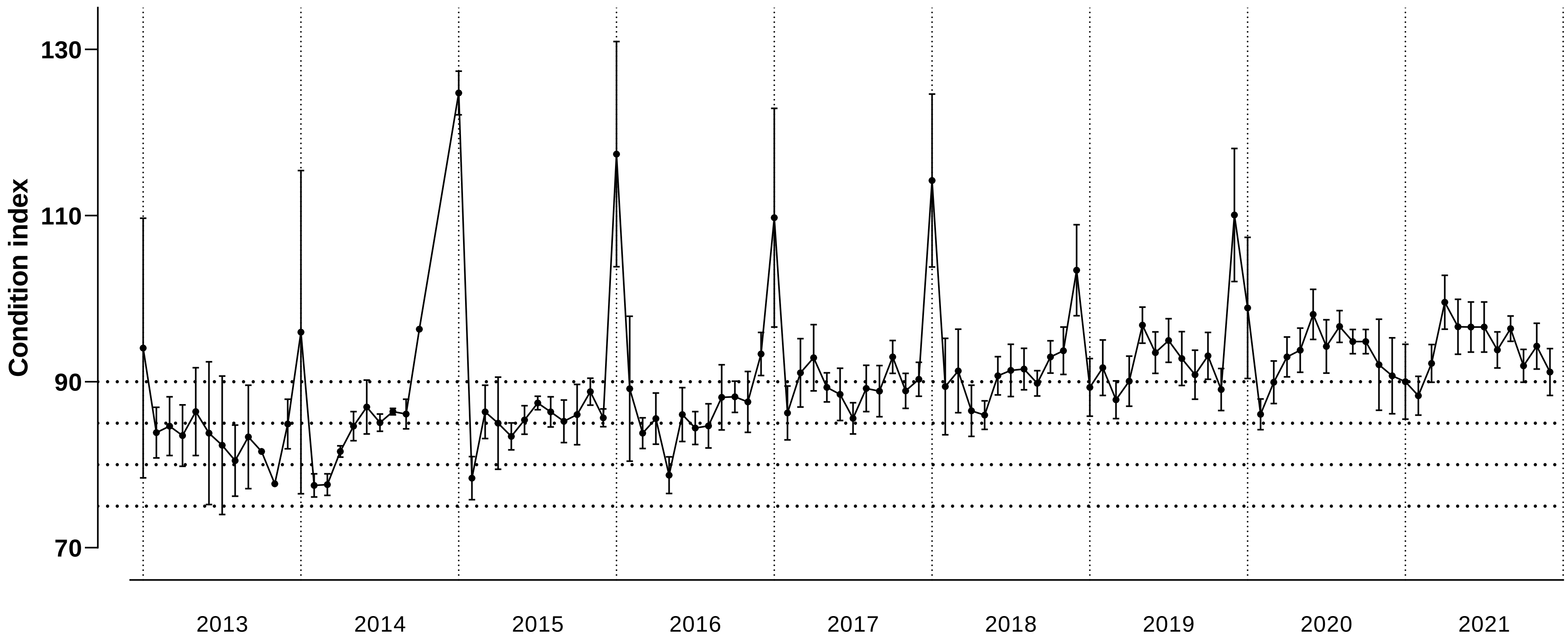

Fig 3.19. Condition indices of all male kororā where the number of birds per year ranged from 2 to 7. Not all male birds were alive or present at the aquarium when the flipper length collection was taken in 2023 at the National Aquarium.

Similar to the female birds we see an increase in CI from 2016 and since, flipper lengths were collected in 2023 and some birds in the study passed away so we do not have a completed representation of condition indices for all female birds. Looking at the birds (Mr.Mac, Mo, Captain) whose flipper lengths we do have an were present at the aquarium from 2015 to 2021 we still see that increase in CI over the years from 2016 (Fig 3.20) similar to the CI of all the male birds (Fig 3.19). Mean condition index (CI) of male birds differed between birds (mixed effects model; $F_{2,33}=3.394$, $p=0.0457$) but did not differ between years ($F_{3.003,92.58}=1.426$, $p=0.2402$), there was no significant interaction between bird and years ($F_{12,185}=5472$, $p=0.8814$). Sidak's multiple comparison tests showed no significance between other successive pairs of other years.

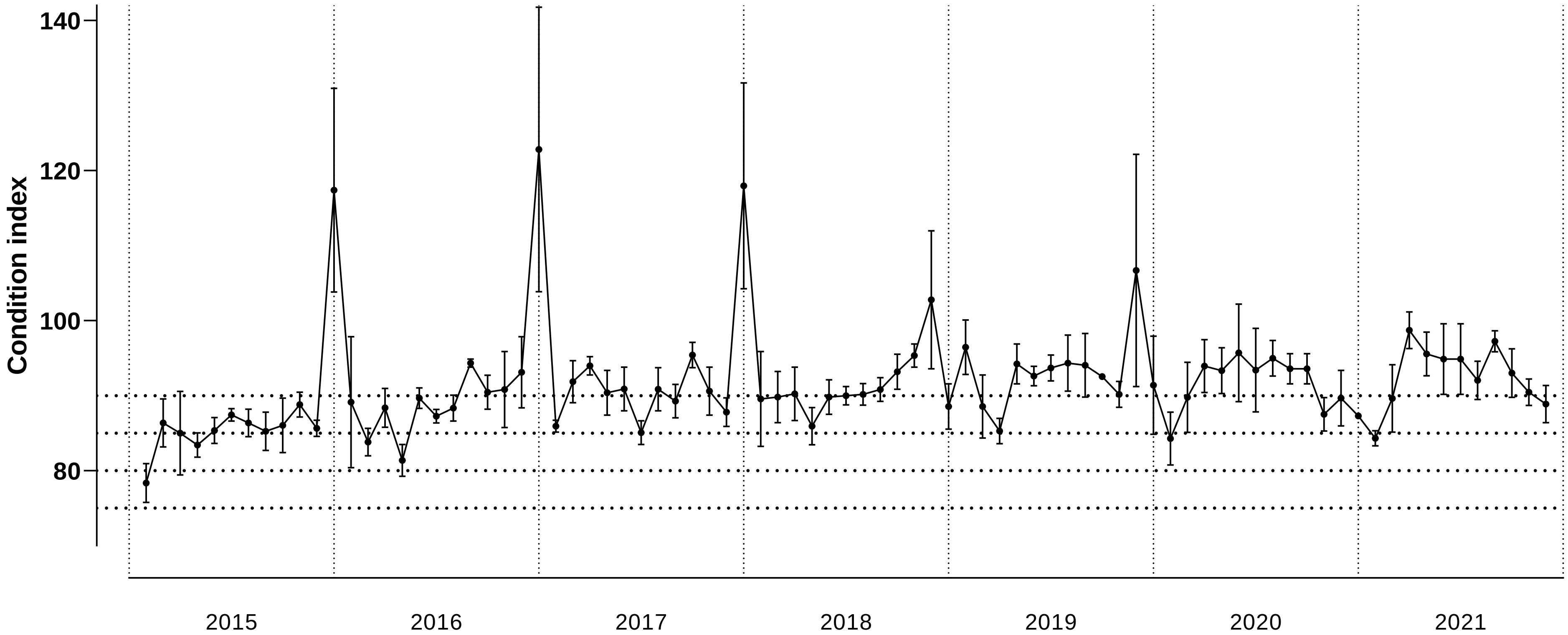

Fig 3.20. Condition indices of all male kororā present from 2015 to 2021 (n = 3).

3.3.2.3 Female and male comparison

Male kororā were in a higher condition index than female kororā (Table 3.6) (Fig 3.21). Both sexes show similar patterns of condition index increasing and decreasing depending on the time of year. Both sexes showed the highest condition during their months of moult. Using an unpaired t-test it showed significant differences (t_{1000}=15.08, $p<0.0001$) in the condition indices between male and female birds used in this study.

Table 3.6. Mean and S.E. of condition indices for female and male birds from 2013 to 2021.

	Mean	S.E.	*n*	Min.	Max.
Female	81.6	0.5	510	63.3	140.8
Male	91.5	0.5	492	71.7	142.2

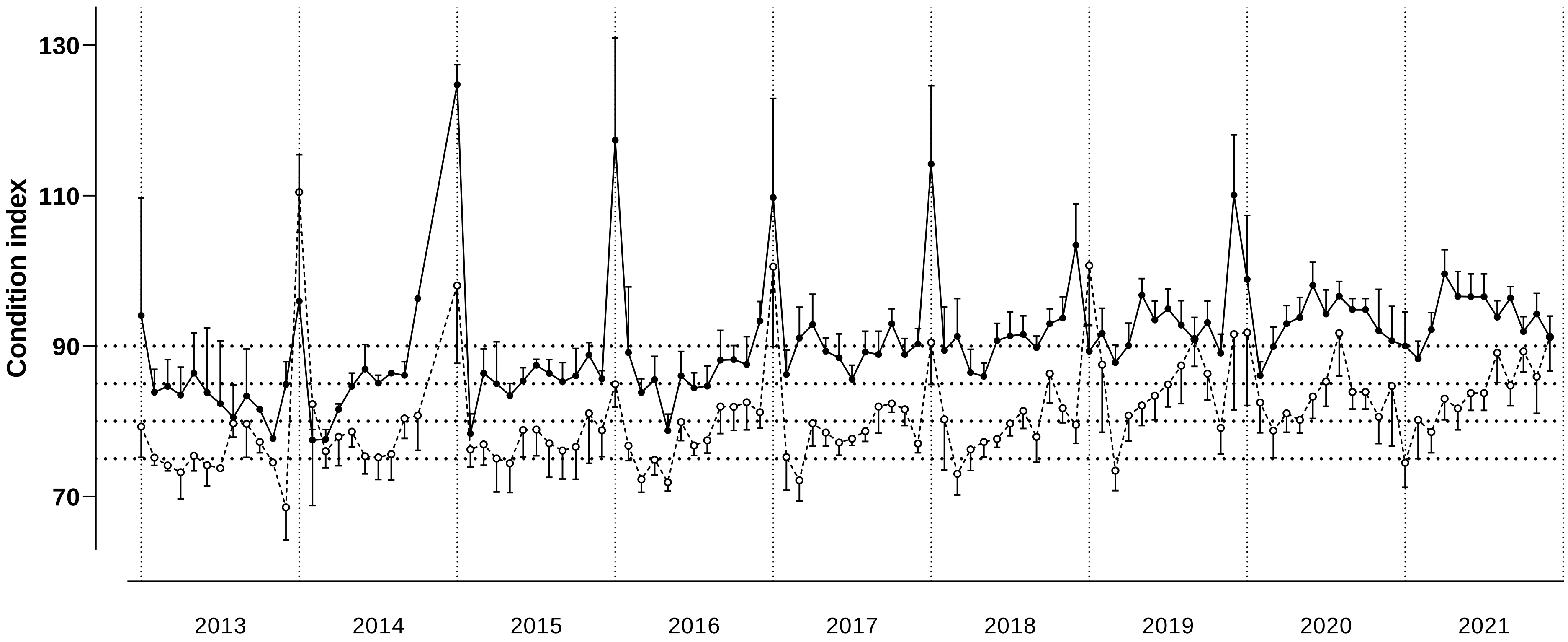

Fig 3.21. Condition Indices (± SE) of all males (— ● —) (birds ranging from 2 to 7) and females (--- ○---) (birds ranging from 2 to 7) birds from 2013 – 2021 (National Aquarium, Napier).

3.3.3 Food intake

3.3.3.1 Female food intake

Fig. 3.22 shows monthly food intake of female penguins from 2013 to 2021. Food intake varied from 0 to 42 fish, with the great majority of food intake between 2 to 16 fish. The highest food intake of individual birds almost always occurred in November to January or in the case of 2015, in September. Having daily food intake gives us more insight into how their food intake varied with the events of their annual cycle

Mean food intake (Fig 3.23) shows peaks at different times depending on the year. We see a high food intake in December in the years 2014, 2018, and 2021. We also see a high food intake in October for 2015, and 2020. 2016, showed and increase in food intake from July and then decreasing in November to then decrease from a peak in December to a minimum in March. Looking at food intake and how it changes from month to month (Fig 3.24), most of the increase in food intake is observed in October. We have one outlier in August, 26 fish being eaten and two outliers in September with 28 and 33 fish being eaten respectively. October reached a peak of 42 fish and then we observe food intake decrease in November and then start to increase again in December followed by a steady decrease from January to March.

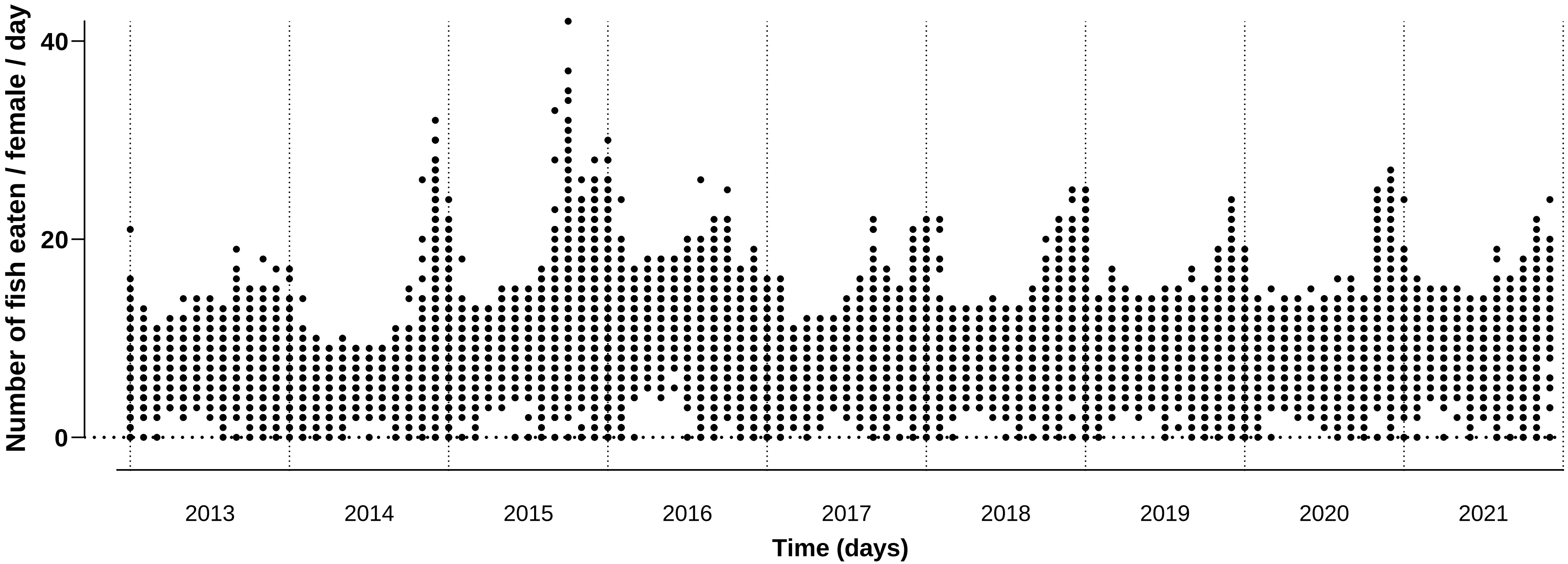

Fig 3.22. Food intake of female kororā. Data are daily measurements. The number of female kororā each year ranged from n = 4 (2013), n = 5 (2014, 2016, 2018), n = 6 (2016 & 2017), and n = 7 (2019, 2020, & 2021) birds. A total of n = 25340 data points. Due to some birds eating the same amount points are overlapped.

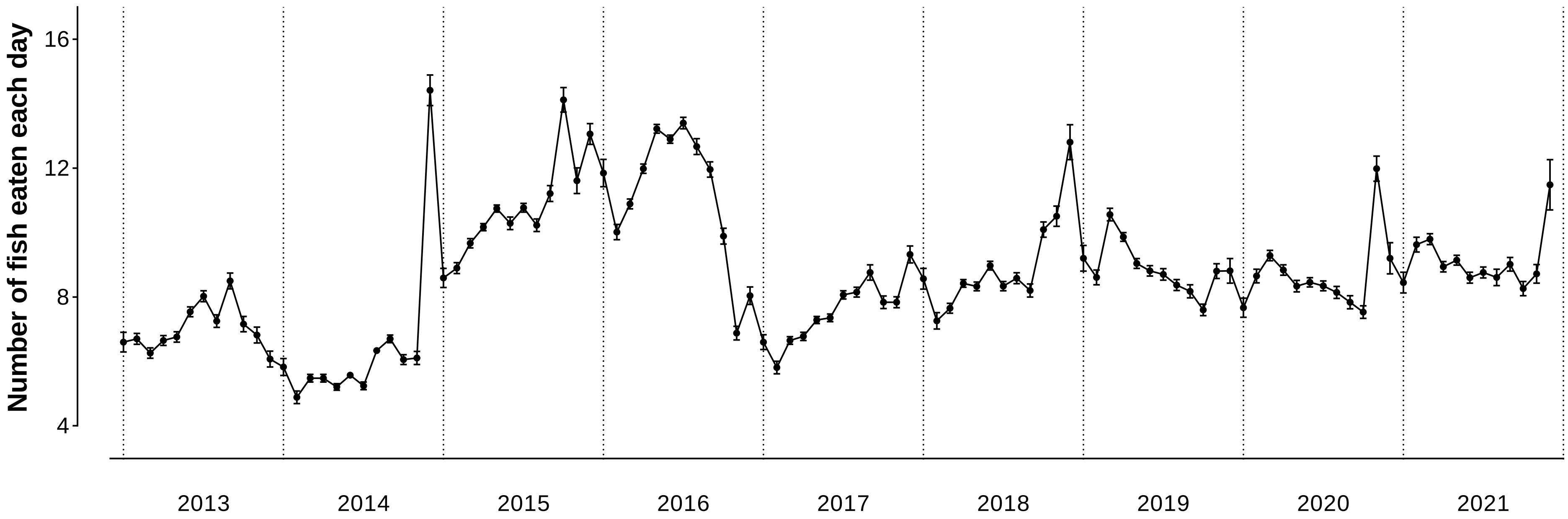

Fig 3.23 Monthly food intake means (± SE) of all female birds from 2013 to 2021 (National Aquarium, Napier)

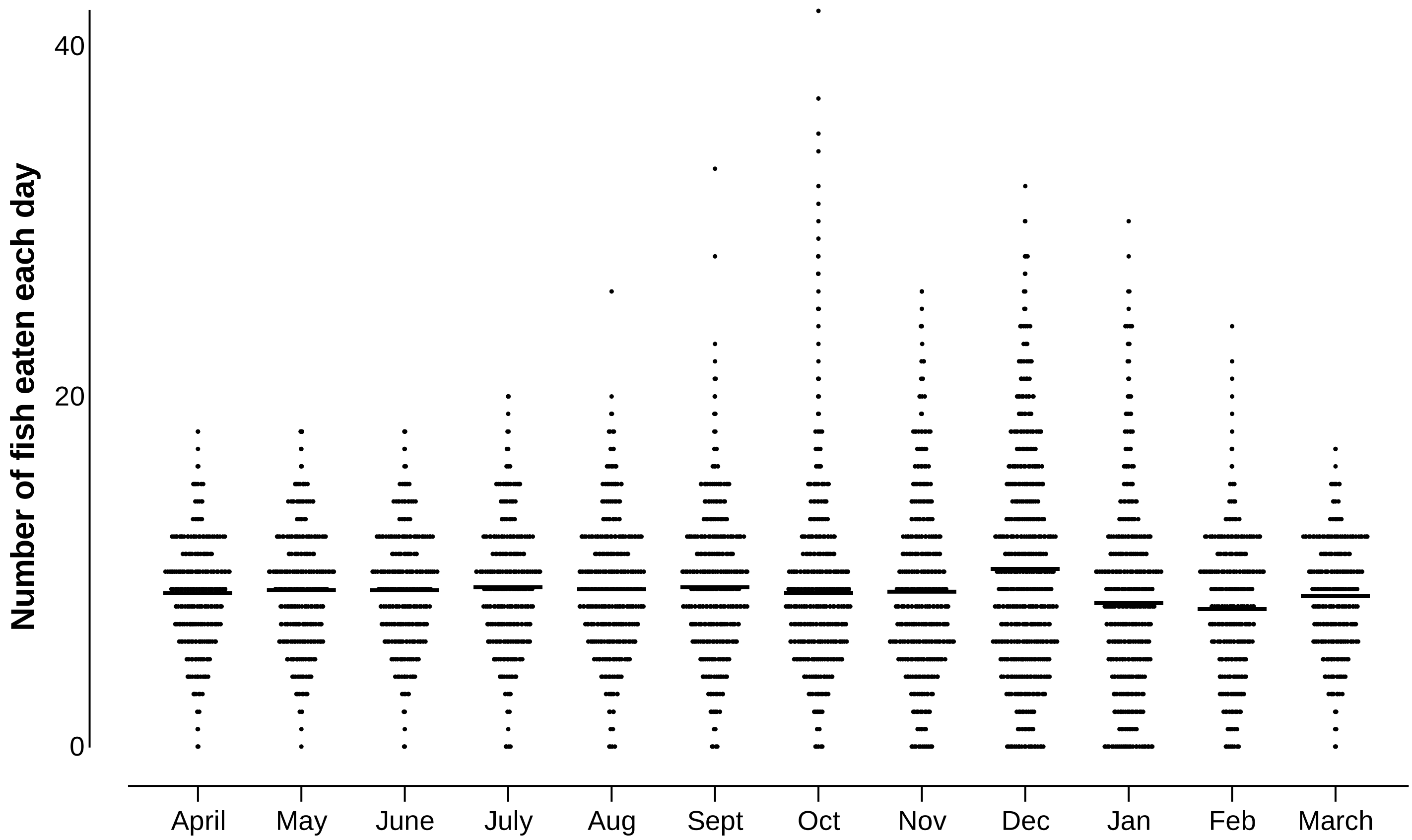

Fig 3.24 Individual food intake of female kororā at the National Aquarium (n = 26389). Data is represented by month and is daily measurements. Some birds have eaten the same number of fish causing the data points to overlap.

