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Sexual size dimorphism in the Black Swan and an alternative to cloacal sexing

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Morphometric data from two study locations, one in South-East Queensland and one in the Australian Capital Territory, were used to establish a non-invasive technique to accurately determine sex in the Black Swan *Cygnus atratus*. Age and breeding status disparities in morphometric variables were also examined. Males were heavier and larger in all other morphometric measurements than females at both locations. There were no significant differences in morphometrics between adults and immature birds, but adult males and females were heavier than immature males and females. Presumed paired males and females were significantly heavier than presumed non-breeding birds of the same sex. Total head length and tarsus and radius lengths each allowed over 80% of birds caught to be sexed, with an accuracy rate of over 90% for birds within 'sexable' measurement ranges. Discriminant function analysis using these three predictor variables increased that accuracy further, with 92.1% of birds being correctly assigned. This approach offers an alternative to the use of cloacal examination in sexing Black Swans for researchers unfamiliar with this technique.

Keywords: Cygnus atratus; sexual dimorphism; morphometric variation.

INTRODUCTION

The Black Swan *Cygnus atratus* is a native of Australia and naturalised in New Zealand (Marchant and Higgins 1990), with introduced populations also occurring in Europe (Banks *et al*. 2008) and Japan (Brazil 2009). The species has been studied in both Australia and New Zealand, with many aspects of its population and breeding biology having been described (e.g. Braithwaite 1981; Williams 1981; Coleman 2014).

As with other members of the genus, the species is sexually size dimorphic, with males typically being larger than females in most metrics, including body mass (Frith 1982; Birkhead and Perrins 1986). Notwithstanding this sexual size dimorphism, the sexes of swan species can show significant overlap in morphometrics (Coleman and Coleman 2002) and, given the importance of assigning sex in many studies, cloacal examination has been the recommended method (Marchant and Higgins 1990; Redfern and Clark 2001).

Cloacal sexing can be difficult to perform accurately, particularly with cygnets, but is considered reliable if done correctly for fully grown birds (Bacon and Andersen-Harild 1989). However, in the Mute Swan *Cygnus olor,* Brown and Brown (2002) established that 89% of cygnets were sexed accurately using this method, but they did not evaluate its accuracy for fully grown birds. As well as being difficult, cloacal sexing is an invasive technique and training is recommended to avoid injuring birds (Redfern and Clark 2001). Therefore, a less invasive technique would be valuable, either as an alternative method of sexing individuals or to evaluate the results of cloacal examination.

In this study, an analysis of morphometric measurements taken on Black Swans that were sexed cloacally is presented to attempt to establish a non-invasive method of sexing members of this species. To account for potential regional variations in size, data are presented from two study populations, one in South East Queensland (SEQ) and one in the Australian Capital Territory (ACT) some 1000 km south of the SEQ study location.

METHODS

The breeding biology of Black Swans has been studied intensively since 2007 in SEQ (Coleman 2010, 2014). The study area extends from Brisbane south to the New South Wales border and inland for approximately 30 km from the coastal strip. The centre of the study area is at approximately -27.827304°, 153.189605°. Every month, the area was surveyed to determine the location of territorial pairs and non-breeding flocks, with attempts being made to catch and band any unbanded individuals. Each captured bird was fitted with an Australian Bird and Bat Banding Schemes metal band on the left leg and a red plastic ring with a unique three-character code on the right leg. In the monthly surveys, as many of these plastic bands as possible were read to provide information on which birds were associating in non-breeding locations and which birds were paired (Coleman 2014). Collection of data in this way facilitated an understanding of the breeding biology of the species and provided information on the factors influencing the breeding success of individual swans. In 2019, a comparative study of Black Swans, with similar objectives and using the same methods, was started in the ACT. The study area encompassed the entire ACT Local Government Area. In both locations, Black Swans bred primarily in suburban areas, usually on artificial water bodies, with birds in SEQ utilising marine canals and lakes in public parks and golf courses, and those in the ACT almost exclusively occurring on lakes in public areas.

