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Capture and handling of saddlebacks during pre-nesting does not affect timing of egg-laying or reproductive success

IAN G. JAMIESON
Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand.
ian.jamieson@stonebow.otago.ac.nz

JEMMA L. GRANT
Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

BRENT M. BEAVEN
Department of Conservation, P.O. Box 3, Stewart Island, New Zealand

Abstract We examine whether mist-netting and handling of birds (including taking blood samples) during the pre-nesting period caused egg-laying to be delayed in a threatened species, South Island saddleback (tïeke) *Philesturnus carunculatus carunculatus*. We used data on egg-laying dates of first clutches for 12 pairs in 2002-03 and 22 pairs in 2003-04, of which 3 (2002-03) and 7 (2003-04) pairs had been caught and handled. There was a significant delay in the peak laying period of first clutches in 2003-04, which was associated with more birds being caught and handled. However, pairs that were handled showed typical laying dates of first clutches for both experienced and inexperienced pairs, and there was no significant correlation between the date when a pair was caught and the date of laying its first clutch. There were also no significant differences between handled and non-handled pairs in the number of chicks raised or fledged. Like saddlebacks, Stewart Island robins *Petroica australis rakiura* monitored at the same site showed a two-week delay in the average laying dates of first clutches in 2003-04. The five inexperienced robin pairs in 2002-03 laid their first clutches earlier in 2003-04, but all three experienced pairs laid later. Weather data indicated it was substantially colder before the nesting period in 2003 compared to 2002, suggesting that colder weather conditions plus a greater number of inexperienced pairs caused a delay in peak egg laying in both species in 2003-04 relative to 2002-03. We conclude that mist-netting, banding and bleeding – standard technique used in present-day research of threatened avian species – did not have any measured short-term effects on nesting behaviour or breeding success of South Island saddlebacks.

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INTRODUCTION

Capture, handling and restraint is used as a standard protocol to measure physiological responses to stress in birds, as it is widely known to produce a stress response (Wingfield 1994; Canoine *et al*. 2002). The stress response to capture and restraint is characterized by a rapid increase of glucocorticosteroids, such as corticosterone, within 5-10 mins reaching a maximum within 30-60 mins (Wingfield 1994). Taking blood samples adds to the cumulative effects of handling stress (Wingfield *et al.* 1997). It is therefore obvious that capture and handling is a stressful event.

Animals have evolved mechanisms to meet the energy needs required for the physiological response to stress (Moberg 2000). However, energy originally used for growth and reproduction could be required by an animal to cope with stress, resulting in a 'biological cost of stress' (Moberg 2000). For most stressors the biological cost will be negligible because the stressors are short-lived, or sufficient reserves exist to cope with the stress without impairing biological functions such as growth and reproduction (Moberg 2000). If there are insufficient resources, or the stress is severe, then biological functions will become impaired and will continue to be impaired until the animal's biological resources are replenished (Moberg 2000).

For decades avian researchers have been mist-netting birds for research purposes. Mist-netting has traditionally been associated with banding and measuring birds, but increasingly researchers are also taking blood samples of captured individuals as part of avian field research studies. Despite capture and restraining clearly causing a measurable stress response (see above), the underlying assumption has always been that such activity does not have

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any lasting effects on the behaviour of the birds once they are released. Several recent studies of passerines and shorebirds have found no effect of mist netting and blood sampling on over-winter survival (Arden *et al.* 1994; Lubjuhn *et al*. 1998) or on return rates to breeding areas the following year (Coldwell *et al*. 1988; Dufty 1988; Hoysak & Weatherhead 1991; Perkins *et al*. 2004). However, no studies of which we are aware have looked for possible short-term effects of capture and handling (including blood sampling), such as delayed egg-laying when birds are caught during the pre-nesting period. Effects of capture and handling on egg-laying dates could be important as offspring that hatch later than the population norm tend to have lower survival or lower recruitment rates (Price *et al*. 1988; Rowe *et al*. 1994)

We had the opportunity to examine these potential short-term effects of mist-netting and handling as part of a long-term study on inbreeding and loss of genetic variation in two threatened species of New Zealand forest passerines, South Island saddleback (*Philesturnus c. carunculatus*) and Stewart Island robin (*Petroica australis rakiura*). For the genetics study, we had to capture and bleed one or both members of several territorial pairs of South Island saddlebacks. Robins exhibit little fear of humans and are relatively easy to catch using baited traps or hand nets; most adult robins and their fledglings were caught after the nesting period. Adult saddlebacks, on the other hand, can only be caught by luring birds into mist nets using playbacks of territorial calls, which are most effective during the pre-nesting period when territorial boundaries are being re-established. Therefore we were able to compare laying dates and subsequent reproductive success of birds that had been caught and handled to those that had not been caught, within the same season. The null hypothesis is that there is no effect of mist-netting and associated handling on laying dates. Our alternative hypothesis was that mist-netting and handling of birds might cause a delay in egg-laying, and/or affect the number of nestlings and fledglings produced.