Mean food intake of individual females ranged from 7.51 ± 0.07 to 9.74 ± 0.08 fish (see Table 3.7). The mean food intake of the bird who average the least (Elmo) was 78% of the food intake of the bird who averaged the most fish (Flip)

Table 3.7. Mean and SE of all individual female birds food intake from 2013 to 2021. Data was collected daily.

	Mean	S.E.	*n*	Min	Max
Elmo	7.51	0.07	2549	0	30
Lulu	7.70	0.09	2814	0	42
Pepper	8.09	0.08	2201	0	25
Jack	8.37	0.09	1913	0	30
Miley	8.93	0.13	1123	0	32
Gonzo	8.98	0.10	1882	0	28
Draco	9.21	0.07	3235	0	30
Pippa	9.27	0.19	485	0	19
Burny	9.37	0.09	1352	0	25
Betty	9.41	0.07	3235	0	28
Marina	9.56	0.13	256	0	15
Dora	9.72	0.07	2505	0	24
Flip	9.74	0.08	2839	0	30

There was an annual cycle of food intake in female penguins and we compare it to the annual cycle observed with their body weight (see Fig. 3.5). Mean food intake were lowest in Febuary, remained relatively constant from April to November, rose to a peak in December, then declined in January and Febuary. Mean, standard error, minimum and maximum food intake each month are shown in Appendix Table 7.3.

Mean food intake of female birds differed between birds (mixed effects model; $F_{3.849,231.6}=3.468$, $p=0.0099$) but did not differ between months ($F_{11,66}=1.395$, $p=0.1965$), with a significant interaction between bird and month ($F_{121,662}=1.249$, $p<0.0001$). Sidak's multiple comparison tests showed that food intake differed between June to July ($p=0.0246$), and February to March ($p<0.0001$) but did not differ between other successive pairs of other months.

Table 3.8. Mean Body weight (g), condition index and food intake of individual female birds from 2013 to 2021. Data for body weight and food intake is monthly and is sorted from the lowest to highest in body weight, condition index, and food intake.

	Body weight			Condition index			Food intake	
Bird	Mean	S.E	Bird	Mean	S.E	Bird	Mean	S.E.
Lulu	759.00	10.40				Elmo	225.20	7.74
Pippa	794.80	21.60				Lulu	230.30	9.85
Dora	808.20	9.20	Dora	77.70	0.90	Pepper	240.60	7.79
Pepper	817.60	13.00	Pepper	78.60	1.20	Marina	244.60	30.70
Betty	848.60	9.80	Betty	79.30	0.90	Jack	254.00	11.90
Jack	866.90	11.00				Miley	270.90	15.87
Draco	967.10	10.10	Draco	79.30	0.80	Gonzo	272.40	12.06
Miley	976.90	17.00				Burny	275.30	10.40
Gonzo	980.80	12.30				Draco	275.90	7.30
Burny	982.00	20.70				Pippa	280.90	22.07
Flip	993.80	10.20	Flip	86.50	0.90	Betty	281.70	7.99
Marina	1033.40	12.50	Marina	88.30	1.10	Dora	289.90	8.94
Elmo	1056.30	11.10	Burny	92.60	1.90	Flip	291.20	8.82

Looking at monthly body weights and food intake, we see most of the birds follow the trend of their body weight corresponding to their food intake rankings but a few exceptions are Elmo sitting at the highest among the female birds at 1056.30 $\pm$ 11.10 g, but in terms of food intake, Elmo has consumed the lowest with a monthly average of 225.20 $\pm$ 7.74 fish. Marina is also the second heaviest bird averaging at 1033.40 $\pm$ 12.50 g but was fourth on the food intake averaging 244.60 $\pm$ 30.70 fish. This could be explained due to body weight being taken monthly there may be limitations to conclusions we can draw from this.

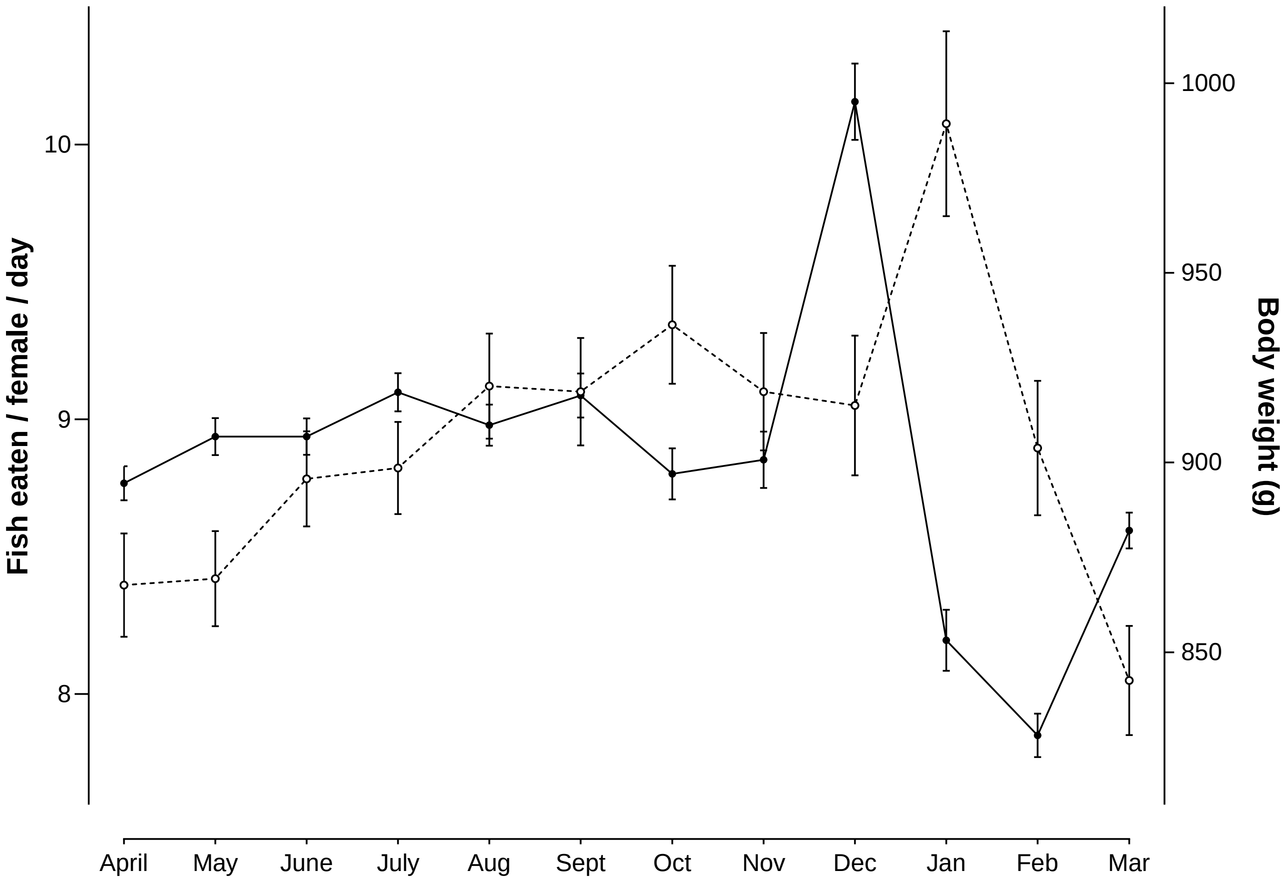

Fig 3.25 Monthly body weight means (+ SE) (--- ○---) (n = 854) and food intake means (+ SE) (—●—) (n = 26389) of all female birds from April to March (National Aquarium). Body weight was taken monthly for each bird and food intake is daily measurements.

3.3.3.2 Male food intake

Fig. 3.26 shows monthly food intake of male penguins from 2013 to 2021. Food intake varied from 0 to 42 fish, with the great majority of food intake between 4 to 18 fish. The highest food intake of individual birds almost always occurred in November to January or in the case of 2015, in October. We also observe an outlier in December of 2014 where Pacino ate 42 fish in one day.

Mean food intake (Fig 3.27) shows peaks at different times depending on the year. We see a high food intake in December in the years 2014, 2015, 2017, 2018, 2020, and 2021. We also see a high food intake in November for 2019. 2016, showed an increase in food intake from July and then decreased in November to then decrease from a peak in December to a minimum in January. Looking at food intake and how it changes from month to month (Fig 3.28), most of the increase in food intake is observed in October and also increases till December. We have four outliers in September with a bird eating 24, 26, 32, and 37 fish and four outliers in December with 34, 35, 38, and 42 fish being eaten respectively. We observed food intake decrease in November and then start to increase again in December followed by a steady decrease from January to March.

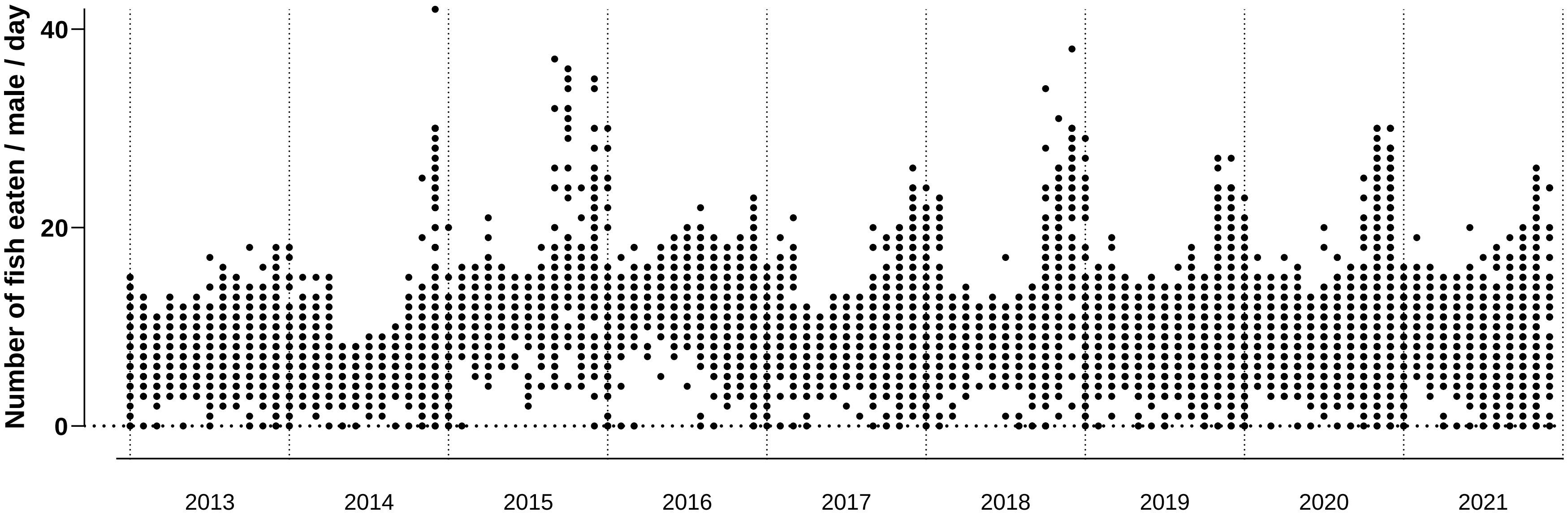

Fig 3.26. Food intake of male kororā. Data are daily measurements The number of male kororā each year ranged from n = 4 (2013), n = 5 (2014, 2016, 2018), n = 6 (2016 & 2017), and n = 7 (2019, 2020, & 2021) birds. A total of n = 18012 data points. Due to some birds eating the same amount points are overlapped.

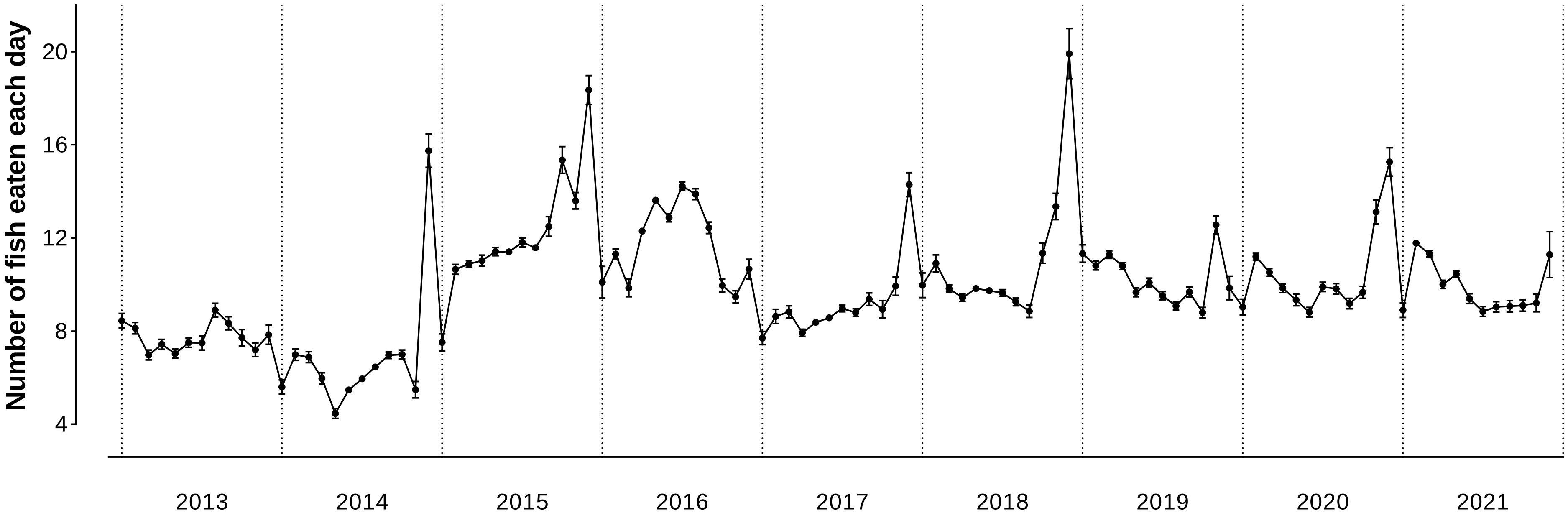

Fig 3.27. Monthly food intake means (± SE) of all male birds from 2013 to 2021 (National Aquarium, Napier)

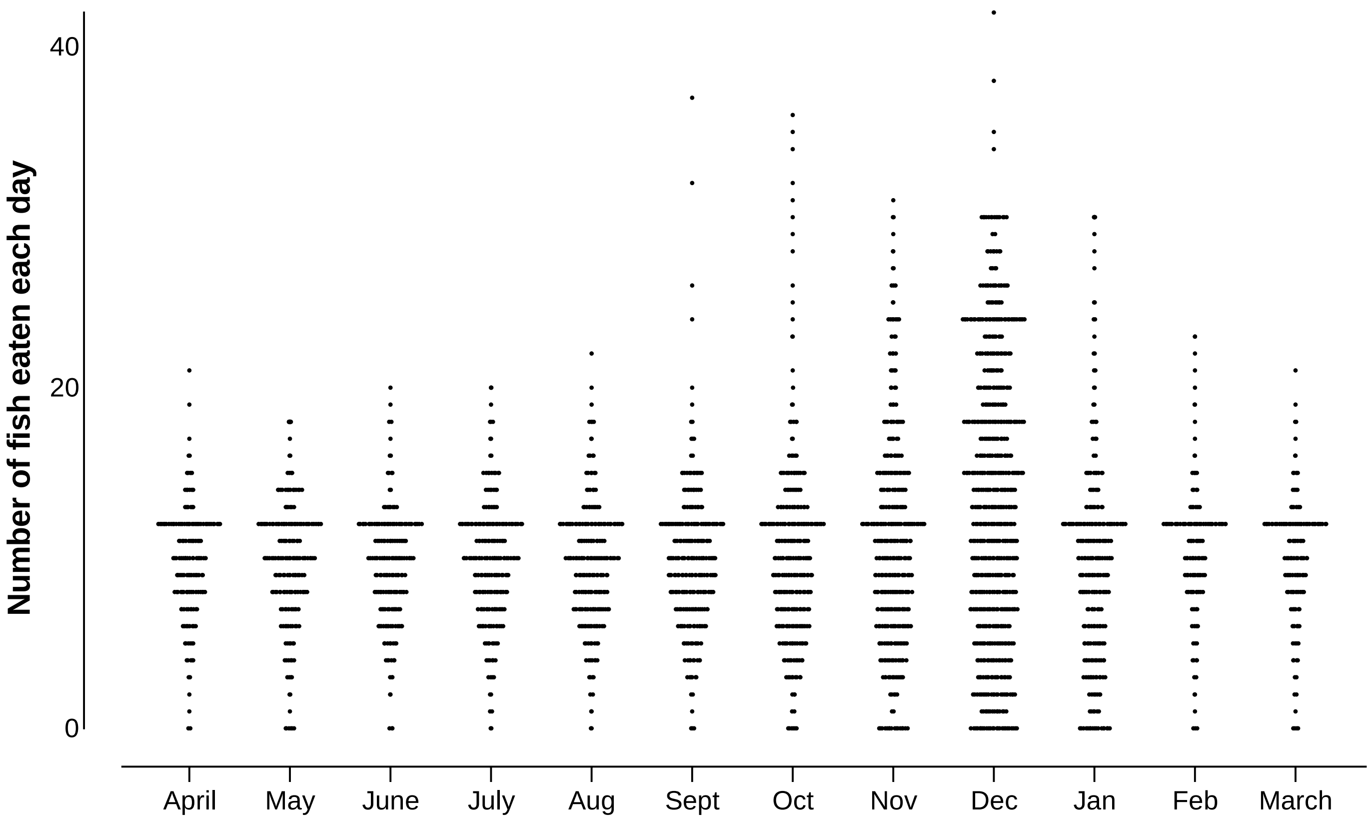

Fig 3.28 Individual food intake of male kororā at the National Aquarium (n = 17902). Data is represented by month and are daily measurements. Some birds have eaten the same number of fish causing the data points to overlap.