In both locations, birds were caught by hand either on land or after being trapped in areas where they could be surrounded on the water. Birds in both locations were habituated to feeding by humans and were easy to approach, with bread or grain being used to attain proximity for catching. All birds caught were measured and weighed (± 0.1 kg). Total head length and tarsus length (± 1 mm) were recorded using the criteria described in Lowe (1989). Radius length was also measured with similar accuracy. All birds were aged using plumage characteristics (Marchant and Higgins 1990), with cygnets and recently fledged young being recorded as age code 1, older immatures up to two years after hatching as age code 2 and 2-, and adult birds at least two years old as age code 2+. All birds were sexed cloacally using the method described in Redfern and Clark (2001). This required the cloaca to be everted and opened, allowing the interior to be examined for the presence or absence of a penis which is well developed and visible from the time juvenile birds leave the natal site (pers. obs.). The method requires the cloaca to be opened fully to see the relevant anatomical features, which less experienced observers often have difficulty doing. Due to concerns about the accuracy of sexing very young individuals (Brown and Brown 2002), birds aged as cygnets or age code 1 were excluded from the analysis due to potential error and because they may have still been growing. Only older juveniles, age codes 2 and 2-, were included in the analysis.

Morphometric measurements were taken by J.T. Coleman and D.S. Braithwaite in SEQ and by J.T. Coleman only in the ACT. All birds were sexed cloacally by J.T. Coleman, who has used this method extensively in Mute Swans and Black Swans over a 30-year period. The two observers tested the comparative accuracy of their measurements regularly in the field to ensure consistency in the metrics taken.

Adult birds when caught and measured were recorded as being either paired and holding territory or as non-breeding. Paired birds were in obvious pairs and exhibiting territorial behaviour, whilst any birds that were alone or in small groups or flocks were considered non-breeding.

Statistical analyses were conducted using *StatsDirect v3.3.3* and *XLSTAT 2020.4.1.* Sex, regional, age and status differences in the morphometric variables were examined individually with single factor analyses of variance (ANOVA). Test statistics for significant ANOVA results in comparisons of males and females are provided in Table 1; all non-significant ANOVA results are summarised in Table 2 (regional and ACT male-female comparisons) and Table 3 (age and status comparisons). These tables are not referred to specifically for individual findings in the Results section. The median value for each variable was used as the cut-off point between males and females when comparing against the results derived from cloacal examination. The structural variables were then used in combination in a Linear Discriminant Function Analysis (*XLSTAT 2020*) to see if accuracy of sex determination could be further improved.

RESULTS

Across both locations, morphometric measurements were taken from 545 birds. In SEQ 467 birds were measured, comprising 75 immature males and 163 adult males (55 of which were recorded as paired and on territory), and 56 immature females and 173 adult females (60 of which were recorded as paired and on territory). In the ACT, 77 birds were measured, comprising two immature females and 36 adult females, (24 of which were recorded as paired and holding territory), and three immature males and 36 adult males (25 of which were recorded as paired and holding territory).

Table 1

Significant ANOVA results for comparisons of morphometric measurements between males and females in ACT and SEQ.

Test Conducted	F	DF	P
SEQ: Total head length – Non-breeding Adult	323.8	1,219	<0.001
SEQ: Tarsus length – Non-breeding Adult	389.4	1,219	<0.001
SEQ: Radius length – Non-breeding Adult	248.9	1,219	<0.001
SEQ: Body Mass – Non-breeding Adult	129.9	1,217	<0.001
SEQ: Total head length – Paired Adult	147.6	1,114	<0.001
SEQ: Tarsus length – Paired Adult	133	1,114	<0.001
SEQ: Radius length – Paired Adult	126.7	1,114	<0.001
SEQ: Body Mass – Paired Adult	47.3	1,112	<0.001
SEQ: Total head length – Juvenile	117	1,126	<0.001
SEQ: Tarsus length – Juvenile	135.4	1,126	<0.001
SEQ: Radius length – Juvenile	174	1,126	<0.001
SEQ: Body Mass – Juvenile	31.6	1,126	<0.001
ACT: Total head length – Non-breeding Adult	19.5	1,21	<0.001
ACT: Tarsus length – Non-breeding Adult	36.9	1,21	<0.001
ACT: Radius length – Non-breeding Adult	25.7	1,21	<0.001
ACT: Body Mass – Non-breeding Adult	5.7	1,20	0.002
ACT: Total head length – Paired Adult	98.6	1,47	<0.001
ACT: Tarsus length – Paired Adult	51.4	1,47	<0.001
ACT: Radius length – Paired Adult	85.3	1,47	<0.001
ACT: Body Mass – Paired Adult	31.7	1,47	<0.001
ACT: Tarsus length – Juvenile	25.8	1,3	0.014

Table 2

Non-significant ANOVA results for comparisons of morphometric measurements between juvenile males and females in ACT (rows 1-3) and between birds measured in ACT and SEQ (rows 4-26).