STUDY AREA AND METHODS

The study took place on Ulva Island (259 ha), a managed 'open sanctuary' located 800 m to the nearest shoreline within Stewart Island's Paterson Inlet (168° 08′ E., 46° 56′ S.) at the south end of the South Island, New Zealand (Beaven 2001). Ulva Island is primarily covered by lowland podocarp forest in its interior, surrounded by coastal scrub. The island has a gentle terrain and a high point of 74 m a.s.l. A detailed description of the island and its vegetation can be found in Oppel & Beaven (2004) and Hooson & Jamieson (2004).

Rats (*Rattus norvegicus*) were eradicated from Ulva Island in 1996. Thirty South Island saddlebacks were reintroduced in April 2000, and 18 Stewart Island robins in September 2000 and January 2001 as part of the island's restoration (Beaven 2001). All released saddlebacks and robins were colour banded, but no blood samples had been taken. In 2001-02, a small number of founders (two saddlebacks) and several nestlings and unbanded yearlings were caught, but only a proportion of active nests were found and closely monitored. The majority of the surviving founders as well as many of the unbanded birds that had fledged in the two previous breeding seasons were caught and bled in the 2002-03 and 2003-04 seasons. First clutches of all known nesting pairs were monitored during these two breeding seasons.

Our analysis focused on breeding saddlebacks, some of which were caught during the pre-nesting period of 0 - 8.7 weeks (mean 5.6) before the earliest pair of saddlebacks in the population laid their first clutch. We also included data on laying dates of robins for comparative purposes. Six metre mist nets were placed in clearings inside known saddleback territories. A pair of speakers was placed on either side of a mist-net and recorded saddleback calls played back through the speakers. Birds caught were removed from the net as quickly as possible and placed in a cloth bag for processing. Processing of birds was normally done in the following order: banding (metal and plastic colour leg-bands), measuring (tarsus, bill and sometimes wattle length), bleeding (brachial vein puncture with 27-gauge needle and blood collected in 0.1 ml capilliary tubes), and weighing (inside bag with Pesola scales). Processing took approximately 20 min and was done by one of two people over the two years of study. We refer to pairs/birds that had been caught in mist nets, banded, measured and bled prior to the egg-laying period as 'handled', and all other nesting pairs/birds in the same year as 'non-handled'. In only four of 13 cases were both members of a pair caught during the pre-nesting period and therefore 'handled' refers to at least one member of a breeding pair.

The reproductive period, divided into the nest building, incubation, nestling and fledgling periods, was monitored continuously from early August until March of each season. If a nest was found with eggs within a week of when the pair was last observed and not known to be nesting (e.g. observed nest building), the laying date was estimated to be the mid-point between the two dates. If the nest was found later in the incubation period, the laying date was estimated by back-dating from the day of hatching (based on incubation periods on Ulva Island of 21 days for saddlebacks and 19 days for robins; I. Jamieson unpubl. data). We did not attempt to estimate laying dates for nests that were found with nestlings of unknown age.

Saddlebacks and robins on Ulva Island lay modal clutches of two eggs (range: 1-2 and 2-3, respectively) (Hooson & Jamieson 2003; I. Jamieson unpubl. data). For each nesting attempt we recorded the number of nestlings when offspring were old enough to band in the nest and the number of fledglings when they were still with their parents. Pairs were scored as 'experienced' if the female had bred in at least one previous nesting season. Results were analysed using general linear models that examined the effects of year, experience, handling and pairs (entered as a random factor) on laying dates. We arbitrarily assigned 1 August and 1 October as the beginning of the breeding period for robins and saddlebacks, respectively, to compare relative differences in egg-laying dates among pairs and between years. Spearman's rank correlation was used to test for evidence of a positive relationship between the date when a pair was caught and the date of laying its first clutch. Student's t-test was used within years to test for differences in laying dates between pairs that were handled and those that were not handled. Daily minimum/maximum temperatures and rainfall data used in the analysis were recorded at Oban approximately 3 km from Ulva Island.