Mean food intake of individual males ranged from 7.27 ± 0.12 to 12.23 ± 0.09 fish (see Table 3.9). The mean food intake of the bird who average the least (Gordon) was 75% of the food intake of the bird who averaged the most fish (Timmy).

Table 3.9. Mean and SE of all individual male bird's food intake from 2013-2021. Data was collected daily.

	Mean	S.E.	*n*	Min	Max
Gordon	7.27	0.12	880	0	22
Pocket	7.89	0.16	730	0	30
Mr.Mac	9.14	0.08	3235	0	37
Pacino	9.67	0.14	1123	0	42
Tux	9.68	0.10	2081	0	30
Dave	9.97	0.12	1064	0	28
Martin	9.97	0.14	1005	0	30
Captain	10.28	0.10	2468	0	38
Mo	10.43	0.07	3235	0	34
Timmy	12.23	0.09	2081	0	30

There was an annual cycle of food intake in male penguins and we compare it to the annual cycle observed with their body weight (see Fig. 3.29). Mean food intake were lowest in January, remained relatively constant from April to October, rose to a peak in December, then declined in January and rose in February. Mean, standard error, minimum and maximum food intake each month are shown in Appendix Table 7.4.

Mean food intake of male birds differed between birds (mixed effects model; $F_{9,42}$=2.229, *p=0.0389*) but did not differ between months ($F_{3.252,128.9}$=2.182, *p=0.0881*), with no significant interaction between bird and month ($F_{99,436}$=0.7937, *p=0.9188*). Sidak's multiple comparison tests showed that food intake differed between June to July (*p=0.0168*), but did not differ between other successive pairs of other months.

Table 3.10. Mean Body weight (g), condition index and food intake of individual male birds from 2013 to 2021. Data for body weight and food intake is monthly and is sorted from the lowest to highest in body weight, condition index, and food intake.

	Body weight			Condition index			Food intake	
Bird	Mean (g)	S.E. (g)	Bird	Mean	S.E.	Bird	Mean	S.E.
Pocket	1039.30	38.40				Gordon	220.40	13.82
Tux	1066.70	12.30	Tux	84.00	1.00	Pocket	250.00	17.72
Pacino	1072.80	14.60				Mr Mac	273.60	9.16
Mo	1078.80	11.00	Mo	88.40	0.90	Tux	287.80	9.62
Mr Mac	1082.30	11.40	Mr Mac	89.50	0.90	Pacino	293.60	15.94
Dave	1128.00	21.30	Dave	94.00	1.80	Dave	294.60	13.32
Gordon	1128.30	19.80				Martin	294.80	12.82
Captain	1147.30	14.40	Captain	95.60	1.20	Captain	305.60	10.35
Martin	1150.40	17.50	Martin	96.70	1.50	Mo	312.30	8.18
Timmy	1205.40	12.40	Timmy	98.00	1.00	Timmy	363.70	9.84

Looking at monthly body weights and food intake, we see most of the birds follow the trend of their body weight corresponding to their food intake rankings but a few exceptions are Gordon sitting at fourth highest among the male birds weight at 1128.30 ± 19.80 g, but in terms of food intake, Gordon has consumed the lowest with a monthly average of 220.40 ± 13.82 fish. Mo, is fourth in body weight with 1078.00 ± 11.00 g but was the second highest in terms of monthly food intake with 312.30 ± 8.18 fish. This could be explained due to body weight being taken monthly there may be limitations to conclusions we can draw from this. Another conclusion could be due to when the bird arrived into the aquarium as to how many food intake enteries are there. Gordon passed away in 2015 so we only have 2 years of food intake enteries, while birds like Mr.Mac and Mo have data from 2013 to 2021.

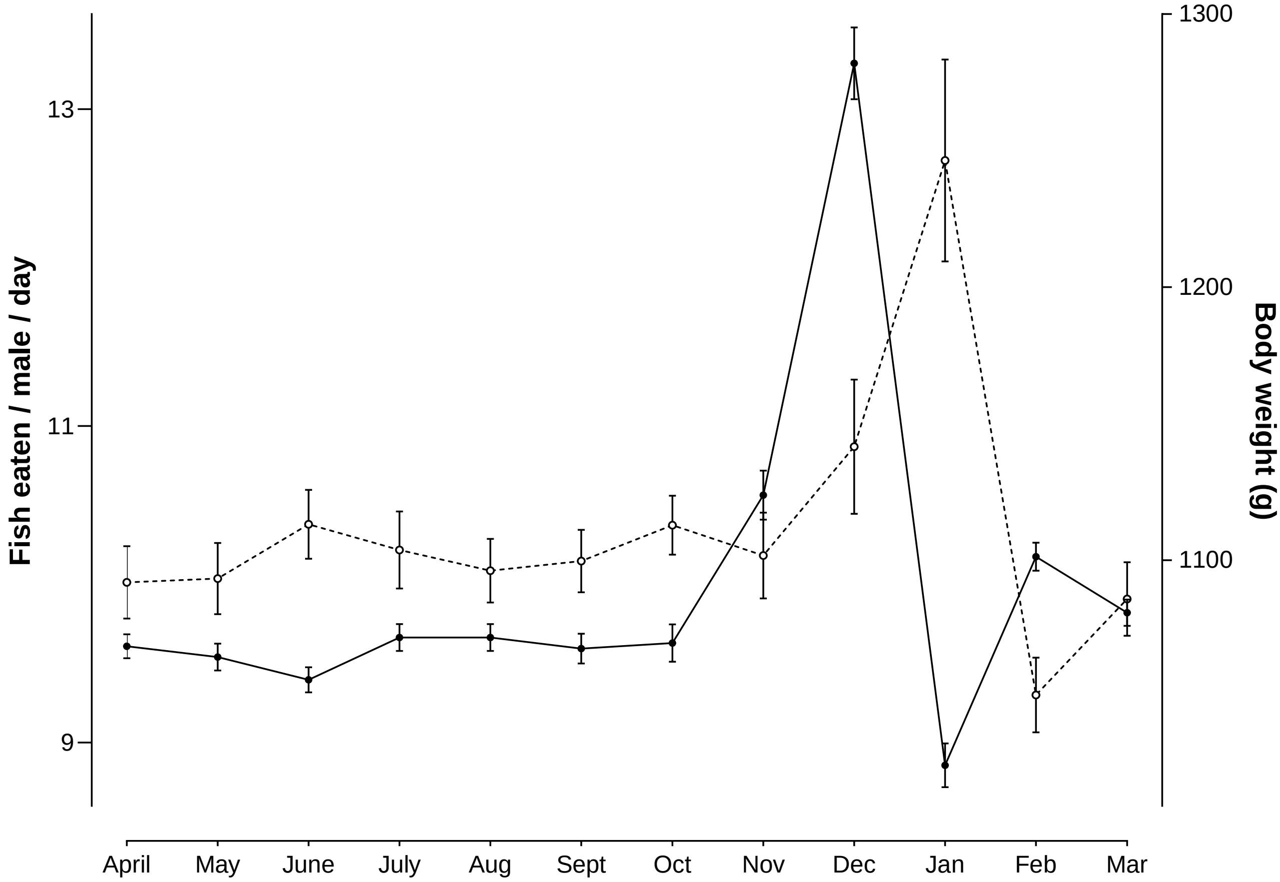

Fig 3.29 Monthly body weight means (± SE) (--- ○---) (n = 577) and food intake means (± SE) (— ● —) (n = 17902) of all male birds from April to March (National Aquarium). Body weight was taken monthly for each bird and food intake are daily measurements.

3.3.3.3 Female and male comparison

Female and male kororā show similar averages of food intake with males only showing an increase of fish eaten by 1-2 fish (Table 3.10). Both sexes show similar patterns of increasing and decreasing food intake depending on the time of year and both sexes are also sitting in a similar range to each other with the male birds are sitting in a slightly higher range (fig 3.30) than the females. The male birds show a higher increase of food intake as they approach their moult. Using an unpaired t-test it showed significant differences (t_{44289}=26.61, $p<0.0001$) in the food intake between male and female birds used in this study.

Table 3.10 Mean and S.E. of food intake for female and male birds from 2013 to 2021.

	Mean	S.E.	*n*	Min.	Max.
Female	8.84	0.03	26389	0	42
Male	9.94	0.03	17902	0	42

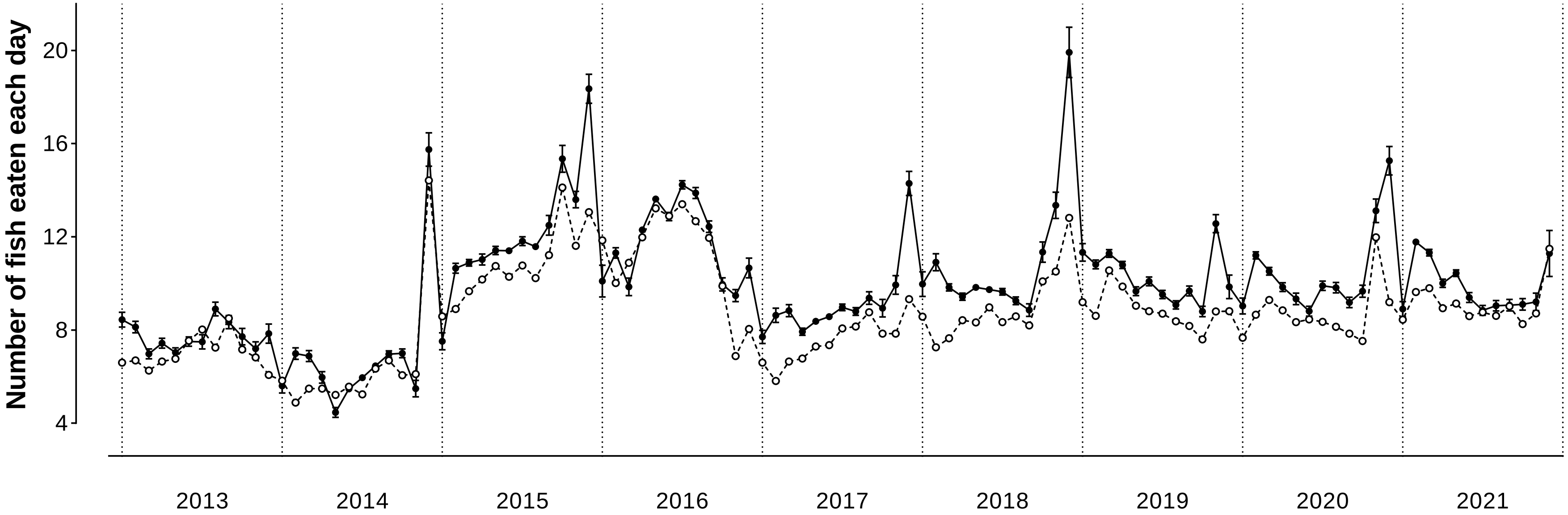

Fig.3.30. Food intake means (+ SE) of all males (— ● —) (n = 17902) and females (--- ○---) (n = 26389) birds from 2013 – 2021 (National Aquarium, Napier

3.4 Discussion

This study is one of the first that looks at the annual variation in body weight for captive little penguins and found that there was an annual pattern of body weight observed in this study from 2013-2021. We observed that birds body weight peaked in January of each year this peak is due to the birds starting their moult. There are still changes in body weight observed from June to December as the birds are going through their breeding season.

Looking at sex differences, we know that female birds are smaller than males (Johannesen *et al.*, 2002; Arnould *et al.*, 2004), and in this study, we can see that female birds sit at a lower weight range than males. The female birds also showed increased weights in January but showed more weight gain and loss from June to December. From the year 2015 to 2021 we see the female birds showing more fluctuations in their annual body weight. In 2019, female birds showed a steady increase in body weight from April to September and then a decreased from September to October, peaking in December. We do observe an outlier in October, with Elmo reaching a peak weight of 1516 g, her body weight was taken as she began her moult.

During the breeding season, female birds are allowed to eat as much fish as they want and their "maximum number of fish per day" is removed to help sustain enough energy to help grow their reproductive organs as well as create the egg (Gales and Green, 1990; Hocken, 2000; Challies and Grau, 2015) hence, the variation can also be due to more female birds coming into the colony but also egg laying doubling from 2015 onwards. The male birds were in a higher weight range than the females and showed an annual pattern with body weight peaking in January in the majority of birds due to the onset of moult. During the months from April to November male birds did not fluctuate in weight as much but that is because unlike the female birds they still had a "maximum amount of fish" they are allowed to eat The male birds in 2018 and 2019 both had their peak

The overall condition indices for female birds were significant with some female birds being able to store more fat than others. Dora was seen with the lowest average condition indices (77.70) among them but Marina on the other hand had a higher average (88.30) than Dora but only a maximum of 94.10 while Dora's maximum was 108.8. One of the reasons we observe this is because we only have nine data points for Burny but eighty-four for Dora. Not all the birds in this study had the flipper lengths measured as some of them had passed away or were relocated to the Antarctic center in Christchurch. Burny was observed with the highest condition index which is consistent with her being a bigger bird than the other females. We observed Timmy have the highest condition indices (98.00) and that is consistent with him also averaging the highest weight among the male birds. Focusing on all the birds whose data we have for condition indices we can see the overall CI increased over the years from 2013 to 2021. One reason for this increase was the addition of newer, younger birds to the colony so, to understand this better we looked at the overall CI for birds that were present from 2015 to 2021 as those birds were still present at the aquarium when flipper length was taken in 2023. We still observed an increase in the CI of these birds and this can be due to the penguin keepers skill in identifying which birds put on the fat required for them to be able to carry out their annual activities.

This study also looked at daily food intake from 2013 to 2021. There isn't an observable annual pattern of food intake but in 2013 and 2014 food intake was lower than the other years. This could be due to having fewer number of birds but also more female birds than males. There are certain years for example, 2014, 2015, and 2018 where food intake peaked in December in preparation of moult.

Looking at sex differences, female birds have shown to have more variation in their food intake from May till November and then peaking in December. Comparing this with their body weight we see that their increase and decrease in food intake is related to their breeding season and the peak observed in December is due to preparing for moult. Male birds on the other hand, average 1-2 more fish than female birds but their food intake is constant from June to October and only starts to increase from October, peaking in December in preparation for their moult. If a bird is approaching their moult, the keepers increase their food by 20% - 30%, and once it starts their moult, the bird stays in its nest box until it's completed. The keepers do not feed the birds while undergoing their moult to prevent them from getting used to being fed on the nest but also to prevent them from overheating. Since these birds do not swim vast distances to get their food, it is important to make sure they are kept at a proper weight and get exercise to prevent issues such as bumblefoot (Wolanin, 2018).

Little penguins in the wild forage for food, leaving the colony before sunrise and returning after sunset (Agnew, 2014). When doing day trips, they usually stay within 25 km of their colony, and every colony has its variation in the length of their foraging trips as it is dependent on the availability of food (Elsom, 2022). Various environmental factors affect their foraging behaviour, and their annual cycle's breeding and moulting stages are crucial and high in energy costs. The energy demands (Buller, 1882) associated with them and other environmental factors make little penguins more flexible with their prey selection (Cullen *et al.*, 1992).

Gales and Green (1990) looked at the annual energetics of the little penguins and found that the highest energy demands for these birds came during their breeding season. They charted that 30% of the bird's yearly energy budget was spent on raising its chicks, even though it only takes up 16% of their annual cycle. While a parent is incubating the eggs, the partner is seen going on foraging trips, which can last a mean of 4.4 days (Collins *et al.*, 1999). In this study, the birds do not need to travel any distances to get food. The penguin keepers food them at the food station but also note who is sitting on the eggs and swap them around to give each partner a chance to come up to the station and to also go in for a swim. A study involving captive birds for 16 months showed birds increasing their body mass by 40% at the start of moult to help with the high energy demands (Baudinette *et al.*, 1986). Evans *et al.* (2015), found that both males and females during the guard phase went down in body weight but increased when they were in the post-guard phase. Both sexes were also seen to increase their weight significantly at the start of moult and then decline at the end of moult. Gales *et al.* (1988b), studied the annual energetics of free-living penguins during moult and found a linear relationship of weight loss during moult. Birds were seen losing 55 g day^{-1} or a total of 46% of their initial weight.

The overall question of this study was to understand if these captive birds experience changes to their body weight and if these changes correspond to any of their annual cycle activities. We can see that during the breeding season, males do not greatly fluctuate in weight whereas female birds are seen eating more to help with the energy expense of egg formation and laying. We then see both male and female birds increase their weight once they begin moult with both sexes weight and condition indices reaching the highest point. Since body weight was taken once monthly using the data in relation to their breeding and moult helps us understand the changes we observed. Looking at food intake we can see a small increase in food eaten by female birds during the breeding season, but it is not significant. But, from the months of October and reaching a peak in December we observed both sexes increase their food intake to reflect the high weight and condition index we observed in January. Using these key dates for when the birds were weighed, when they started to lay eggs and moult from the diaries have helped us understand the changes we observed and also how their body weight reflects their annual cycle.

4. Timing of egg laying

4.1 Introduction

The breeding season in little penguins in the wild is normally from July to December (Department of Conservation, 2023). The birds start coming to shore from July onwards to start nest building and developing new pair-bonds or to meet up with their already formed partners (Agnew *et al.*, 2014). This is then proceeded with the laying of their 1st clutch of the season and the timing of egg laying in wild birds varies depending on location and other factors such as food availability, sea surface temperature, chlorophyll-a etc., (Bool *et al.*, 2007; Agnew *et al.*, 2015; Berlincourt and Arnould, 2015). But in general, we can observe that wild birds start laying from August to December in New Zealand (Rowe *et al.*, 2020). Wild birds generally lay two eggs in a single clutch though one egg clutches are not uncommon (Agnew *et al.*, 2014) and depending on the breeding season, they may lay replacement clutches if the first one failed or even because the first one was successful and they are capable of producing another clutch (Agnew *et al.*, 2014). Once the eggs are laid both pair of birds take turns incubating the eggs, but it is not uncommon for the eggs to become abandoned if the parents are unable to care for them or they are predated on during a forage (Cannell *et al.*, 2012). The incubation period last from 36 to 38 days in the wild (Croxall, 1982; Kemp and Dann, 2001a).

In terms of the captive birds, they are provided with food every day, most of the birds in this study have a bonded partner and these birds are not affected by any external factors like predation or prey availabilty. The birds in this study are still affected by weather due to having an outside enclouse and even with the presence of veterinary care they can encounter diseases such as bumblefoot (Wolanin, 2018). The majority of birds in this study also lay two egg clutches but there are some birds that may lay only one egg in the clutch and there are cases of replacement clutches being laid. This study will look at the timing of egg laying from 2013-2021 and also the outcomes for birds that were able to naturally incubate their eggs for more than 32 days. Using the information we gather from the diaries, we looked at when timing of egg laying occurred in the captive little penguin. We then focused on eggs that were incubated for more than 32 days to calculate the average incubation period for birds who were allowed to incubate till hatch. The following section 4.2 follow through the methods of how we extracted the information from the penguin diaries that were provided from 2013-2021. The next section is 4.3 which go through our results and the discussion and answers to our questions can be found in section 4.4

4.2 Methods

4.2.1 Penguin diaries

The Aquarium provided us with their "penguin diaries" from 2013 to 2021. They had daily entries in the diaries that included egg-laying data. The penguin keepers write down who laid an egg, how many eggs were laid and whether it was a 1^{st}, 2^{nd} or even 3^{rd} clutch. The keepers also made note of who sat on the eggs occasionally. The outcome of the eggs was also noted whether it was removed due to being "kicked out", "cracked/broken", "abandoned", or "infertile". If the eggs were fertile in most cases, they were allowed to be naturally incubated for more than 32 days and the keepers noted if the eggs "hatched" or removed them after the eggs were overdue. Most of the birds have formed a pair bond with the opposite sex, but there are some cases of same-sex pair bonds (e.g., Jack and Gonzo), and they laid infertile eggs. We will not include Jack and Gonzo's eggs in this study because even though the penguin diaries tell us who laid the eggs, the penguin keepers noted it was just a guesstimate, and they can't be completely sure who laid which egg in this case.