Test Conducted	F	DF	P
Male/Female Total head length – Juvenile	9.8	1,3	0.052
Male/Female Radius length – Juvenile	0.7	1,3	0.465
Male/Female Body Mass – Juvenile	3	1,3	0.181
Non-Breeding Adult Male: Total head length	0	1,118	0.968
Non-Breeding Adult Male: Tarsus length	0	1,118	0.995
Non-Breeding Adult Male: Radius length	0.2	1,118	0.655
Non-Breeding Adult Male: Body Mass	1.3	1,115	0.26
Non-Breeding Adult Female: Total head length	3.2	1,122	0.074
Non-Breeding Adult Female Comparison: Tarsus length	0	1,122	0.958
Non-Breeding Adult Female Comparison: Radius length	0.8	1,122	0.363
Paired Male: Total head length	0.7	1,79	0.42
Paired Male: Tarsus length	0.1	1,79	0.807
Paired Male: Radius length	0.2	1,79	0.697
Paired Male: Body Mass	3.1	1,79	0.085
Paired Female: Total head length	3.7	1,82	0.057
Paired Female: Tarsus length	0.6	1,82	0.447
Paired Female: Radius length	0.2	1,82	0.635
Paired Female: Body Mass	1.7	1,82	0.198
Juvenile Male: Total head length	0.8	1,69	0.387
Juvenile Male: Tarsus length	0.1	1,69	0.802
Juvenile Male: Radius length	0.9	1,69	0.337
Juvenile Male: Body Mass	0.6	1,69	0.447
Juvenile Female: Total head length	0.1	1,49	0.928
Juvenile Female: Tarsus length	0.1	1,49	0.76
Juvenile Female Comparison: Radius length	0.8	1,49	0.384
Juvenile Female Comparison: Body Mass	0.3	1,49	0.562

Table 3

Non-significant ANOVA results for comparisons of morphometric measurements between adult and juvenile birds and paired and non-breeding adults in ACT and SEQ.

Test Conducted	F	DF	P
SEQ Adult Male vs Juvenile Male: Total head length	0.1	1,241	0.938
SEQ Adult Male vs Juvenile Male: Tarsus length	0.7	1,241	0.651
SEQ Adult Male vs Juvenile Male: Radius length	0.5	1,241	0.797
SEQ Adult Female vs Juvenile Female: Total head length	0	1,221	0.949
SEQ Adult Female vs Juvenile Female: Tarsus length	0.5	1,221	0.475
SEQ Adult Female vs Juvenile Female: Radius length	1.8	1,221	0.184
ACT Adult Male vs Juvenile Male: Total head length	0.1	1,37	0.87
ACT Adult Male vs Juvenile Male: Tarsus length	0	1,37	0.921
ACT Adult Male vs Juvenile Male: Radius length	2	1,37	0.176
ACT Adult Female vs Juvenile Female: Total head length	0	1,36	0.954
ACT Adult Female vs Juvenile Female: Tarsus length	0.1	1,36	0.797
ACT Adult Female vs Juvenile Female: Radius length	0.2	1,36	0.658
SEQ Paired Adult Male vs Non-Breeding Adult male: Total head length	1.2	1,165	0.221
SEQ Paired Adult Male vs Non-Breeding Adult male: Tarsus length	1.3	1,165	0.3
SEQ Paired Adult Male vs Non-Breeding Adult male: Radius length	0.2	1,165	0.56
SEQ Paired Adult Female vs Non-Breeding Adult Female: Tarsus length	0.3	1,169	0.707
SEQ Paired Adult Female vs Non-Breeding Adult Female: Radius length	0.5	1,169	0.447
ACT Paired Adult Male vs Non-Breeding Adult male: Total head length	0.2	1,28	0.872
ACT Paired Adult Male vs Non-Breeding Adult Male: Tarsus length	0.1	1,34	0.737
ACT Paired Adult Male vs Non-Breeding Adult Male: Radius length	0	1,34	0.928
ACT Paired Adult Male vs Non-Breeding Adult Male: Body Mass	1.6	1,34	0.292
ACT Paired Adult Female vs Non-Breeding Adult Female: Tarsus length	0.5	1,34	0.497
ACT Paired Adult Female vs Non-Breeding Adult Female: Radius length	0.7	1,34	0.426
ACT Paired Adult Female vs Non-Breeding Adult Female: Total head length	4.1	1,34	0.052
ACT Paired Adult Female vs Non-Breeding Adult Female: Body Mass	0.6	1,34	0.429

Sex comparisons

Males were significantly heavier and larger in all other morphometrics than females in all age and status categories (Figures 1, 2, 3 and 4) in both ACT and SEQ, with the exception of juveniles in the ACT. For this age category, only tarsus length differed significantly between males and females, although the sample size in ACT was very small (n=5).