The study was conducted under Department of Conservation banding and research permits and University of Otago Animal Ethics permits and with the approval of local consultative Maori group Kaitiaki Roopu.

RESULTS

We had data on saddleback egg-laying dates of first clutches for 34 pair-years, 12 in 2002-03 and 22 in 2003-04. Three pairs were handled during the pre-nesting period in 2002-03, and seven different pairs in 2003-04 (Fig. 1). Additionally, we had data on reproductive success, but not egg-laying dates, for two pairs (neither were handled) for 2002-03 and for five pairs (three were handled) in 2003-04.

Saddleback pairs laid first clutches, on average, two weeks later in 2003-04 compared to 2002-03 (Fig. 1), and inexperienced breeders laid later clutches than experienced breeders in each year (Fig. 2) (Year: $F = 15.3$, 1 df, $P = 0.004$; Experience: $F = 11.2$, 1 df, $P = 0.01$). The later breeding in 2003-04 could have been partly due to a larger proportion of pairs being handled, a larger proportion of inexperienced breeders, or a combination of these factors; a delay in laying could also have been a consequence of harsher weather conditions (see below).

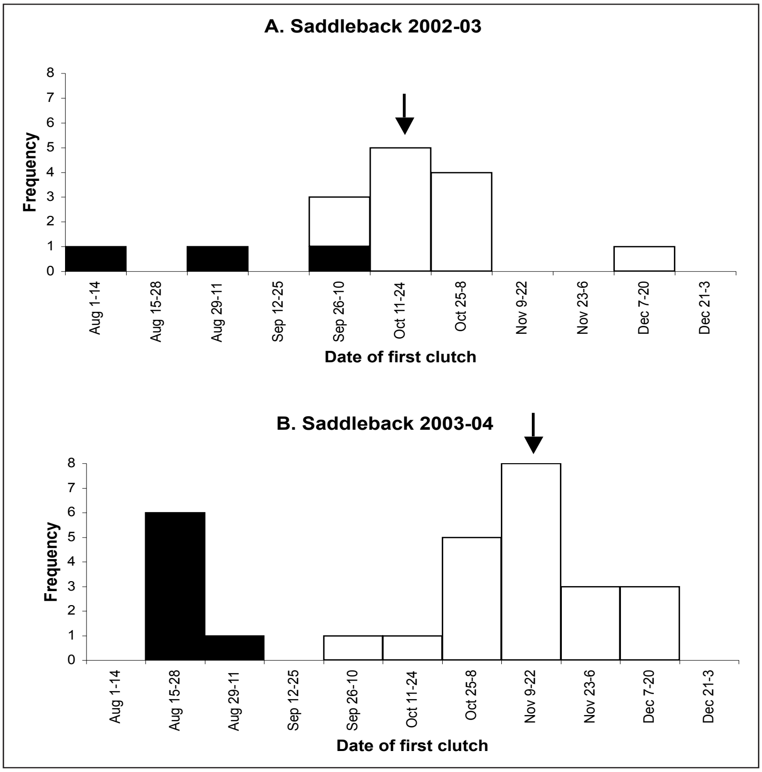

Figure 1 Frequency histogram of dates of capture (black bars) and dates of first clutches (open bars) for breeding pairs of South Island saddlebacks on Ulva Island in 2002-03 (A) and 2003-04 (B). Arrow indicates median date of first clutches.

We first analysed the effect of handling within each year. Although the number of handled pairs was small in 2002-03, there was no effect of breeding experience on egg-laying date (F = 0.19, 1 *df*, P = 0.68), whereas the effect of handling approached significance (F = 4.68, 1 *df*, P = 0.059). However, these two factors were confounded, as all three of the handled pairs were also inexperienced. Furthermore, most of the difference in time of egg-laying between handled and non-handled pairs could be attributed to one handled pair that laid their first clutch twice as late as any other pair, including the other two handled pairs (Fig. 2A); this late laying pair was caught the earliest of the three handled pairs (Fig. 1A). The three handled pairs all laid their first clutch at an earlier date the following season, which is not unexpected for more experienced pairs. However, all seven pairs that were not handled and were experienced breeders, laid their first clutches later in 2003-04, suggesting that there might have been something unusual about that season.