4.2.2 Data analysis

Data were analysed using Prism (GraphPad Software, LA Jolla, CA). The penguin diaries were scanned and made into a digital format for each respective year. The data included the date of when the egg was laid, who laid the egg, and when the 2^{nd} egg was laid for the clutch. It was also noted if any bird had replacement clutches (2^{nd} or 3^{rd} clutches). This data was all assigned a day number, which was done by assigning 1st of April = day 1 and 31st of March = 365/366 days of the year. This was created in Excel, and a VLOOKUP function was used to assign the respective day to the dates. "Removal of clutch" is an umbrella term to indicate the end of an incubation period. The eggs have had various outcomes, such as being abandoned/ or broken, replaced with a dummy egg, removed, infertile, incubated naturally, hatched, etc. This study focuses on the eggs naturally incubated for over

32 days and the associated outcomes. “Natural” incubation in this study refers to the original egg staying in the nest and having at least one bird incubating it.

4.3 Results

4.3.1 Timing of egg laying

1st clutch eggs

The average 1st egg laid was at 157.03 (September) ± 4.78 days (n = 39), and the 2nd egg laid was at 157.23 (September) ± 4.78 days (n = 35); they range from day 95 (July) to day 227 (November) (Fig 4.1). Individual bird 1st clutch egg laying mean can be found in Table 4.1 below.

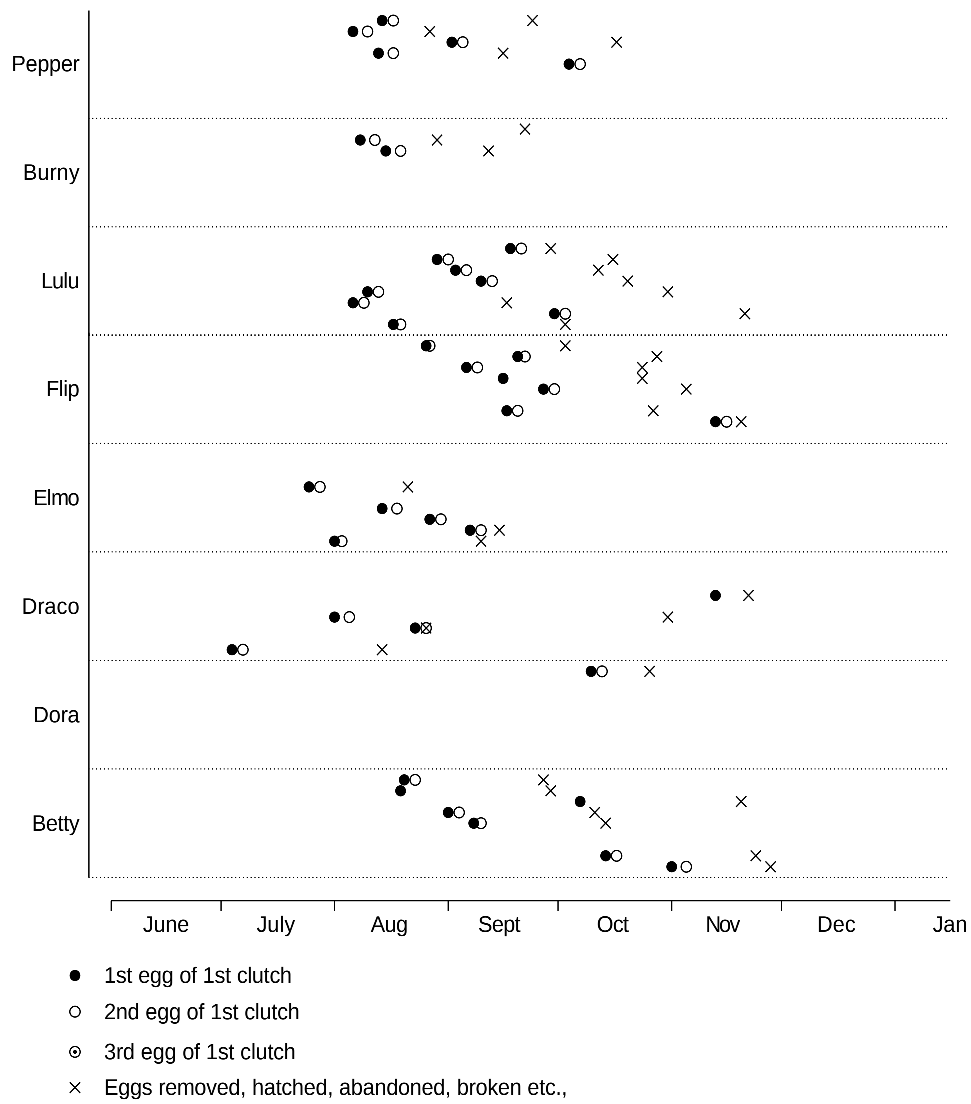

Fig 4.1. Egg laying dates for all 1st clutches laid from 2013 to 2021. The data progresses in an upward manner from 2013 to 2021

Table 4.1. The mean and SE of individual birds 1st clutch eggs laid from 2013-2021

	Mean (day)*	Associated month	S.E.	*n*	Min (day)*	Associated month	Max (day)*	Associated month
Betty								
1st egg	171.43	September	11.02	7	141	August	215	November
2nd egg	176.80	September	13.99	5	145	August	219	November
Burny								
1st egg	133.50	August	3.50	2	130	August	137	August
2nd egg	137.50	August	3.50	2	134	August	141	August
Dora								
1st egg	193.00	October	0.00	1	193	October	193	October
2nd egg	196.00	October	0.00	1	196	October	193	October
Draco								
1st egg	147.50	August	28.41	4	95	July	227	November
2nd egg	124.33**	August	14.50	3	98	July	148	August
Elmo								
1st egg	136.80	August	8.10	5	116	July	160	September
2nd egg	139.80	August	8.18	5	119	July	163	September
Flip								
1st egg	175.14	September	9.49	7	148	August	227	November
2nd egg	178.67	September	11.34	6	149	August	230	November
Lulu								
1st egg	152.88	August	6.81	8	128	August	183	September
2nd egg	155.75	September	6.85	8	131	August	186	October
Pepper								
1st egg	148.20	August	10.68	5	128	August	187	October
2nd egg	151.60	August	10.53	5	132	August	190	October

**Day 1 = 1st of April and day 365/366 = 31st of March. Since the annual penguin year begins in April, we run the dates from 1st of April to 31st of March*

*** Draco only laid one egg in 2018 causing the mean of her 2nd egg mean value to be lower*

Replacement clutches

The penguins in this study have been seen to lay replacement 2nd and even, on some occasions, 3rd clutches (Fig 4.3). The average 1st egg of the 2nd clutch was 195.50 (October) ± 8.29 days (n = 12), and the 2nd egg it was 201.27 (October) ± 8.60 (n = 11); they ranged from day 164 (September) to day 240 (November) for the 1st egg and day 167 (September) to day 243 (November) for the 2nd egg. The average 1st egg of the 3rd clutch was 232.50 (November) ± 17.50 (n = 2), and for the 2nd egg, it was 236.00 (November) ± 17.00 (n = 2). Individual mean and SE for replacement clutches can be found in table 4.2.

Table 4.2. Mean and SE for individual bird's 2nd clutch from 2013-2021

	Mean (day)	Associated month	S.E.	*n*	Min (day)	Associated month	Max (day)	Associated month
Burny								
1st egg	174.00	September	0.00	1	174	September	174	September
2nd egg	176.00	September	0.00	1	176	September	176	September
Draco								
1st egg	165.50	September	0.50	2	165	September	166	September
2nd egg	169.00	September	0.00	1	169	September	169	September
Flip								
1st egg	221.00	November	0.00	1	221	November	221	November
2nd egg	225.00	November	0.00	1	225	November	225	November
Lulu								
1st egg	212.40	October	13.74	5	164	September	240	November
2nd egg	215.40	November	13.74	5	167	September	243	November
Pepper								
1st egg	186.00	October	10.58	3	166	September	202	October
2nd egg	189.00	October	10.58	3	169	September	205	October

Table 4.3. Mean and SE for individual bird's 3rd clutch from 2013-2021

	Mean (day)	Associated month	S.E.	*n*	Min (day)	Associated month	Max (day)	Associated month
Draco								
1st egg	215	November	0	1	215	November	215	November
2nd egg	219	November	0	1	219	November	219	November
Lulu								
1st egg	250	December	0	1	250	December	250	December
2nd egg	253	December	0	1	253	December	253	December

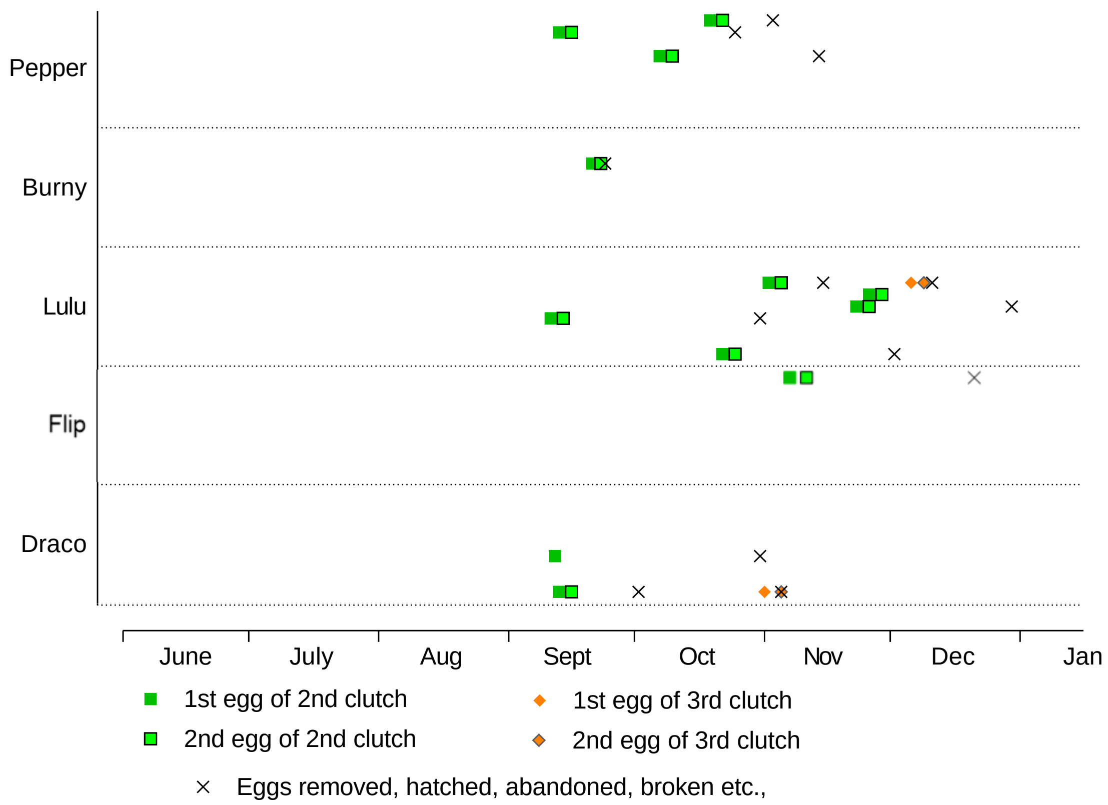

Fig 4.2. Egg laying dates for all replacement clutches laid from 2013-2021. The data progresses in an upward manner from 2013 to 2021.

4.3.2 Eggs incubated naturally for more than 32 days

This study only focuses on the naturally incubated eggs for over 32 days. Of those 46 eggs, 22 did not hatch (fig 4.3) and had a mean incubation period of 42.23 $\pm$ 1.32 (n = 22), and 23 (fig 4.4) did hatch with a mean incubation period of 36.77 $\pm$ 0.51 (n = 23). A 2nd egg by Flip in 2016 didn't have an outcome written about. It was only the 1st egg. Individual mean and S.E. can be found in Table 4.3.

Table 4.4. Individual Mean and S.E. for eggs naturally incubated for more than 32 days and whether they hatched or not.

	Mean	S.E.	*n*	Min.	Max.
Betty					
Eggs that hatched	35.33	0.88	5	34	37
Eggs that didn't hatch	39.67	2.19	4	37	44
Draco					
Eggs that hatched	-	-	-	-	-
Eggs that didn't hatch	49.00	0.00	1	49	49
Elmo					
Eggs that hatched	-	-	-	-	-
Eggs that didn't hatch	38.00	0.00	2	38	38
Flip					
Eggs that hatched	36.50	0.29	7	36	37
Eggs that didn't hatch	42.50	2.50	4	40	45
Lulu					
Eggs that hatched	38.20	0.86	9	36	41
Eggs that didn't hatch	43.20	2.65	9	34	49
Pepper					
Eggs that hatched	35.00	0.00	2	35	35
Eggs that didn't hatch	42.00	0.00	2	42	42

Table 4.5. Mean and S.E. for eggs naturally incubated for more than 32 days and whether they hatched or not.

	Mean	S.E.	*n*	Min	Max
Hatched	36.77	0.51	23	34	41
Did not hatch	42.23	1.32	22	34	49

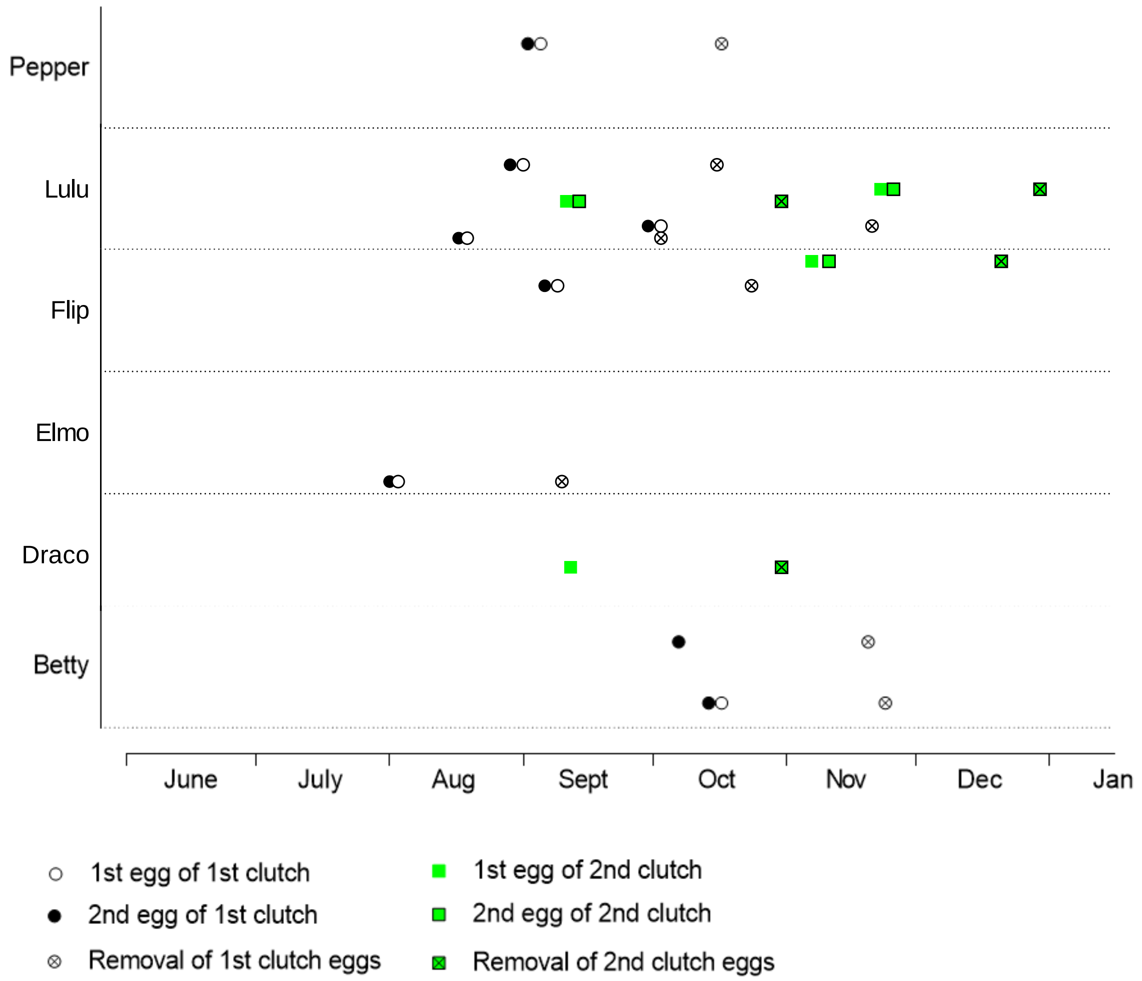

Fig 4.3. Eggs that were naturally incubated for over 32 days but did not hatch from 2013-2021. The data progresses in an upward manner from 2013 to 2021.

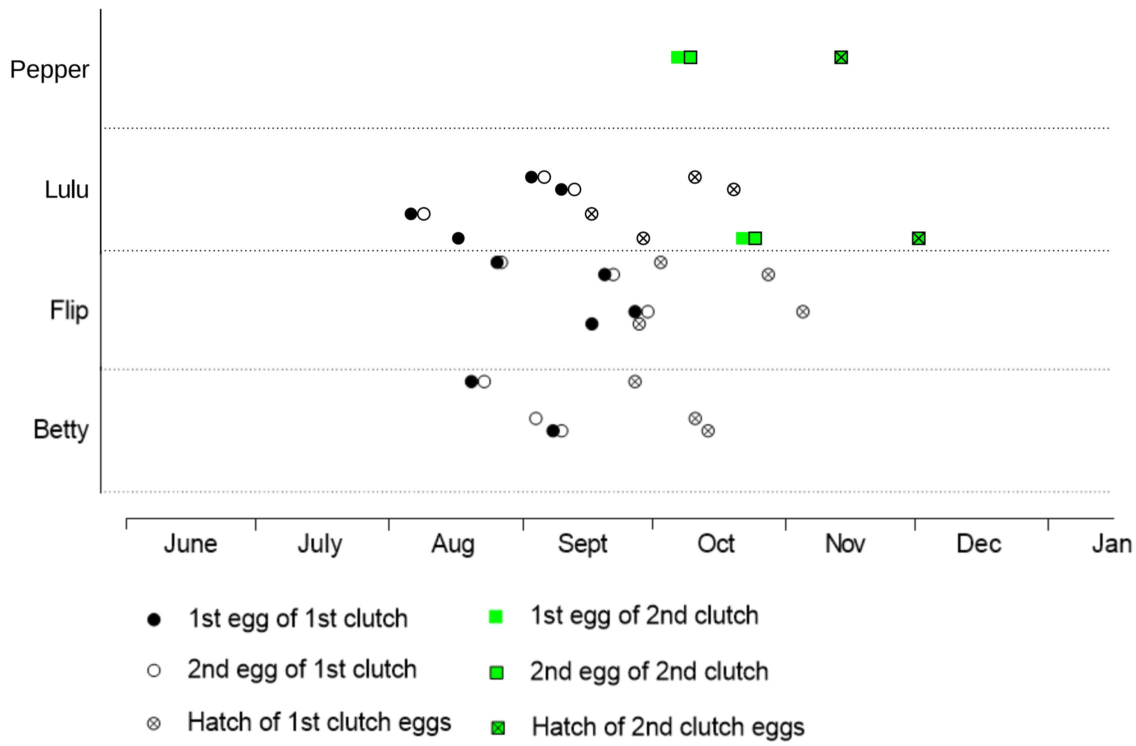

Fig 4.4. Eggs that were naturally incubated for over 32 days and hatched from 2013-2021. The data progresses in an upward manner from 2013 to 2021.