Regional comparisons

There was no significant difference for any morphometric variable between all birds in ACT and all birds in SEQ. There were also no significant differences between ACT and SEQ in the body mass of either sex, except that ACT non-breeding females were, on average, 0.6 kg heavier than their SEQ counterparts ($F_{1,122}$ =4.6, P=0.034).

Age comparisons

In SEQ, adult males were heavier than immature males by an average of 0.6 kg ($F_{1,236}$ =36.3, P<0.001) and immature females were, on average, 0.3 kg lighter than adult females ($F_{1,219}$ =4.24, P=0.04). Sample sizes were inadequate to make similar comparisons for the ACT for either sex. There were no significant differences in total head length, radius length or tarsus length between immature and adult males or between paired, territorial adult and non-breeding adult males in either SEQ or ACT. There were also no significant differences in any of these three morphometric variables between immature and adult ACT and SEQ females.

Status comparisons

Within the adult cohort, paired, territorial males were, on average, 0.3 kg heavier than their non-breeding counterparts in SEQ ($F_{1,160}$ =7.5, P=0.007), but a comparable significant disparity was not evident in the ACT. Paired, territorial females in SEQ were, on average, 0.6 kg heavier than non-breeding females ($F_{1,169}$ =14.4, P<0.001), but again a comparable significant difference was not evident in ACT. There were no significant differences in total head length, radius length or tarsus length between paired, territorial adult and non-breeding adult males in either SEQ or ACT. There was no significant difference in radius length or tarsus length between paired, territorial adult and non-breeding adult females in either study area. However, total head length differed significantly between paired, territorial and non-breeding females in SEQ, the former being, on average, 1.9 mm longer in this metric ($F_{1,169}$ =5.6, P=0.044), but this disparity was not evident in the ACT, albeit based on a much smaller sample size.

Given the minimal differences in morphometrics between juvenile and adult birds and between the two geographical locations, all data, except those for birds aged as age code 1, were combined for further analysis to better establish whether any one of the structural measures provided a viable parameter for sexing Black Swans. However, using this combined data set a small percentage of birds still could not be sexed, as their measurements were intermediate between those for the two sexes. For total head length with 139 mm taken as the cut-off value, 4.2% (n=23) of birds could not be assigned a sex, as their value for this metric was at the cut-off level. For radius length (240 mm cut off) the percentage of unassignable birds was 4.8% (n = 26) and for tarsus length (98 mm cut off) it was 7.3% (n = 40) (Table 4). The proportion of birds incorrectly assigned a sex using the amalgamated morphometric data set varied between 9.5% and 9.7%. However, although sexing by cloacal examination is very accurate for adult birds, when conducted by an experienced operator, its accuracy was not independently verified in this study, so this percentage may actually have been slightly higher or lower. Overall, total head length was the most accurate morphometric predictor of sex, with 86.3% of all birds caught being sexed accurately, only 9.5% inaccurately and 4.2% being unassignable using this metric (Table 4).

A Linear Discriminant Function Analysis with all three predictor variables included increased the accuracy of sexing using morphometrics, with 92.1% of birds being accurately assigned (91.7% of females and 92.5% of males). This was a substantial improvement on the use of any single predictor variable, which only allowed 83.0 to 86.3% of birds to be assigned correctly (Table 4).

DISCUSSION

Sexual size dimorphism is well documented in the six true swan species (Birkhead and Perrins 1986; Kraaijeveld *et al*. 2004; Miller *et al*. 1988), but due to overlaps between the sexes in morphometric measurements, cloacal examination has remained the preferred method of determining sex in swans (Redfern and Clark 2001). Molecular techniques, although accurate, are largely unavailable to researchers lacking access to appropriate laboratory facilities. Our analysis has demonstrated that, at least in the Black Swan, there is a less invasive method of sex determination available using morphometric measurements. Measurements allowed over 80% of all birds to be accurately sexed, and yielded an accuracy rate exceeding 90% for birds with measurements larger or smaller than the cut-off values.