For 2003-04, there was no effect of handling on laying dates for first clutches (F = 0.011, 1 *df*, P = 0.91), but there was a significant effect of experience (F = 4.48, 1 *df*, P = 0.048). Pairs that were handled showed typical laying dates of first clutches for both experienced and inexperienced pairs (Fig. 2B), and there was no significant correlation between the date when a pair was caught and the date of laying its first clutch (both years combined, r_s = -0.23, n = 10, P > 0.10). We further analysed the possible effects of handling on subsequent reproductive success using 2003-04 data only and including the five additional pairs (three of which were handled) for which we did not have laying dates. There were no significant differences between non-handled and handled pairs in the number of chicks raised (mean difference = 0.42, 95% CIs = -0.31-1.14, t = 1.18, 25 *df*, P = 0.25) or in number of chicks fledged (mean difference = 0.54, 95% CI = -0.18 – 1.26, t = 1.54, 25 *df*, P = 0.13).

Although only one pair of robins (out of 29 pair-years) was handled during their pre-nesting period, we also examined date of laying of their first clutches over the same two seasons, for comparative purposes. Like saddlebacks, robins showed a two-week delay in the average laying dates of first clutches in 2003-04 (Fig. 3), and inexperienced breeders laid their first clutches later than experienced breeders (Fig. 4). The effect of year on laying date was not significant (F = 4.29, 1 *df*, P = 0.08), but the effect of experience was (F = 28.95, 1 *df*, P < 0.01). Also similar to saddlebacks, the five inexperienced pairs in 2002-03 laid their first clutches earlier in 2003-04, but all three experienced pairs laid later.

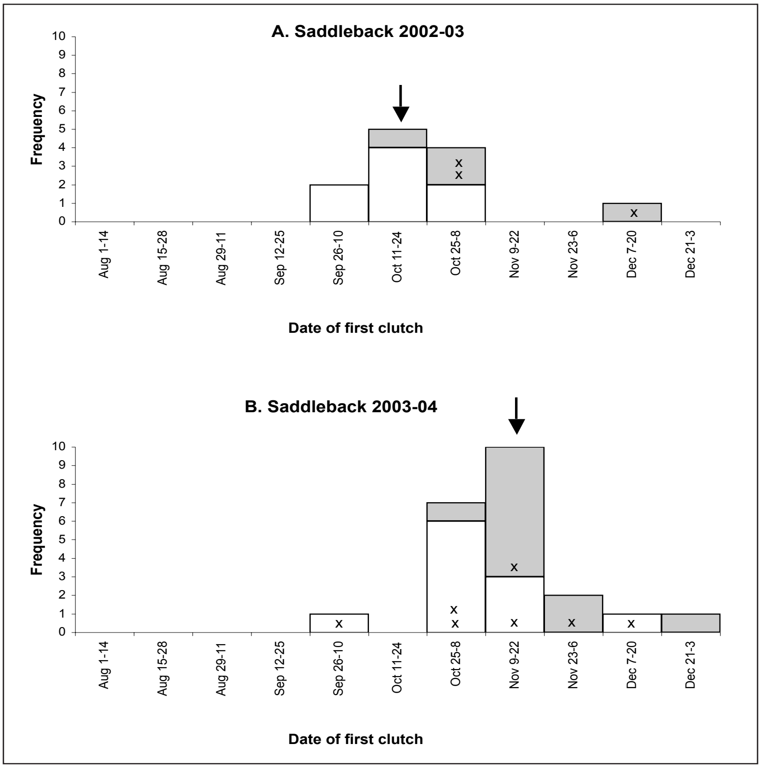

Figure 2 Frequency histogram of dates of first clutches for experienced (open bars) and inexperienced (grey bars) breeding pairs of South Island saddlebacks on Ulva Island in 2002-03 (A) and 2003-04 (B). Arrow indicates overall median date of first clutches and 'x' within bars indicates the number of pairs that were handled.