4.4 Discussion

This study focuses on the timing of egg laying in the captive little penguins from 2013-2021 and found that on average egg laying started from September. But there were cases like Draco in 2013 who started her egg laying as early as July and this clutch was naturally incubated for 38 days before it was found broken and removed. We also have cases like Lulu in 2019 who laid her 3rd clutch of the season in December. Most of the clutches laid in this study were two egg clutches but there were some cases, for example, Betty's egg laying in 2019 and 2020, she laid single egg clutches, and the one in 2019 was incubated for 44 days before it was removed due to being infertile while the other in 2020 was replaced with a dummy egg after 12 days of being incubated to then be removed after 20 days adding up to a total of 32 days of incubation. Draco laid a one egg clutch in 2018 which was removed after 9 days due to being infertile. In Fig 4.2 we can see that in 2016, Flip has only one egg, but the diary did mention this being a two-egg clutch but it was uncertain when the 2nd egg was laid. This study also found that replacement clutches were not uncommon.

This study focuses on the eggs that were naturally incubated for more than 32 days to then be able to make comparison with wild little penguins. This study looked at the breeding season from 2013-2021 and we had a total of 46 eggs that were incubated for more than 32 days. The average incubation period for the eggs that hatched was 37 days. This was similar to that observed by the Phillip Island colony in Australia in the 1995/96 season and 2000/01 by Chiaradia and Kerry (1999) and Kemp and Dann (2001) respectively. It is higher than the colony on Tiritiri Marangi Island in New Zealand by Boyer (2010) and the colony of the West Coast of South Island (Heber et al., 2008). The little penguins in captivity still show similar incubation period compared to their wild counterparts. Table 4.5 has a summarized comparison of other studies compared to this study.

The birds in captivity sometimes are not able to incubate their eggs for more than 32 days for various reasons such as abandonment/being broken or cracked and in the wild, there are cases of birds abandoning their eggs for reasons such as survival (Colombelli-Negrel, 2015) or decrease in resources, age or inexperience of birds (Numata *et al.*, 2000; Geraldene, 2020). But the eggs that were incubated naturally and hatched from 2013-2021 had a 51% successful hatch rate which was lower than observed in the Phillip Island colony from 1968-69 and 1998-99 (Kemp and Dann, 2001a), the Oamaru colony from 1994-2012 (Agnew *et al.*, 2014), the Otago colony in 1982 (Gales, 1985), and the West coast colony in 2006 (Heber *et al.*, 2008). Interestingly, the Wellington colony from 1954 was seen to have a hatching success (Kinsky, 1960) similar to this study but, in 1956-1957 and 1995-1996 we see the hatching success go up for the Wellington colonies (Kinsky, 1958; Bull, 2000a). Table 4.5, lists other studies comparisons to hatching success with this study.

Table 4.5. Comparison of other studies looking at incubation period of hatched eggs with this study.

Location	Mean	SE	n	Min	Max	How was incubation period defined	Reference
Summerland Peninsula, Phillip Island, AUS	35.7	1.39	38	31	40	From the date of laying to the date of hatching of the 1st egg.	(Chiaradia and Kerry, 1999)
West Coast, South Island, NZ, 2006	33.9	4	14	30	38	Not defined	(Heber *et al.*, 2008)
Summerland Peninsula, Phillip Island, AUS	35.39	0.14	142	33	44	From the laying of the 2nd egg to hatching	(Kemp and Dann, 2001)
Wellington, NZ, 1958	-	-	-	33	47	From the laying of the 2nd egg to hatching	(Kinsky, 1958)
Banks Peninsula	38	-	-	-	-	Not defined	(O'Brien, 1940)
Tiritiri Matangi Island, NZ	35.5	0.4	16	33	39	Assumed to begin on the day the egg was laid if parent was present on the nest.	(Boyer, 2010)
The National Aquarium, NZ 2013-2021	36.77	0.51	23	34	41	From the laying of the 2nd egg to hatching.	This study

5. Moult

5.1 Introduction

Moult is crucial in the little penguin's (*Eudyptula minor*) annual cycle. It is the complete replacement of their old plumage and can also be classified as a "catastrophic moult" (Davis and Renner, 2010). This replacement is necessary as their feathers wear with time and to ensure efficiency they are replaced annually. This process normally occurs once their breeding season has ended (Groscolas and Robin, 2001). This time they spend foraging to fatten up so when they come ashore, they can progress with their moult and avoid starving to death (Reilly and Cullen, 1979; Croxall, 1982). The moult in wild birds is estimated to occur from January to March and lasts 14-17 days (Department of Conservation, 2023). The process leaves the bird is completely immobilized on land and cannot go to sea to forage due to their feathers losing their waterproofing features during this period. Another reason the birds do not forage at seas when they are going through their moult besides the waterproffing issues is the factor of increased drag, making them to slow to catch their prey (Wilson, 1995). During this period, even though the penguins are sedentary and keep their activity levels low they are still undergoing a highly energetic process that is more than their maintenance costs. This nutrient and energy requirement is normally met when the birds in the wild spend weeks in the sea foraging for food also known as the pre-moult foraging period (Reilly and Cullen, 1983; Brown, 1985; Cherel *et al.*, 2005).

Captivity allows the birds to be predator-free without worrying about starving to death. Wild birds undergo an increase in their energy metabolism due to a decrease in their thermal regulation and need to increase their foraging efforts during their "pre-moult" feeding phase (Baudinette *et al.*, 1986; Davis and Renner, 2010). This leaves wild birds to focus on building their reserves so they may focus their foraging efforts when they have increased in food availability and this effort reflects local areas variation in food availability (Croxall, 1982; Gales *et al.*, 1988b). In this chapter, we look to answer when the captive penguins of this study begin their moult. We also want to know how long their moult lasts. We look to see if there is any relationship between female birds' egg laying and the timing of moult. The section 5.2 and 5.3 go through the methods and the results of the study. We then answer our questions and discuss our results in section 5.4 below.

5.2 Methods

5.2.1 Penguin diaries

The National Aquarium provided us with their “penguin diaries”, yearly diaries that held day-to-day information about the little penguins in the colony. The diaries contained the information for which and when a penguin started their moult and when they finished. The diaries also had the dates for when the penguins were weighed, and using this data, we were able to go back to the body weight Excel file provided and see how many days before the start of moult, during moult, and after the end of moult, the keeper weighed the birds. It should also be noted that only dates that specifically said “bird x started moult” and “bird x finished moult” were considered. There were a few cases of birds only moulting either just their head or flipper which is considered “a partial moult” and those data points were not used in this study. “

5.2.2 Data analysis

Data were analysed using Prism (GraphPad Software, LA Jolla, CA). The penguin diaries were scanned and uploaded to a digital format and made into PDFs for their respective years. Then, the data, such as the start and end of the moult, were extracted from this, and a day number was assigned. This was done by associating the 1st of April = day 1 and the 31st of March = 365/366 (if a leap year is used only for 2016 and 2020). We decided on starting the year in April since that is when the little penguins begin their annual cycle. For the sex comparison, we use an unpaired t-test to determine any significance in the start and end of moult dates between females and males. Simple linear regression was used to see if there was a relationship between the last egg laid for the season and start of moult, it was also used to see if there was a relationship with the time between last egg laid for the season and start of moult, and also the duration of moult and age of the bird. Data are presented as individual values or as mean ± S.E.

5.3 Results

5.3.1 Timing of moult

Females

In this study, the mean start of moult in the female captive little penguins was 270.23 (December) ± 5.68 days (n = 53). The females' start dates ranged from 191 days (October) to 320 days (February). The mean end of moult for these penguins was 288.56 (January) ± 4.11 days (n = 41). The females' end dates ranged from 213 days (October) to 336 days (March). Table 5.1 and table 5.2 show the individual birds mean start and end dates respectively, and Fig 5.1 shows us visually the start and end dates of the female birds' moult.

Table 5.1. The mean and SE of the individual start dates for female birds at the National Aqaurium from 2013 to 2021.

Start of moult					
	Mean*	S.E.	*n*	Min (days)	Max (days)
Betty	272.00	9.70	4	244	286
Dora	298.33	8.09	3	284	312
Draco	273.67	4.91	3	265	282
Elmo	241.75	11.30	4	222	274
Flip	274.20	3.73	5	263	286
Gonzo	277.50	13.50	2	264	291
Jack	303.75	4.80	4	297	318
Lulu	284.00	5.77	3	274	294
Miley	216.67	13.22	3	191	235
Pepper	266.75	7.74	4	244	277
Pippa	301.00	0.00	1	301	301

**Day 1 = 1st of April and day 365/366 = 31st of March. Since the annual penguin year begins in April we run the dates from 1st of April to 31st of March*

Table 5.2. The mean and SE of individual end dates for female birds at the National Aquarium from 2013-2021.

End of moult					
	Mean*	S.E.	*n*	Min (days)	Max (days)
Betty	286.25	7.85	4	263	297
Dora	313.33	8.76	3	297	327
Draco	287.33	5.24	3	279	297
Elmo	256.00	10.52	4	238	286
Flip	288.20	3.53	5	280	300
Gonzo	301.50	15.50	2	286	317
Jack	323.25	4.39	4	317	336
Lulu	298.33	6.69	3	286	309
Miley	233.67	10.73	3	213	249
Pepper	281.75	6.34	4	263	291
Pippa	318.00	0.00	1	318	318

**Day 1 = 1st of April and day 365/366 = 31st of March. Since the annual penguin year begins in April we run the dates from 1st of April to 31st of March*

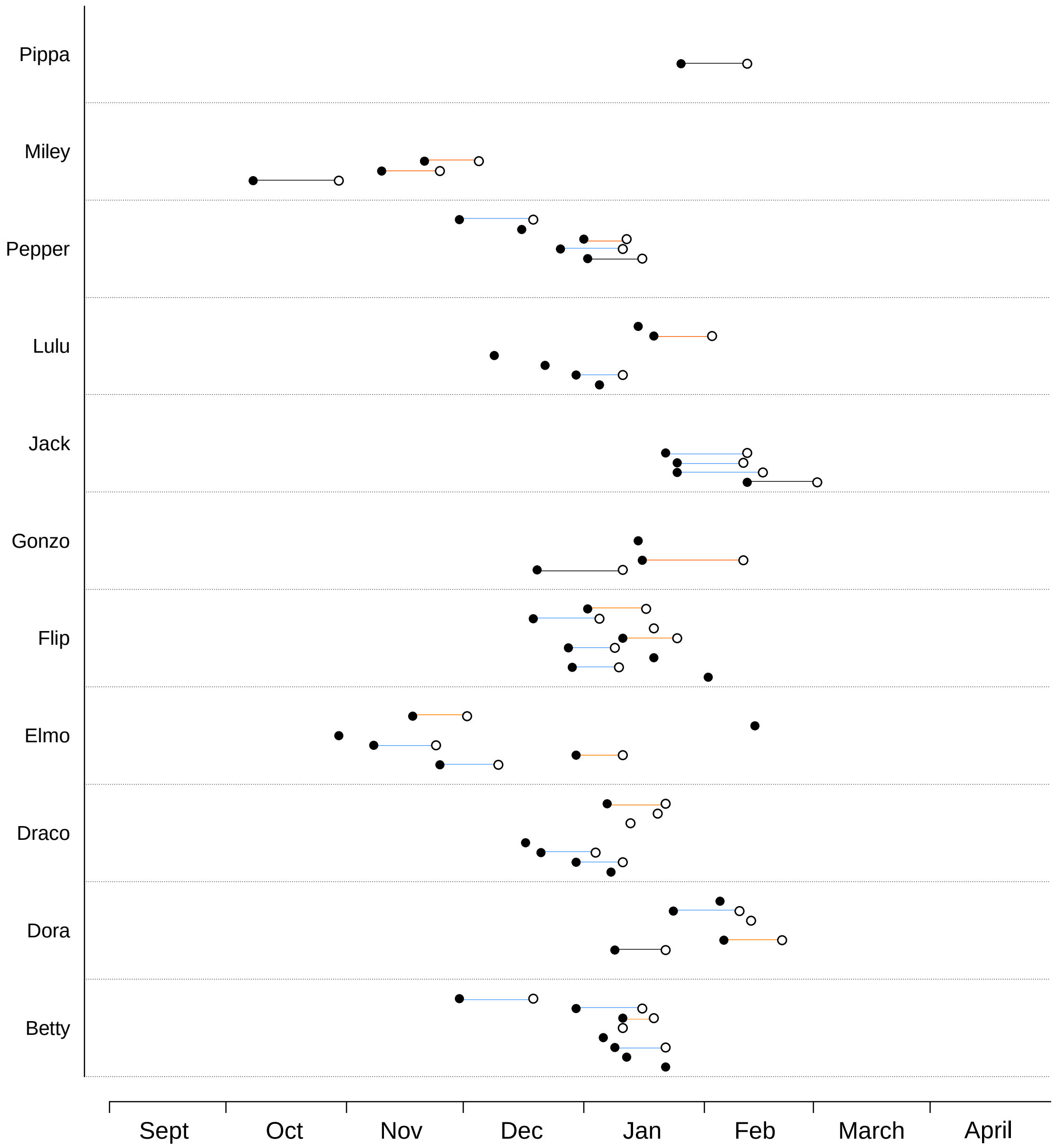

Fig 5.1. Start of moult (n = 53) and end of moult (n = 41) of all female birds at the National Aquarium, Napier from 2013-2021. ● – represents the start of moult and ○ represents the end of moult. The data progresses in an upward manner from 2013 to 2021. A black line represent the first moult duration observed. A blue line represents if the onset of moult was early and the orange line represents if the onset of moult was late from year to year.

Males

In this study, the mean start of moult in the captive little penguins is 268.26 (December) ± 4.03 days (n = 41). The males' start dates ranged from 198 days (October) to 325 days (February). The mean end of moult for these penguins was 285.14 (January) ± 4.28 days (n = 29). The males' end dates ranged from 210 days (October) to 339 days (March). Individual means for the birds' start and end dates can be found in Table 5.3 and table 5.4, and Fig 5.2 shows us visually the start and end dates of the male birds' moult.

Table 5.3 The mean and SE of the individual start dates for male birds at the National Aquarium from 2013-2021.

Start of moult					
	Mean*	S.E.	*n*	Min (days)	Max (days)
Captain	268.50	5.07	4	254	276
Dave	275.50	6.50	2	269	282
Gordon	261.50	63.50	2	198	325
Mr Mac	270.25	1.25	4	267	273
Mo	281.00	3.53	6	271	294
Pacino	284.33	19.84	3	264	324
Timmy	264.67	0.88	3	263	266
Tux	253.25	4.27	4	244	261

**Day 1 = 1st of April and day 365/366 = 31st of March. Since the annual penguin year begins in April we run the dates from 1st of April to 31st of March*

Table 5.4 The mean and SE of the individual end dates for male birds at the National Aquarium from 2013-2021.

End of moult					
	Mean*	S.E.	*n*	Min (days)	Max (days)
Captain	284.50	3.30	4	276	292
Dave	290.00	5.00	2	285	295
Gordon	274.50	64.50	2	210	339
Mr Mac	285.50	1.66	4	281	288
Mo	297.17	3.75	6	286	310
Pacino	299.33	19.84	3	279	339
Timmy	280.00	0.00	3	280	280
Tux	269.00	3.83	4	263	279

**Day 1 = 1st of April and day 365/366 = 31st of March. Since the annual penguin year begins in April we run the dates from 1st of April to 31st of March*

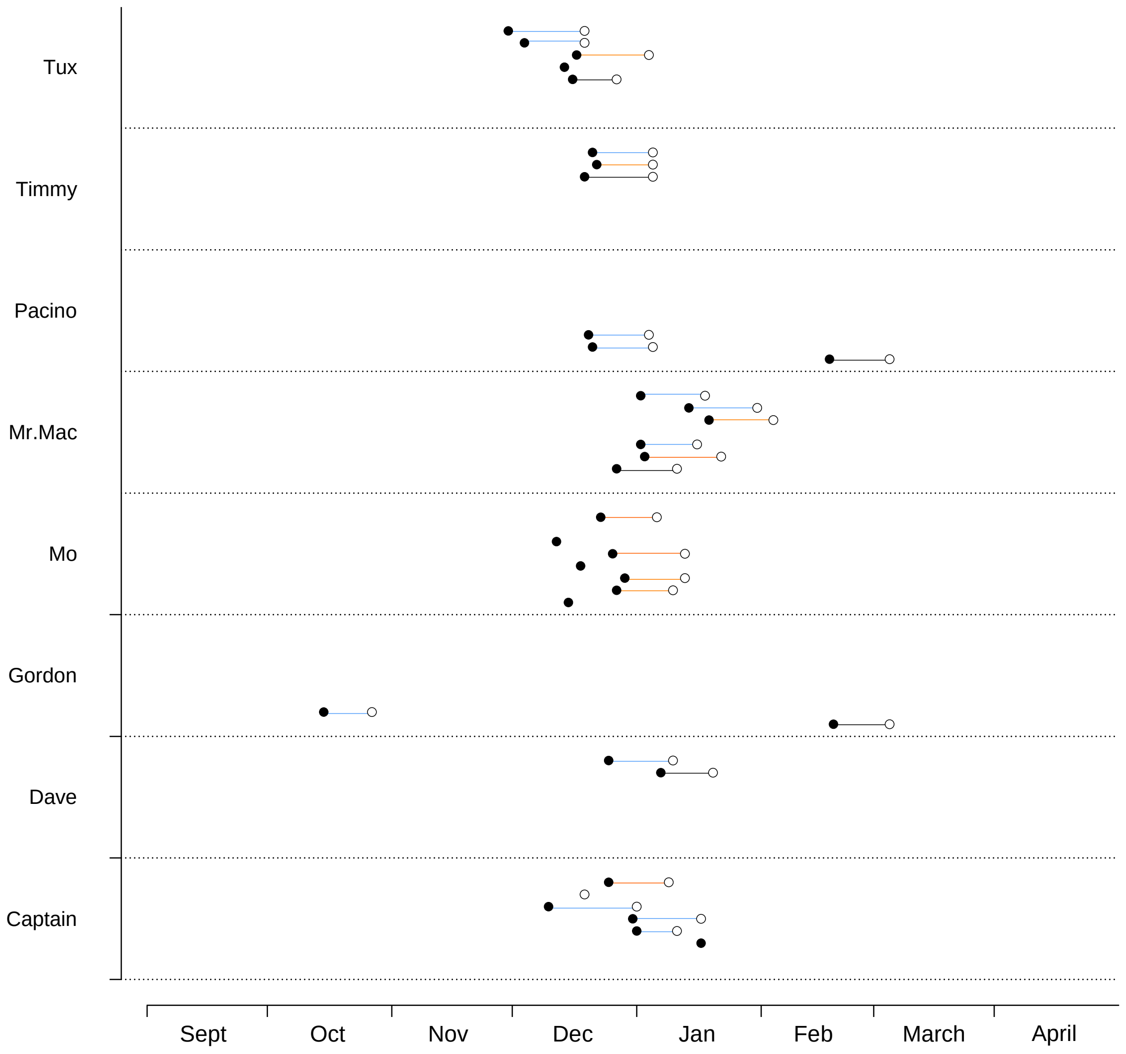

Fig 5.2. Start of moult (n = 41) and end of moult (n = 29) of all male birds at the National Aquarium, Napier from 2013-2021. ● – represents the start of the moult, and ○ represents the end of the moult. The data progresses in an upward manner from 2013 to 2021. A black line represent the first moult duration observed in the study. A blue line represents if the onset of moult was early and the orange line represents if the onset of moult was late from year to year.

Female and male comparison

Both male and female little penguins in this study show similar starting and ending dates for moult. There is an outlier with Gordon, who started his moult in February 2013 and ended it in March; the following year, in 2014, he started his moult early in October and ended it in October after 12 days. However, the males still exhibit more consistency in their range of moult as compared to the females, who are not as consistent (Fig 5.3). Using an unpaired t-test, neither sex showed significance in their start of moult dates (t_{83}=0.8509, p=0.3973) and the end of moult dates (t_{68}=0.5637, p=0.5748).

Table 5.5 shows the mean and S.E. of the start and end dates for female and male birds at the National Aquarium from 2013 to 2021.