Cloacal examination requires training to achieve accuracy and safeguard the bird's welfare, and it is important that the cloaca is opened enough for the presence or absence of the penis to be accurately assessed. However, whilst the penis is only visible as a small button in cygnets and can remain undetected, it is well developed when birds leave the natal site (pers. obs.). As a result, if the cloaca is opened fully, cloacal sexing can be considered extremely accurate in fully grown birds, although no studies verifying the accuracy of sexing fully-grown birds using this method have been published. Researchers unfamiliar with the technique often do not open the cloaca enough (pers. obs.) and may miss important anatomical features as a result. For those unfamiliar with this technique, morphometrics provide an alternative enabling the sex of most Black Swans to be accurately determined. However, for individuals that fall within the overlap range for sexing using individual morphometrics, and that cannot be further categorised using Discriminant Function Analysis incorporating multiple morphometric measurements, cloacal examination to determine sex is still required.

Morphometric examination also provided an opportunity to compare measurements between two regions and among

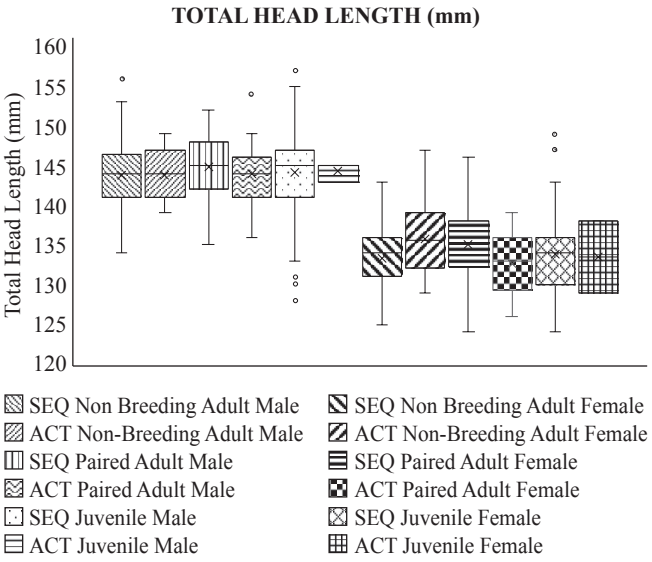

Figure 1. *Total head length (mm) of male and female Black Swans measured in SEQ and ACT. For figures 1-4, X represents the mean and the central line within the upper and lower quartile box limits represents the median. Open circles show outlier values which are beyond the limits of the upper and lower whisker ranges.*

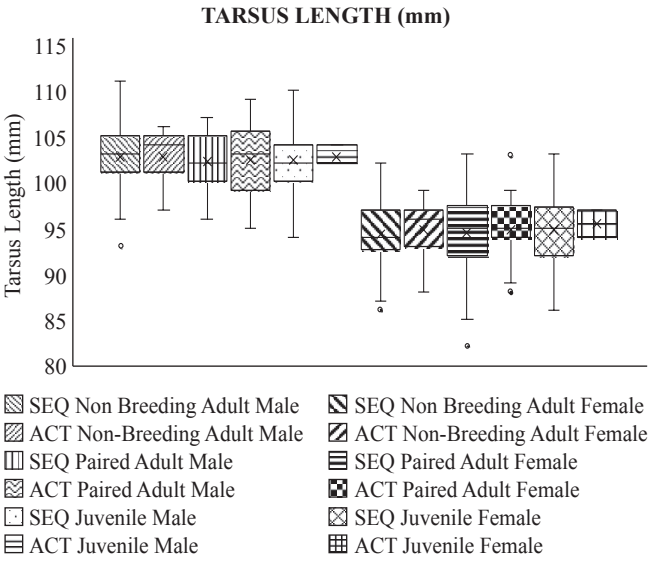

Figure 2. *Tarsus length (mm) of male and female Black Swans measured in SEQ and ACT.*