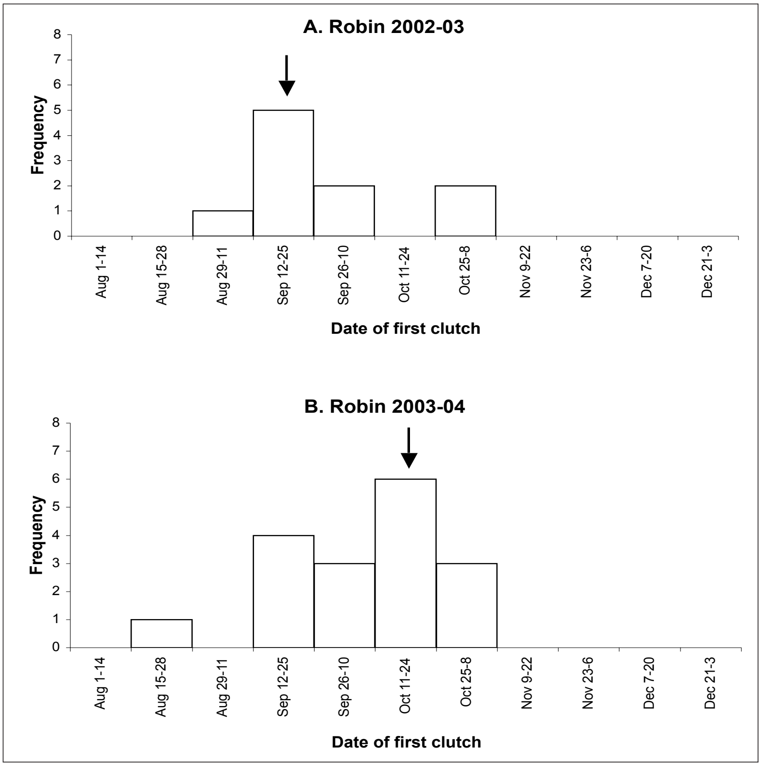

Figure 3 Frequency histogram of dates of first clutches for breeding pairs of Stewart Island robins on Ulva Island in 2002-03 (A) and 2003-04 (B). Arrow indicates median date of first clutches.

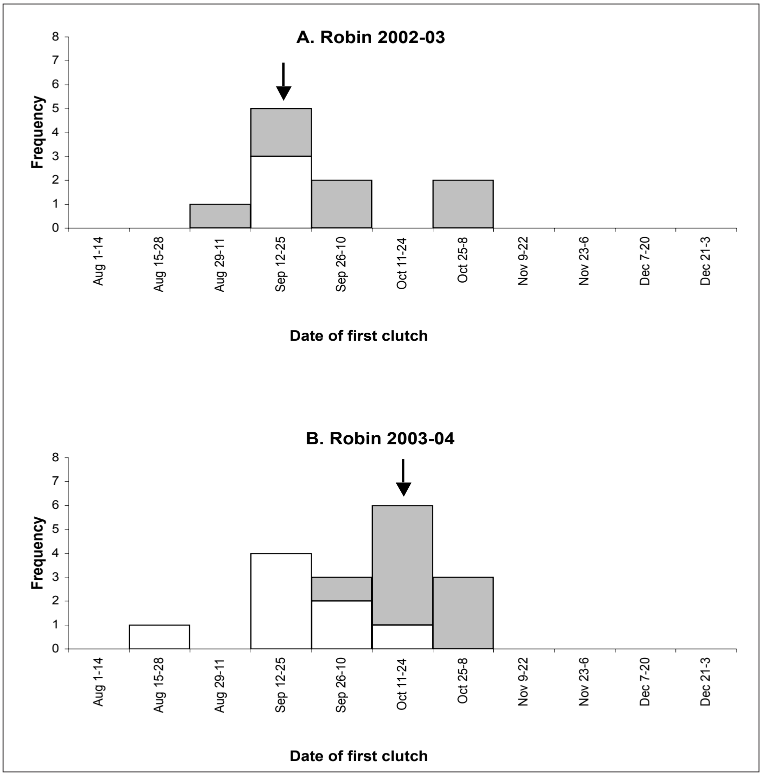

Figure 4 Frequency histogram of dates of first clutches for experienced (open bars) and inexperienced (grey bars) breeding pairs of Stewart Island robins on Ulva Island in 2002-03 (A) and 2003-04 (B). Arrow indicates overall median date of first clutches.