	Mean*	S.E.	*n*	Min (days)	Max (days)
Start of moult					
Females	274.5	3.8	52	191	320
Males	269.8	3.8	33	198	325
End of moult					
Females	288.6	4.1	41	213	336
Males	285.1	4.2	29	210	339

**Day 1 = 1st of April and day 365/366 = 31st of March. Since the annual penguin year begins in April, we run the dates from the 1st of April to the 31st of March*

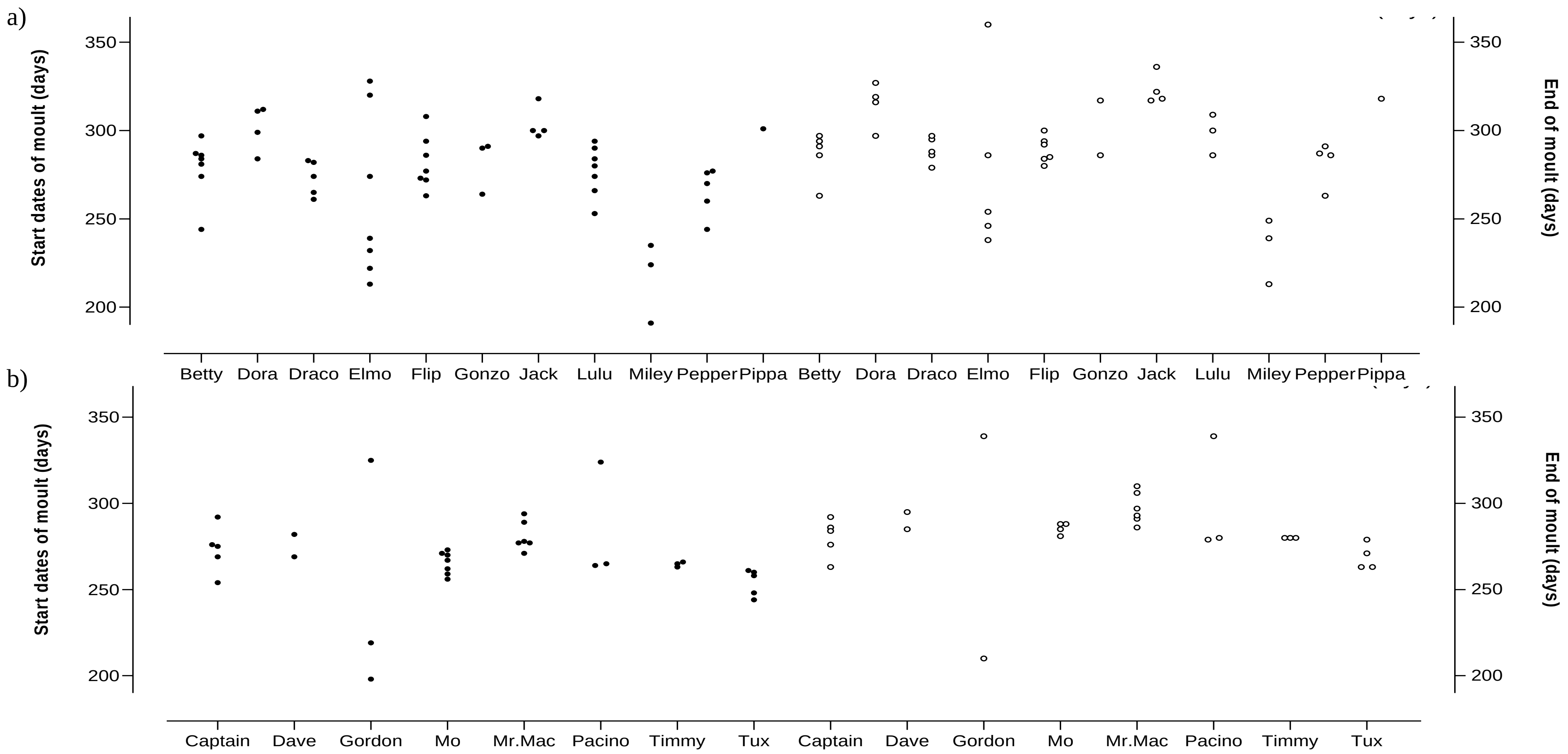

Fig 5.3. a) Scatter dot plot of female captive little penguins start and end dates from the National Aquarium, Napier. b) Scatter dot plot of male captive little penguins start and end dates from the National Aquarium, Napier. Day 1 = 1st of April and day 365/366. ● – represents the start of the moult, and ○ represents the end of the moult.

5.3.3 Duration of moult

Females

The mean moult duration in the female captive little penguins was 15.75 ± SE 0.61 days (n = 36) and ranged from 8 to 26 days. The individual duration of the moult is represented in Table 5.6 below, and Fig 5.4 visualize the duration of the moult for the individual birds.

Table 5.6 The individual mean and SE of the duration of moult for the female birds.

	Mean	SE	n	Min	Max
Betty	14.25	2.43	4	8	19
Dora	15.00	1.15	3	13	17
Draco	13.67	0.88	3	12	15
Elmo	14.25	0.85	4	12	16
Flip	14.00	0.95	5	12	17
Gonzo	24.00	2.00	2	22	26
Jack	19.50	1.19	4	17	22
Lulu	14.33	1.20	3	12	16
Miley	17.00	2.52	3	14	22
Pepper	15.00	1.68	4	11	19
Pippa	17.00	0.00	1	17	17

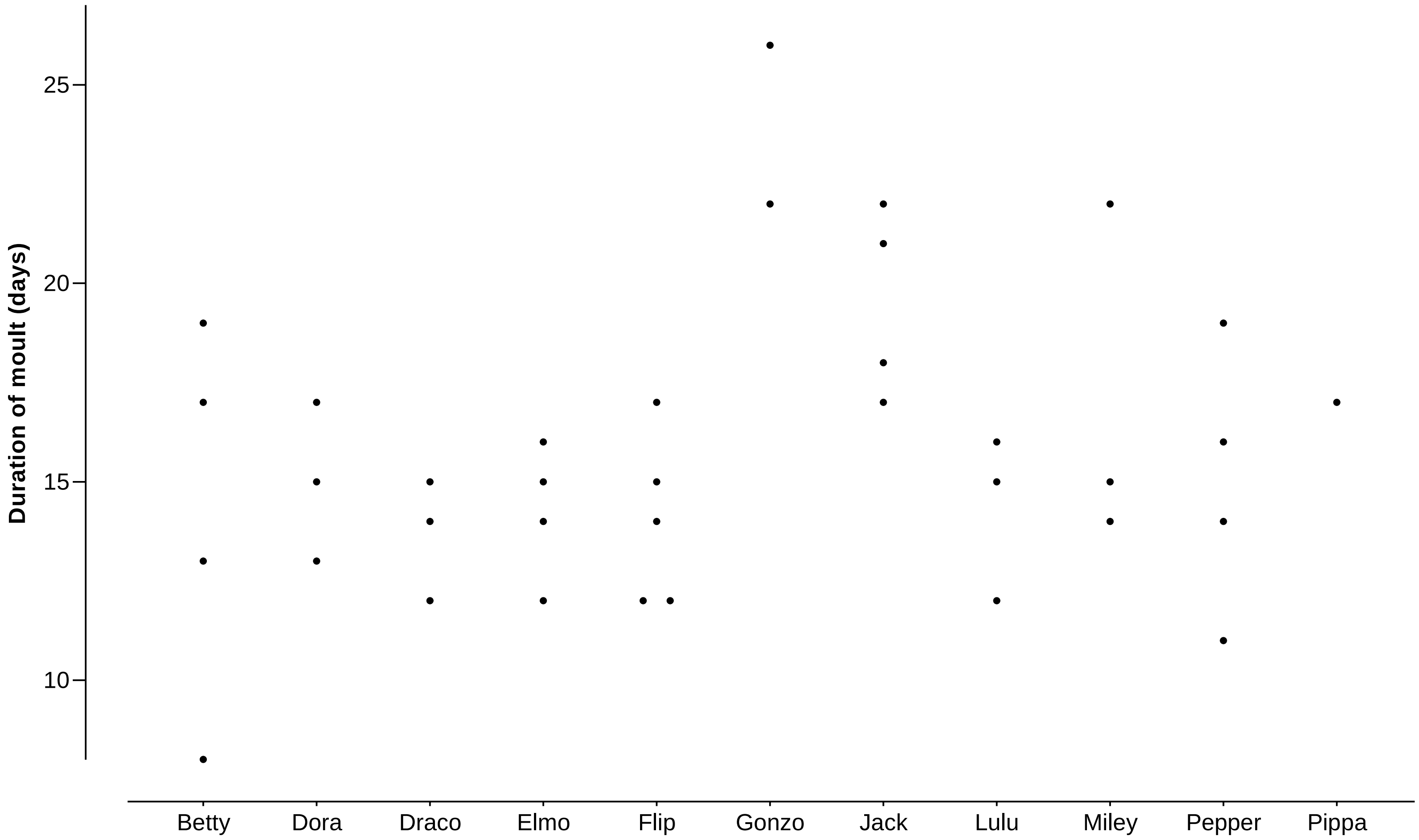

Fig 5.4. Scatter dot plot of the duration of moult for the captive female little penguins at the National Aquarium, Napier from 2013 – 2021.

Males

The mean moult duration for the male birds in this study was 15.39 ± SE 0.47 days (n = 28) and ranged from 10 to 22 days. The individual duration of the moult is represented in Table 5.5 below, and Fig 5.7 visualize the duration of the moult for the individual birds.

Table 5.7. The individual mean and SE of the duration of moult for the male birds

	Mean (days)	S.E.	*n*	Min (days)	Max (days)
Captain	16.00	2.48	4	10	22
Dave	14.50	1.50	2	13	16
Gordon	13.00	1.00	2	12	14
Mo	15.25	0.95	4	14	18
Mr. Mac	16.17	0.70	6	14	19
Pacino	15.00	0.00	3	15	15
Timmy	15.33	1.20	3	14	17
Tux	15.75	1.53	4	11	19

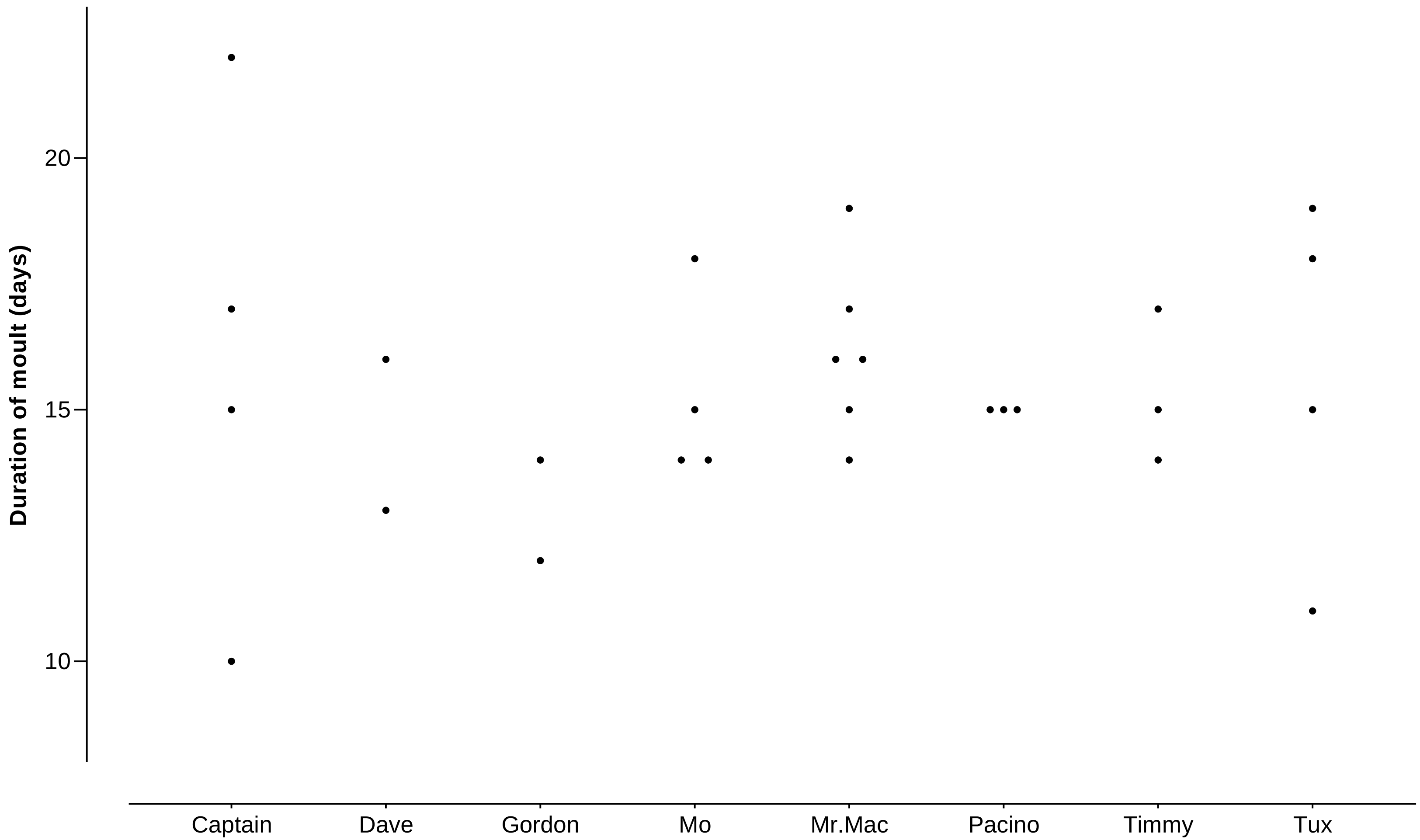

Fig 5.5. Scatter dot plot of the duration of moult for the captive male little penguins at the National Aquarium, Napier from 2013 – 2021.

5.3.3 Moult and body weight

The birds increased their body weight as they approached their moult, and once they reached their target weight, they began their moult in which they were not fed for the duration of their moult. The pattern observed in Fig 5.6 shows us the bird's weight increasing as they approach their moult and it peaks at day = 0 and then their weight starts to decrease as their moult progresses and once the moult is completed we can see their weight increasing as they start eating again. The birds' mean weight before the start of the moult was 1034.13 ± 24.54 g (n = 79); during the moult it was 1256.08 ± 39.68 g (n = 36), and after the end of the moult, it was 932.95 ± 17.37 g (n = 60). The highest weight observed was Dave at 1706 g just four days before his moult started and Captain at 1702 g just one day after his moult. We also observed Betty, who had a lower weight of 779 g just 4 days before the start of her moult, but due to not having the data for her weight during the moult, it is unsure how much her weight peaked during the moult.

Looking at just their body weight the scatterplot is very dispersed and it does show us a an upward trend as the birds approach their moult and a decrease in body weight as they start their moult but the association looks weak. So, we look at the % of mean weight with the start of moult in Fig 5.7. This scatterplot is less dispersed and shows us a more defined upward trend as the birds approach their moult and a downward trend as they finish up their moult. The bird's mean body weight (% of mean weight) before the start of the moult was 104.15 ± 1.68 (n = 79); during the moult, 127.93 ± 3.32 (n = 36), and after the end of the moult, 93.03 ± 0.86 (n = 60). Pepper reached 170% of her mean weight when she started her moult which was the highest observed. We also observed two outliers in 2021, Betty and Tux, who were only 100.13% and 94.22%, respectively, after two days from starting their moult.

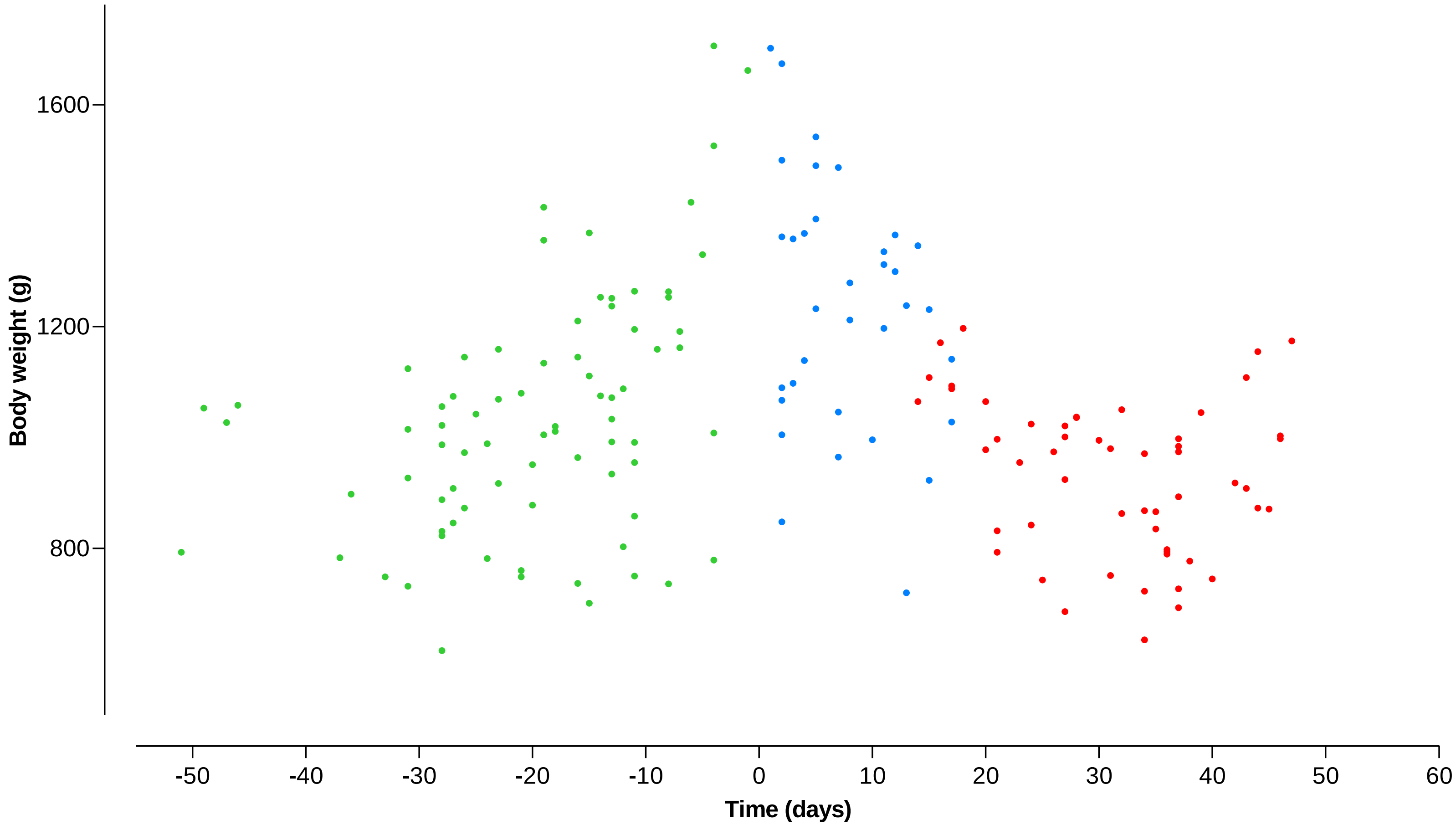

Fig 5.6. Body weight (g) v time since the start of moult where -ve = days before the start of moult, 0 = moult start date, and +ve = days after a moult. ● – represents the last body weight (g) before the moult started, ● – represents the body weight (g) during the moult, and ● – represents the first body weight after the moult ended.