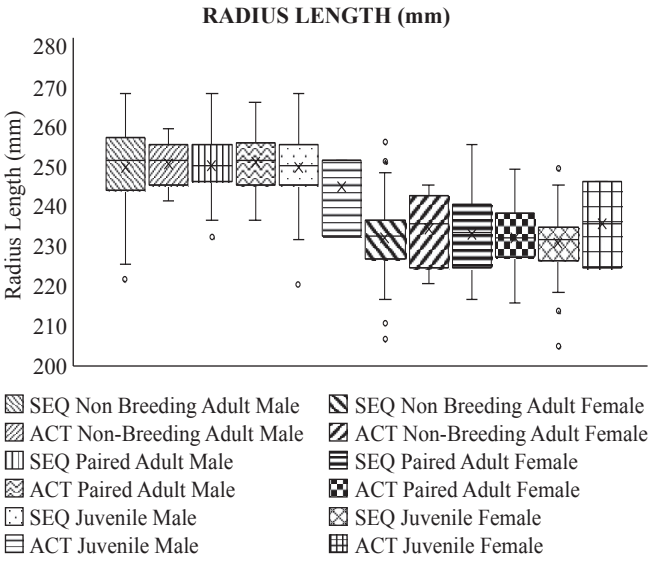

Figure 3. *Radius length (mm) of male and female Black Swans measured in SEQ and ACT.*

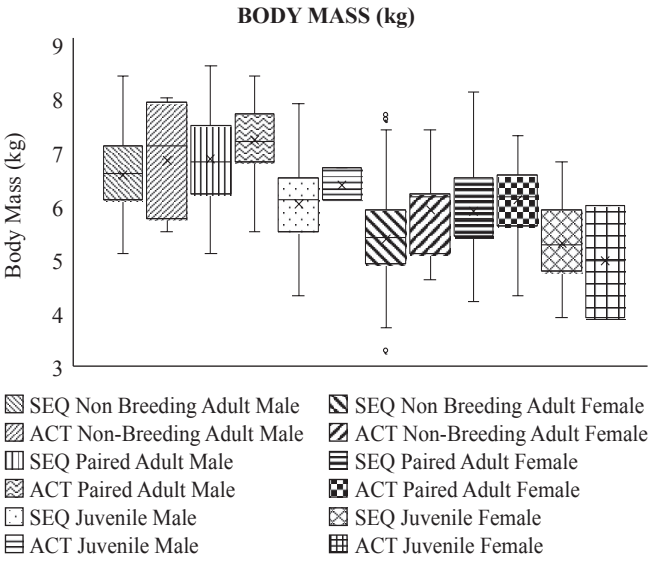

Figure 4. *Body mass (kg) of male and female Black Swans measured in SEQ and AC.*

Table 4

Assignment of sexes of Black Swans caught in SEQ and ACT based on cut off values for total head length, tarsus length, radius length and body mass.

Variable	Cut Off value	Sex criteria compared to cloacal examination results		
		Correctly Sexed	Incorrectly Sexed	Unknown Sex
Total head length	F<139>M	86.30%	9.50%	4.20%
Tarsus length	F<98>M	83.00%	9.70%	7.30%
Radius length	F<240>M	85.50%	9.70%	4.80%
Body mass	F<6>M	70.20%	24.80%	5.00%

age and status cohorts. The lack of size differences between the two study areas indicates that the sexing criteria identified here may be widely applicable and not restricted to a limited geographical region. In many bird species, morphometric sexing is complicated by clinal variations in size (e.g. Andrew *et al*. 2008), but this study indicates that this consideration may not be important for this species.

Differences in size between male and female swans probably reflect the differing roles of the sexes (Scott 1988). Black Swans are primarily territorial breeding birds, although colonial breeding is common in some parts of their range (Frith 1982). Males assume the primary role in territorial defence of resources (Kraaiijeveld *et al*. 2004), but also play a significant role in incubation (Brugger and Taborsky 1994). Larger size is almost certainly advantageous in competition, and greater body mass is probably also important in ensuring ongoing dominance and the necessary resources for incubation and territory protection (Kraaiijeveld *et al*. 2004). It is therefore not surprising that breeding males were significantly heavier than their non-breeding counterparts and immatures, and this has also been demonstrated in other swan species (e.g. Coleman and Coleman 2002).

Large body size in breeding females may sometimes actually be disadvantageous, with more resources needing to be allocated to maintaining body condition than to breeding activity (Kendeigh 1970). Females in this study were typically smaller than males and whilst paired females were not significantly larger than their non-breeding counterparts, they were significantly heavier. The resources required for egg production and incubation are significant where this has been studied in other swan species (Beekman 1991). In those studies, breeding females were significantly heavier than their non-breeding counterparts and put on body mass prior to breeding. The finding in this study, that many paired females were significantly heavier than their non-breeding counterparts, is therefore not surprising.

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