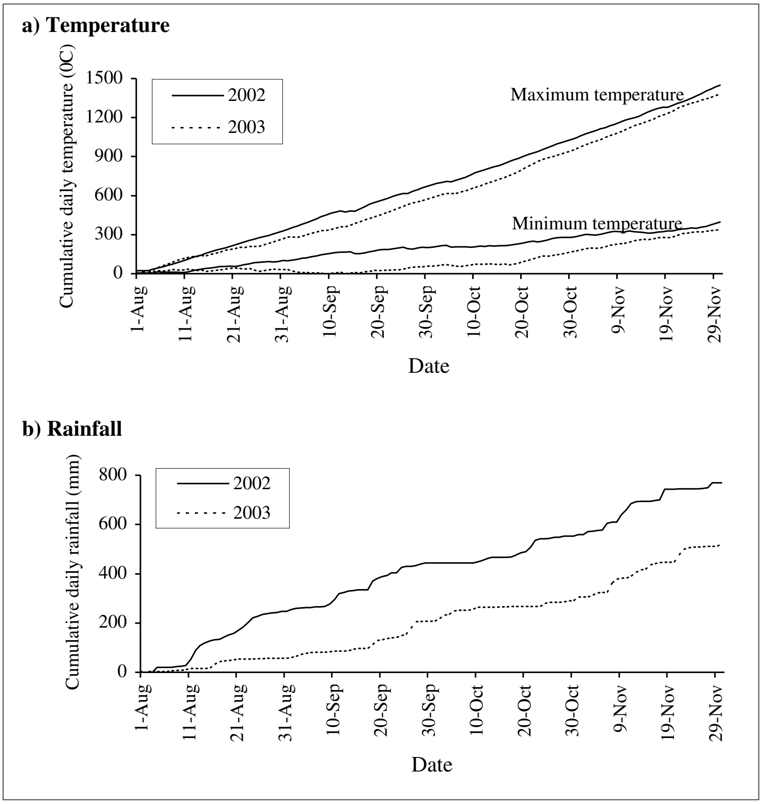

Figure 5 Comparison of the cumulative daily minimum/ maximum temperatures and rainfall between 2002 and 2003, for the period from 1 August to 30 November (i.e. leading up to and during the egg-laying period of South Island saddlebacks and Stewart Island robins).

Finally, we examined daily minimum/ maximum temperatures and rainfall data immediately before and during the egg laying period in 2003 compared to 2002 (Fig. 5). After 22 August, the cumulative daily minimum and maximum temperatures were consistently lower in 2003 compared to 2002. By contrast, substantially less rain fell prior to the egg-laying period in 2003 compared to 2002. This pattern suggests very cold but dry days in 2003. Between 22 August and 22 September, there were 19 days when the minimum temperature was below zero (mean –5.0°C) and 12 days with less than 0.5 mm of rain in 2003 compared to only 5 days below zero (mean –4.3°C) and 5 days with less than 0.5 mm of rain for the same period in 2002.

DISCUSSION

There is little doubt that being caught in a mist-net, held in a human hand and having blood extracted can cause stress to a bird, but avian researchers generally assume that these effects will be short-lived. Recent studies have shown that this sort of handling of birds for research purposes does not have any long-term effects such as reducing over-winter survival or return rates to breeding sites (Perkins *et al*. 2004). In our retrospective analysis, we asked whether the stresses associated with capture and handling of South Island saddleback were significant enough to cause behavioural changes with respect to the timing of egg-laying. If capture and handling does cause significant stress to birds we might expect to detect such effects earlier rather than later in the breeding cycle. One potential criticism of the study was that in only a few cases were both members of a pair caught and handled. However, for all seven pairs handled in 2003-04, it was the female that was caught and we expect the time of laying to be most influenced by the female's behaviour.

We did find a significant delay in the peak laying period of first clutches in the second season of our study which was associated with more birds being caught and handled during the pre-nesting period, 4-7 weeks before the earliest pair in the population laid their first clutch. However, pairs that were caught and handled did not delay their egg-laying relative to pairs that were not caught and handled. There were more pairs breeding for the first time in 2003-04, and inexperienced breeders lay first clutches later than experienced breeders, but this was unlikely to be the sole cause of the delay because pairs that were experienced breeders in 2002-03 also laid later in 2003-04. A similar late-breeding pattern was also shown by nesting robins in 2003-04 suggesting that there might have been an unusual weather event that caused birds to delay their nesting. Analysis of differences in weather data (daily minimum/ maximum temperatures and rainfall) indicate that it was substantially colder (but drier) for a sustained period before the normal nesting period in 2003 compared to 2002. We were unable to obtain Stewart Island weather data from previous years to determine what the normal weather pattern is for this time of year. It would be interesting to know whether other birds species in other parts of the South Island experienced delayed breeding or whether this was a localised effect peculiar to Ulva Island and/or to saddlebacks and robins.

In conclusion, it is reassuring that mist-netting, banding and bleeding – standard technique used in present-day research of threatened avian species – did not have any apparent short-term effects on nesting behaviour or breeding success of South Island saddlebacks. Nevertheless, this does not mean that efforts to minimise handling and disturbance should be ignored. Field researchers can still minimise disturbance by mist-netting adult birds outside the breeding period, limiting handling time during capture, and receiving adequate training in banding and bleeding techniques to maximise efficiency when these sorts of techniques need to be employed.

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