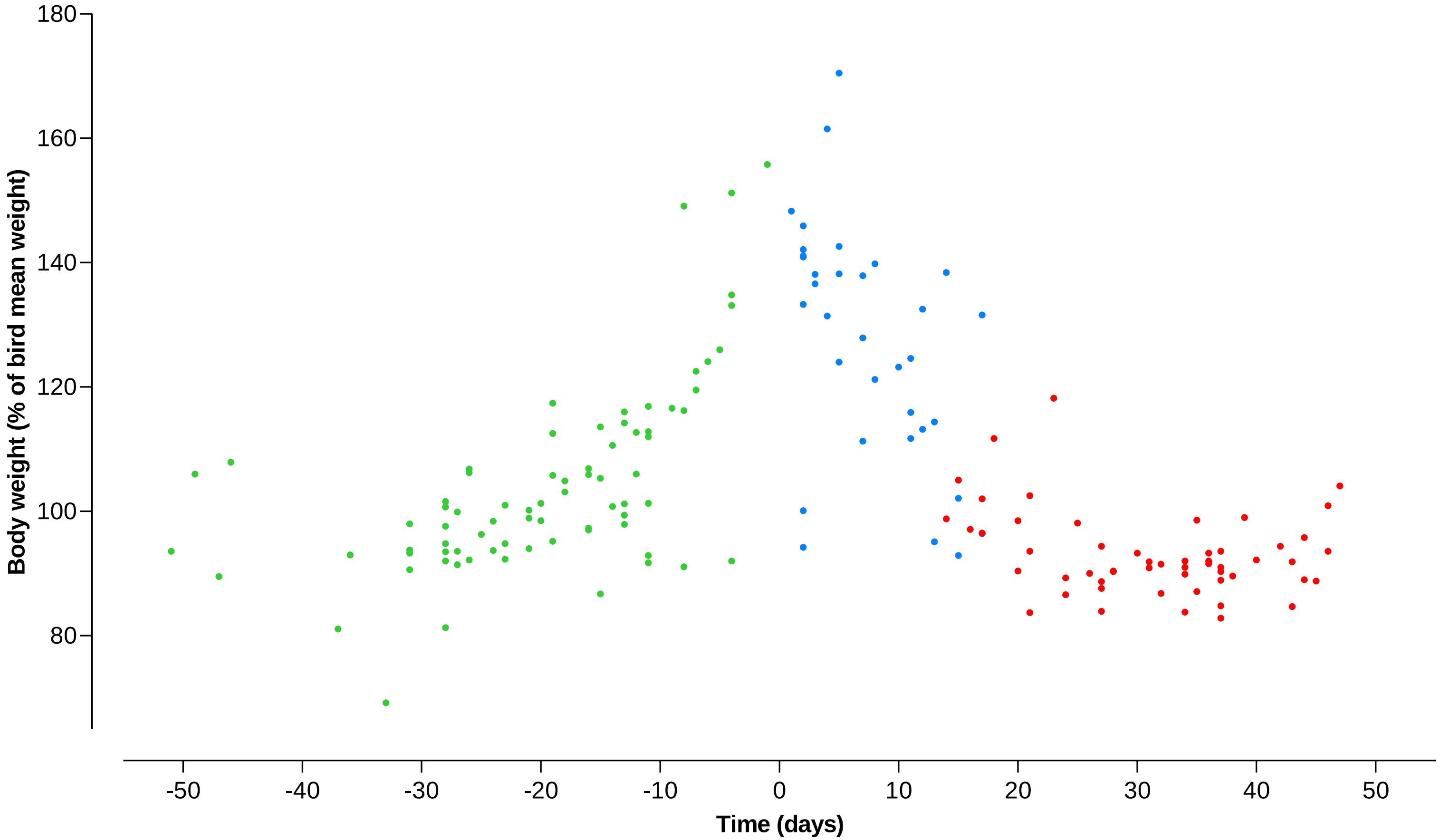

Fig 5.7. Body weight (% of mean weight) v time since the start of moult where -ve = days before the start of moult, 0 = moult start date, and +ve = days after the moult. ● – represents the last body weight (% of birds mean bwt) before the moult started, ● – represents the body weight (% of birds mean bwt) during the moult, and ● – represents the first body weight (% of birds mean bwt) after the moult ended.

5.3.4 Timing of moult and timing of egg laying

As discussed in section 5.3.1, female birds were not as consistent in their start of moult as compared to the males. This may result from their breeding; if they lay eggs early, they may end their breeding season early and begin their moult. However, it was observed that in most cases, the birds started their moult around December-January, regardless of egg laying. For example, in Fig 5.8, Elmo 2014 laid her eggs in September and started her moult in late November. Then, the following year, she laid earlier in late August and started her moult later in December. A similar case is Betty; in 2014, she started her egg laying later in the year in October and began her moult in late January. In 2018, she laid much earlier in September but only started her moult in January. Running a simple linear regression (Fig 5.9) for the date of last egg laid in the season and the start date for moult showed a significant relationship (r_s=0.5573, p<0.0001). As the birds laid their last egg for the season earlier their moult started earlier. We also ran a simple linear regression for the time between the last egg laid for the season and the start of moult (in days) versus date of last egg laid for the season and found a significant relationship (r_s=0.7280, p<0.0001).

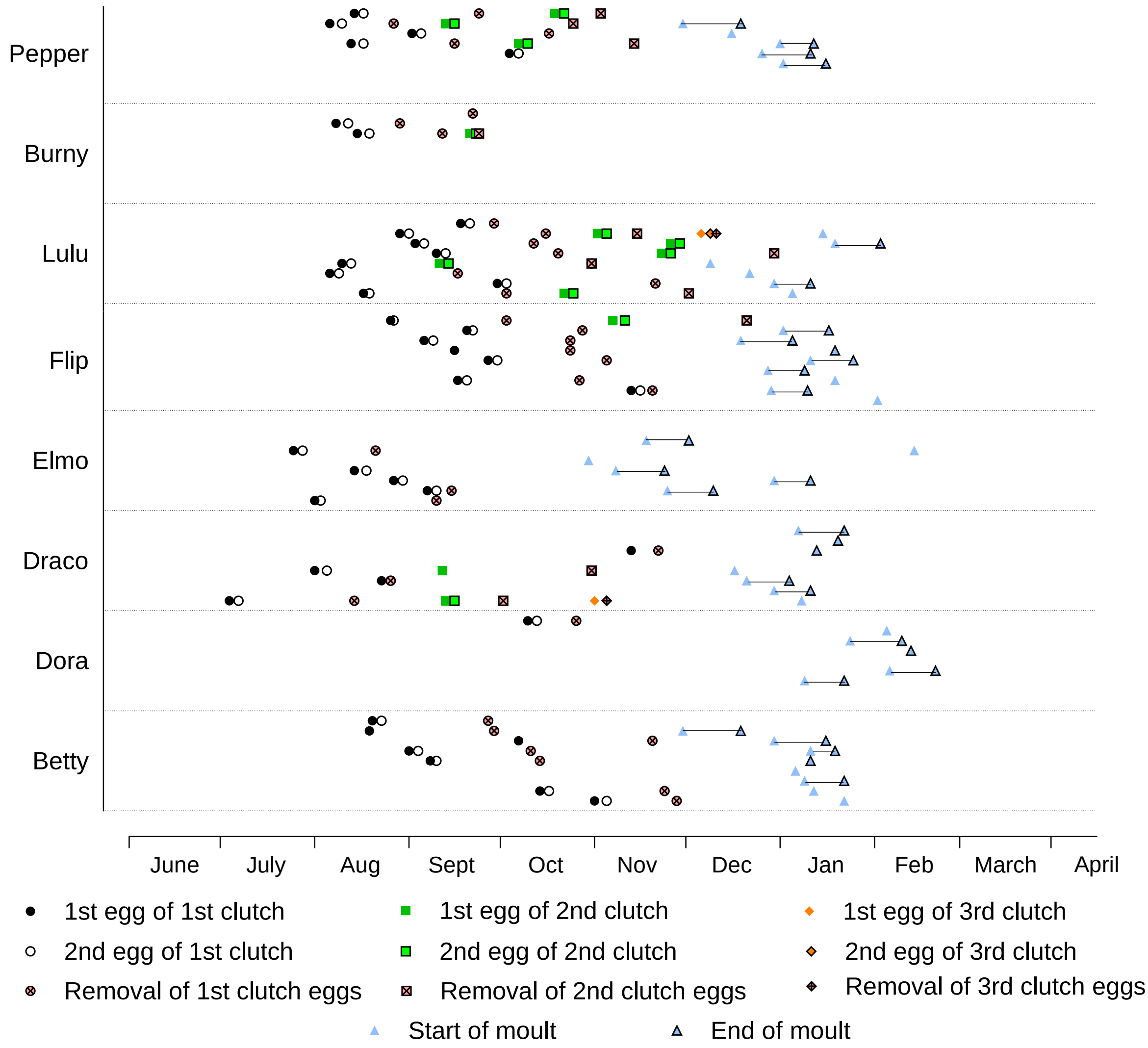

Fig 5.8. Egg laying dates (1st, 2nd and 3rd clutch) and moult start and end dates of female birds at the National Aquarium, Napier, from 2013-2021.

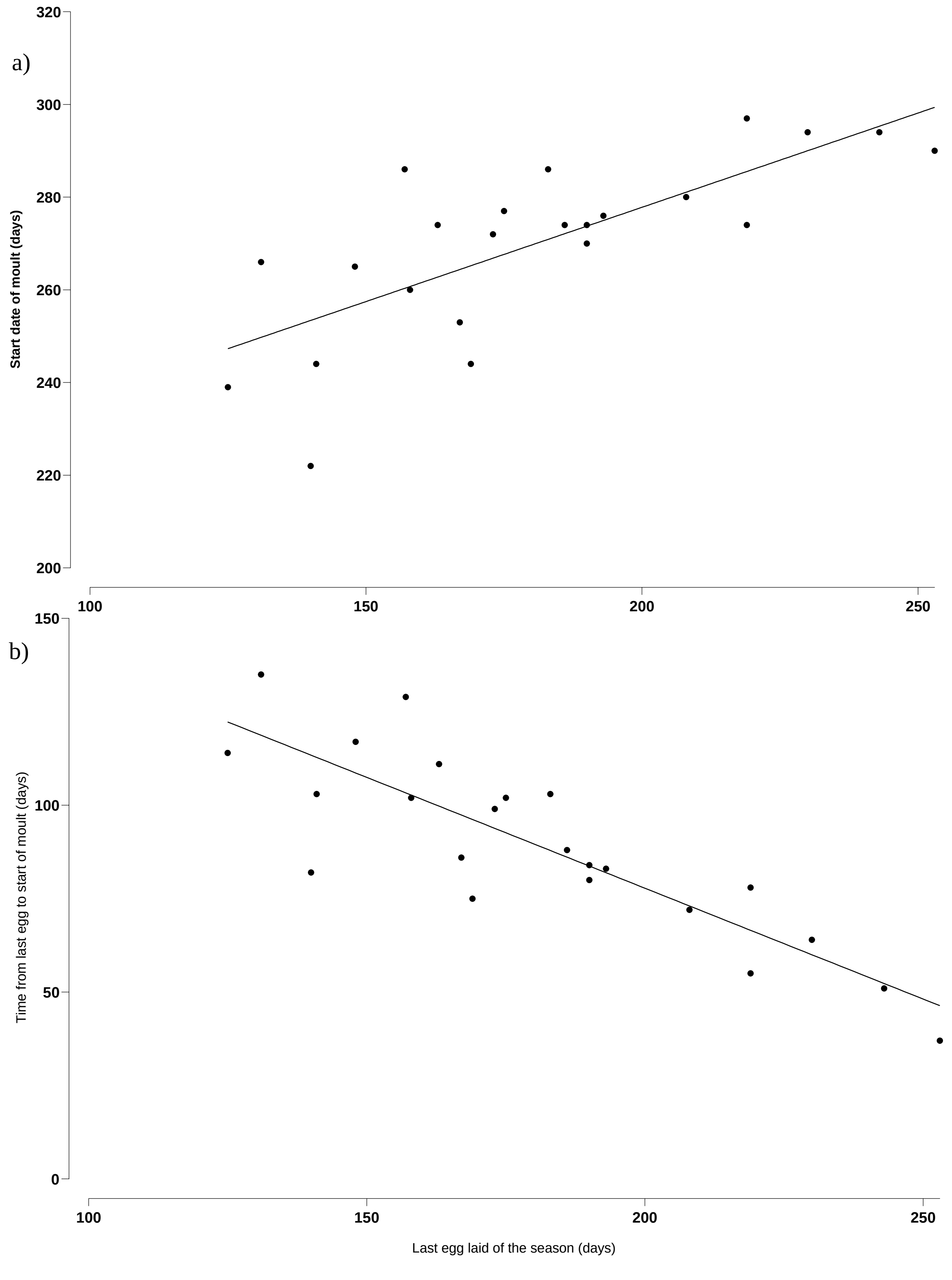

Fig 5.9. a) Start of moult (days) vs last egg laid of the season (days). b) Time from last egg laid to start of moult (days) vs last egg laid of the season (days).

5.3.5 Duration of moult with age

A simple linear regression was used to test the relationship between age and duration of moult for all female birds. The overall regression was statistically not significant for all female birds (r_s=0.08, p=0.0946) (Fig 5.10)

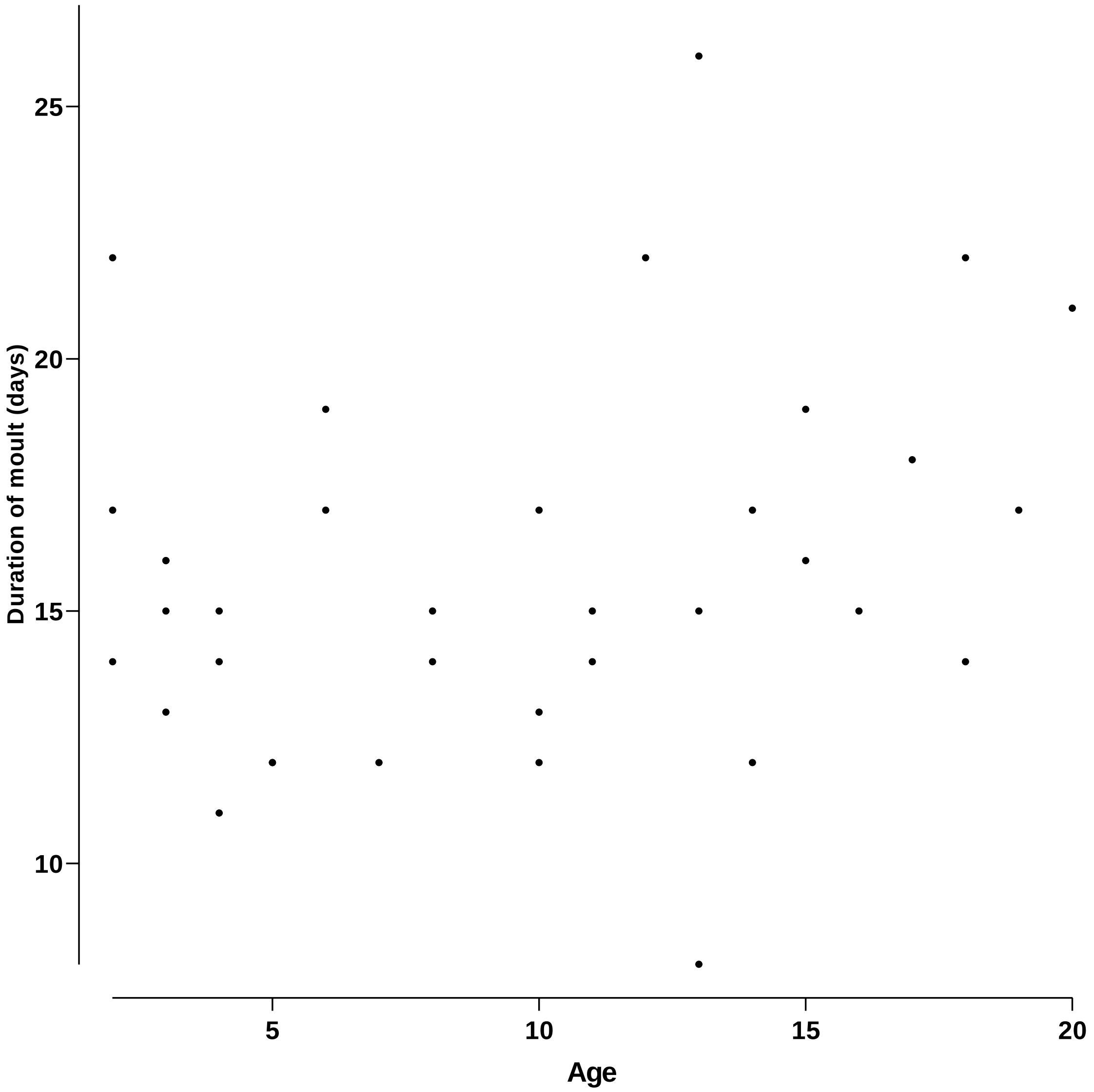

Fig 5.10. Duration of moult of female kororā vs their age.

A simple linear regression was used to test the relationship between age and duration of moult for all male birds. The overall regression was statistically not significant for all male birds (r_s=0.07, p=0.1870) (Fig 5.11)

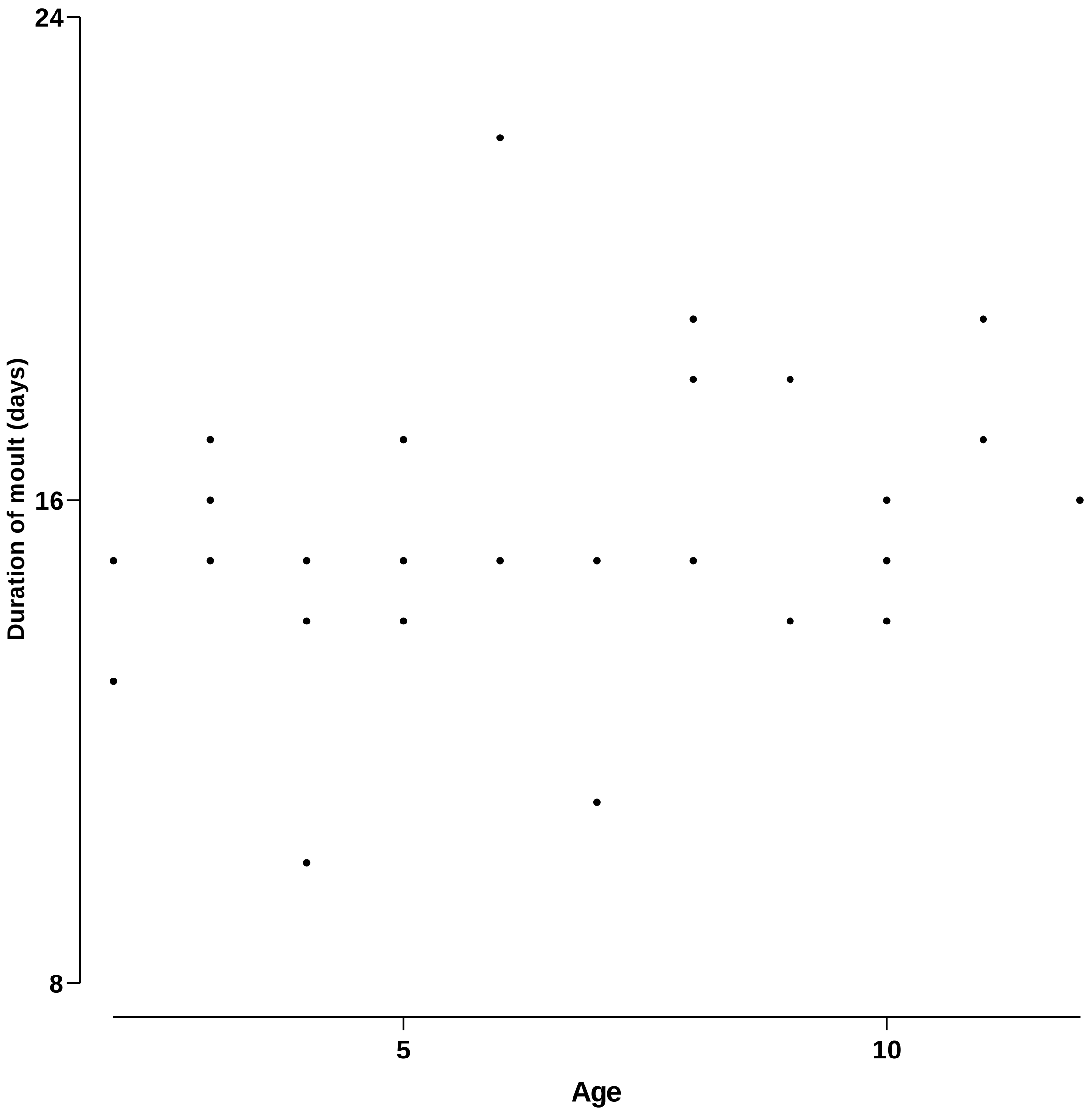

Fig 5.11. Duration of moult of male kororā vs their age.

5.4 Discussion

The little penguins at the National Aquarium in Napier also undergo a full-body moult annually. They also seem to follow a similar moult timing pattern compared to their wild counterparts. During the moulting part of their annual cycle, the penguin keepers increase their diet 20-30% and allow them to eat more than their maximum if they seem “very hungry”. A lot of this increase in their diet occurs in December, and the onset of moult was observed in many birds in January with some birds going through their moult as late as March. The birds are seen to increase an average of 120% of their mean body weight when they start to moult, and we also have one bird (Pepper), who reached 170% of her mean weight during one of her moults.

In the wild, timing of moult occurs from December to March in New Zealand (Kinsky, 1960; Department of Conservation, 2023; New Zealand Birds Online, 2023) and in this study of captive birds ranged from October to March, with a mean from December to January for both male and female birds. Male birds show more consistency with their timing of moult each year. However, there were some outliers including Gordon, who began moulting in February 2013 and was observed moulting in October 2014, but only had two moulting events before he died, and Pacino, who began moulting in February 2014, but in 2015 and in 2016 he started moulting in December. For the female outlier, Elmo, who began moulting in November in 2014, but in 2018 she started her moult in February. The duration of moult is around 2 to 3 weeks for New Zealand birds (Gales *et al.*, 1988b; Marchant and Higgins, 1990a; New Zealand Birds Online, 2023). This study records the average duration of moult at 15 days for both female and male birds but can range from 8 to 26 days for females and 10 to 22 days for males.

We observed in this study that female penguins were not as consistent in their start of moult compared to the males and wanted to see if timing of egg laying played a role in start of moult. Moult occurs at a relatively fixed time every year for little penguins and in this study it was observed during October to March. There are cases depending on the breeding season where if the birds laid their eggs late, they would start moult later but, there are also cases like Elmo who have instances where she laid early but still moulted later. Reilly and Cullen (1983), also observed that the timing of moult was less variable and only ranged within two weeks for each season while start of egg laying ranged from two months for each season. They concluded that there was an urgency to start moult since it was observed that the breeding season that started later was shorter and stopped when it was time to moult. In the case of the captive birds in this study, there is a pattern of when they stop their egg laying to start their moult but, in most cases the birds do not raise chicks and also do not have to forage to gain the weight needed to have a successful moult. Therefore, we do not see any significant pattern when it comes to timing of egg laying and start of moult but there are individual differences.

When we look at all the bird's duration of moult versus their age it is statistically significant but, looking at male and female separately it is not. One question brought up was whether the older birds moult for longer but there may be a season or two where a bird may moult for longer but both sexes were seen to average a moult duration of 15 days. Overall, when combined older birds moulted for longer but when we split them into their sexes, we don't observe any correlation between age and duration of moult in males and females. Female birds moult varied from year to year in terms of their onset as well as duration more than males and the oldest birds in this study from 2013 were Elmo, Gonzo and Jack who were all over 10 years old and both Gonzo and Jack had moult durations that were over 20 days. But, we also have Captain who was around 6 years of age when he moulted for 22 days. There may have been other factors which need to be explored besides age that may have played a role in determining how long the birds in this captive study took to moult. Since the birds in this study are not in danger of being starved if they do not finish their moult in those two weeks, we see individual variation with birds moulting for either longer periods or shorter periods some seasons.

6. General discussion and conclusions

This study focused on the little penguins at the National Aquarium, Napier from 2013 to 2021. There have been other studies conducted on captive little penguins, but it involved more the impact of a zoo setting or a breeding management (Dunn, 1986; Chiew *et al.*, 2019; Chiew *et al.*, 2022). A lot of zoos and aquariums that house these birds keep records of important dates such as egg laying and moult, but they also have body weight data for their records to ensure their birds are healthy. I wanted to use this data that has been meticulously collected over several years to help understand whether the little penguins in captivity were still undergoing their annual cycle and if there were any changes to timing of egg laying or moulting. There is an understanding that there are other external factors that can play into the annual cycle of wild little penguins, one important factor is changes to prey availability causing them to forage longer and farther away leading to poorer breeding outcomes (Bastille-Rousseau *et al.*, 2018; Cavallo *et al.*, 2020). Other factors are changes to sea surface temperatures, chlorophyll-a, human disturbances, predators etc (Mickelson *et al.*, 1991; Agnew *et al.*, 2015; Berlincourt and Arnould, 2015; Cleeve, 2017; Robson, 2018). But in this instance of captivity, we see that these penguins are fed three times a day, all have ample housing and space. They were a very small colony of birds ranging from 14 to 17 birds from 2013 to 2021. These birds in captivity also experience the day/night cycle similar to the wild which also plays a part in understanding the timing of their annual cycle (Cockrem, 1995b).

6.1 Body weight, condition index and food intake

In the wild, the use of body condition (Cairns, 1988; Monaghan, 1996), breeding success (Cairns, 1988) and even egg laying dates (Monaghan, 1996) have been used as indicators for food availability. Studies that look at body condition of wild birds have found that there is an increase prior to the breeding season i.e., in preparation. Robinson *et al.* (2005), found that their birds in 2000 increased their body condition into incubation and suggested that this increase may be due to the food supply being lower in the following year when they were comparing. In this study we found that both male and female birds showed a distinct cyclic pattern of annual fluctuations in their body weight. The female birds were smaller than the male birds and as they went through the breeding season the female birds had their “maximum” cap of fish removed while the males did not. We see more variation with their changes in weight from the months of August to November compared to the males. But in both cases male and females body weights increased as they started their moult. In this study we had female birds that were increasing over 1300 g and male birds increasing over 1500 g as they approached their moult. This result is similar to that of the little penguins studied by Evans *et al.* (2015). Johannesen *et al.* (2002), saw their birds increase over 1200 g as they approached their moult and this increase is similar to Otago birds being studied by Gales (1987). Looking at the non-moulting period the birds in this study the females had a mean weight of 900 g and the male birds were around 1100 g. Finger *et al.* (2016), did not determine the sex between the birds they were studying but the compared two different colonies and the birds at St. Kilda had a mean weight around 1200 g while the Phillip Island birds sat at 1120 g during the non-moulting period. Gales and Green (1990), found that these birds spent a lot of their energy budget during the breeding season, from courtship to chick fledging they use up 44.8% of their annual energy budget.

In the wild, food availability plays an important role in little penguins breeding and moulting outcomes for the season. These birds are considered generalist feeders (Gales and Pemberton, 1990) and eat a variety of prey items. Studies looking at their diet found that pilchards (*Sardinops sagax)* and anchovies (*Engraulis australis)* constitute the majority of their diets (Montague and Cullen, 1985; Cullen *et al.*, 1992) and some studies even attributed to an increase in pilchards availability to be the catalyst for the early egg laying in that season (Cullen *et al.*, 2009). In 1995, Dann *et al.* (2000), found that the predominant cause of death for first-year little penguins was the widespread mortality of pilchards which is one of the little penguins prey species (Griffin *et al.*, 1997; Whittington *et al.*, 1997) and it started in April and then more followed during late June and July.

This loss of prey species, studies found, caused long-term population declines for the little penguins on Phillip Island, Australia. But, with our birds in captivity, they are fed three times a day and have a minimum amount of food they need to eat per week to maintain them at a healthy weight as established by the team at the aquarium. With the monthly body weight data for nine consecutive years and the fact that they do not undergo any food availability or predation pressures, we still observe these annual patterns of changes that reflect where in their annual cycle they are.

6.2. Timing of egg laying

Breeding season in this study started when egg laying started and ended either after eggs were removed or hatched which concluded the incubation period. Egg laying in this study averaged a start in September for the 1st clutch. We also have birds that produced replacements clutches which averaged a start in October for 2nd clutches and November for the two 3rd clutches. This study looked at the egg laying season for 9 years. We also noticed that some birds were laying earlier each season such as Betty, Flip and Burny. Our mean start to egg laying is similar to those observed by the wild bird colonies of Phillip Island in 2000 to 2001 (Robinson *et al.*, 2005), South Bay, Kaikoura from 2006-2019 (Rowe *et al.*, 2020), and for the London Bridge breeding colony at Port Campbell National Park in 2012 (Berlincourt and Arnould, 2015), but was a month earlier compared to the 18 year study of the Phillip Island at the Summerland Peninsula which had a mean laying in October (Nisbet and Dann, 2009) and also the birds at Gabo Island in October of 2011 and London Bridge in October of 2013 (Berlincourt and Arnould, 2015). The 19 year-long monitoring for the wild birds at the Oamaru colony from 1994-2012 saw a mean egg laying date in July which much earlier than that observed by the birds in this study (Agnew *et al.*, 2014).

The main focus of the National Aquarium is rehabilitation and is not a breeding center. But, penguin keepers will still candle each egg and check if they were fertile or not. They also documented the outcome of each egg and noted if any eggs were abandoned, broken, replaced with dummy eggs or incubated naturally. So, due to all of these different circumstances that occur in captivity this study looked at focusing on eggs that were incubated naturally for ≥ 32 days as that is the average incubation period (Department of Conservation, 2023) for little penguin eggs. We had a total of 129 eggs laid for the 9 years and of those eggs 46 of them were naturally incubated for more than 32 days. 23 of those eggs hatched and they had an average incubation period of 37 days. This is similar to other studies that looked at incubation periods of wild colonies. Our average incubation period in a day higher than that of the Summerland Peninsula colony birds of Australia (Chiaradia and Kerry, 1999; Kemp and Dann, 2001a) and the Tiritiri Matangi Island colony in New Zealand (Boyer, 2010). Chiaradia and Kerry (1999), found that their birds had shorter incubation spells compared to previous studies done on the Phillip Island birds of Australia (Reilly and Cullen, 1981) and attributed some of the wide range of 31-40 days in the incubation period to interrupted incubation. They had nests that were left unattended for one to six days. Kemp and Dann (2001a), birds showed an incubation range of 33 – 44 days. The variation in incubation period may be due to foraging trips need to be coordinated by the parents so has to have one parent present when the chick hatches and depending on food availability the bird may spend a longer time foraging which could lead to the incubating partner also leaving the nest to forage (Agnew *et al.*, 2015). But, our birds in captivity do not experience any food availability issues nor do they have to forage for their food. The keepers also check on the bird that is incubating and if needed may feed them in the nest. But, the birds in this study also showed a range of incubation period from 34 - 41 days. Internal (hormonal) and external (environment) may play a role in determining how long these birds incubate their eggs for. The onset of moult followed after the end of the breeding season. For the birds in this study, the end of the breeding season was either due to eggs being removed due to various circumstances (abandonment, kicked out of the nest, or replaced with dummy eggs) or after being incubated and hatched till the chicks were either removed or introduced into the colony. The birds in this study do not experience a full chick-rearing to fully fledge phase as these chicks are captive born.

6.3. Timing of moult

When we look at the timing of moult in this study it ranges from October to March for both female and male birds, though males seem to start and end their moult earlier than females by 3 to 4 days. This maybe due to not having the same breeding costs involved with having to forage for both themselves and their chicks. Reilly and Cullen (1983), gathered data on the colony at Phillip Island, Australia from August 1968 to June 1971 and their birds moulted from January to March. They also observed that overall timing of moult was relatively fixed to the range, individuals moult start dates were affected by the end of their parental responsibilities. In this study we also found that the male birds were more consistent in their start and end of moult than females, looking at when the females laid their last egg of the season showed that it affected when they started their moult. Evans *et al.* (2015), found that their birds spent 3-4 weeks fasting on land during the moult period, and found their birds were significantly heavier during the start of moult than any other phases and the lowest at the end of moult. Our study also observed that as birds were approaching their moult their body weight increased significantly and most of our birds were averaging 128% of their mean body weight and by the end of their moult, they dropped to 98% of their mean body weight. During the time our birds are moulting they are not fed and undergo a fasting period. The duration of the little penguin's moult is around 16-18 days (Reilly and Cullen, 1983; Gales *et al.*, 1988b) and in this study the females averaged 16 days and the males average 15 days. We also found that age did not play a factor in the duration of their moult for the birds in this study.

The birds in this study showed us similar timings to moult and also duration of moult compared to their wild counterparts. The female birds have more parental responsibility than the males in this captive situation since neither parent has to leave the nest for too long to forage for food as they are looked after by the penguin keepers. Regardless, the keeper may still get involved in swapping the parents on incubation duty but when the female bird has laid her last egg for the season is a reason we've observed that individual females may not be as consistent in the start of their moult as males.

During the moult period though, the birds will stay in their burrow and will not be fed until their moult process is complete so to avoid any birds getting too overweight and causing health problems but also to avoid these birds from getting used to being fed on the nest.

6.4. Conclusions

Captivity provides a novel setting for research. This study had daily entries from penguin keepers about each individual bird. Using this data, we could look at the timing of egg laying, when replacement clutches occurred and the hatching success rate. We are also able to see when their moult starts and the duration. Their monthly body weight and daily feed intake data were provided. Exploring the birds' body weight, we saw annual changes occurring from 2013 to 2021. The birds would sit in a consistent body weight range from April to October, but from October to January, we see their body weight increase, peaking in January, and then we see a decline in body weight from January to February. They then start to increase their weight from February to March. This is attributed to the birds approaching their moult. When we look at sex differences, we do see female birds increasing and decreasing their weight as the breeding season is going on but then peaking in January along with the males during moult. So, yes, we do see annual changes in the captive little penguin's body weight. From the daily feed intake data provided, we can see how the bird's body weight changes with respect to an increase or decrease of food. We do see female birds increase their feed intake a month before we see the increase in their body weight. We see feed intake peak in December but start increasing from November. This increase in December is attributed to the increase in body weight in January. During January to February, we see a decrease in feed intake which is due to the birds being in a state of starvation as the aquarium does not feed them as they go through their moult.

The timing of egg laying ranged from July to December for these captive birds. Their 1st clutch eggs ranged from July to November and the replacement clutches ranged from September to November for 2nd clutches and November to December for 3rd clutches. This is similar to wild birds who are seen laying eggs from August to December. The hatching success of eggs naturally incubated for more than 32 days was 51.11% which is lower than observed in the wild. But it is important to remind ourselves that the National Aquarium is a rehabilitation center and not a breeding program. Successful eggs were incubated for a mean of 37 days which is similar to that seen in the wild.

Moult ranges from October to March for the captive little penguins in this study and averaged around 15 days for both male and female birds. Females were seen to vary more than males when it came to start of moult, and we looked at if timing of egg laying played a role. There was no observable pattern observed but there were individual cases were if a bird laid later in the year she moulted later and if the birds laid earlier, she moulted earlier but, the converse was also observed where a bird laid earlier in the year but still moulted later or laid later in the year and still moulted earlier.

In conclusion, from the results we see that the birds in captivity experience their annual cycle of egg laying and moult similarly to the birds in the wild. In this study, their timing of egg laying ranges from July to December and breeding season for the wild birds is normally seen to begin from August to December. This study has not looked at chick rearing and only focused on egg laying and incubation aspect of the birds breeding cycle. In the wild, breeding is an integral part to the survival of the colony and the species hence, we see higher hatching success. But the National Aquarium is a rehabilitation center, it houses penguins that are not strong enough to return to the wild but also helps penguins that need it and then release them back to the wild. Their primary focus is not breeding its rehabilitation. We also observed that the moult period occurs from October to March for these captive birds and averages 15 days which is also similar to that of their wild counterparts.

There is a lack of research focusing on the annual cycle patterns on little penguins in captivity and the purpose of this study was to start addressing this gap by investigating the little penguins housed at the National Aqaurium in New Zealand. These finding will hopefully provide a baseline for the comparison of biological patterns of captive little penguins across other facilities. It also seeks to facilitate communication and collaboration among other facilities that house captive little penguins to contribute to improving animal welfare outcomes.

6.5 Further research

Wild studies are important in broadening our understanding of how certain species interact with the environment around them but also helps us understand certain chain of events that occur. Studies have looked at using penguins as indicators of food supply by studying their body condition (Robinson *et al.*, 2005), egg laying (Dann, 1994; Agnew *et al.*, 2015), and reproductive success (Bull, 2000b; Cullen *et al.*, 2009). But also looking at how these birds respond to changes and their sensitivity to change should also be considered (Chiaradia and Nisbet, 2006). Another interesting research opportunity would be to look at their energy intake since we know the type of fish they eat, how much of the fish they eat and the nutrient content of their food. Wild studies have looked at the energetics of the annual cycle for the little penguins but captivity provides us with a novel circumstances to be able to conduct this type of research.

Facilities such as rehabilitation center, zoos, aquariums, etc that house little penguins most often also house many different species. Importantly, these centres record a lot of day-to-day information that they keep for their records. Captive studies in little penguins are not studied well and it is important in the context of helping us understand their wild counterparts to an extent. This study looks at opening more researchers to not only partnerships with these facilities but also providing insight into captive management.

7. Appendix

Appendix table 7.1. Mean ($\pm$ S.E.) monthly body weights of female kororā. Data are from 2013 to 2021.

	Mean	S.E.	*n*	Min	Max
April	867.7	13.7	74	647	1076
May	869.4	12.6	73	681	1101
June	895.7	12.6	73	665	1098
July	898.5	12.2	73	717	1094
August	920.2	13.9	71	702	1222
September	918.6	14.2	73	682	1210
October	936.3	15.6	71	696	1516
November	918.7	15.5	70	688	1330
December	915.0	18.4	70	616	1493
January	989.3	24.4	72	625	1500
February	903.8	17.7	73	593	1291
March	842.5	14.4	76	599	1279

Appendix table 7.2. Mean (± S.E.) monthly body weights of male kororā. Data are from 2013 to 2021.

	Mean	S.E.	*n*	Min	Max
April	1091.9	13.3	51	930	1390
May	1093.3	13.1	50	911	1308
June	1113.1	12.6	49	917	1327
July	1103.7	14.2	50	903	1403
August	1096.2	11.7	50	907	1302
September	1099.7	11.5	50	911	1253
October	1112.8	10.8	47	882	1308
November	1101.7	15.7	48	948	1526
December	1141.6	24.6	49	855	1662
January	1246.4	37.0	42	933	1714
February	1050.7	13.7	49	893	1279
March	1085.8	13.5	49	931	1335

Appendix table 7.3 Mean (± S.E.) of all female bird's monthly food intake from April to March

	Mean	S.E.	*n*	Min	Max
April	8.8	0.1	2190	0	18
May	8.9	0.1	2228	0	18
June	8.9	0.1	2190	0	18
July	9.1	0.1	2256	0	20
August	9.0	0.1	2263	0	26
September	9.1	0.1	2183	0	33
October	8.8	0.1	2244	0	42
November	8.9	0.1	2220	0	26
December	10.2	0.1	1952	0	32
January	8.2	0.1	2276	0	30
February	7.9	0.1	2118	0	24
March	8.6	0.1	2269	0	17

Appendix table 7.4. Mean (± S.E.) of all male bird's daily food intake (number of fish per day) from April to March

	Mean	S.E.	*n*	Min	Max
April	9.6	0.1	1530	0	21
May	9.5	0.1	1546	0	18
June	9.4	0.1	1500	0	20
July	9.7	0.1	1543	0	20
August	9.7	0.1	1544	0	22
September	9.6	0.1	1493	0	37
October	9.6	0.1	1550	0	36
November	10.6	0.2	1500	0	31
December	13.3	0.2	1275	0	42
January	8.9	0.1	1457	0	30
February	10.2	0.1	1383	0	23
March	9.8	0.1	1581	0	21

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