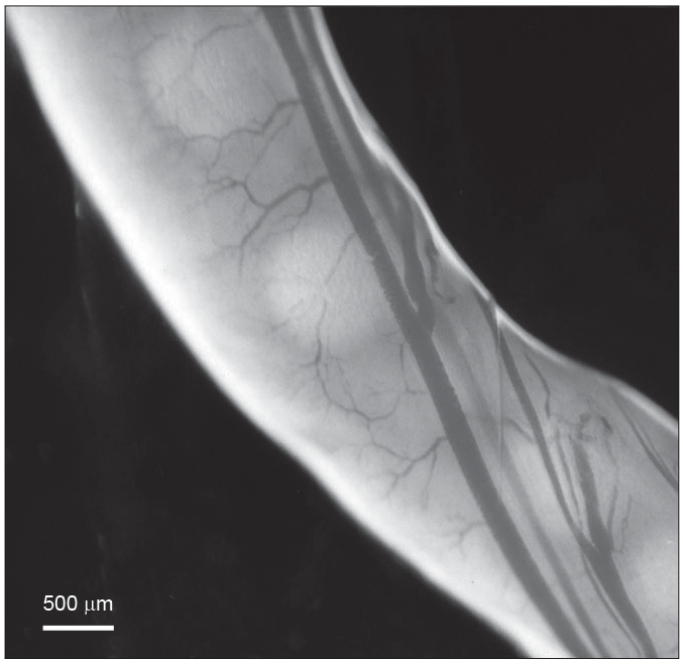

Figure 2. View of blastocysts within the uterine wall of a stoat (*Mustela erminea*) during diapause.

3.3 ASSESSMENT OF REPRODUCTIVE FUNCTION

Techniques to assess the reproductive physiology of captive, acclimatised stoats were investigated throughout a natural breeding season. Female stoats were visually assessed for physical changes that have previously been associated with oestrus in stoats and other mustelids (vulva swollen, changes in vulva colour and vaginal discharge; King & Murphy 2005). A new technique for detecting oestrus, based on a protocol that was developed for mink (*Mustela vison*) (European Mink Biology and Conservation 1995), was also trialed. This method used changes in the cell population in vaginal washes to confirm the onset of oestrus in stoats. Female stoats were anaesthetised using halothane in oxygen. A fine tube was inserted into the vaginal opening and 100 µL of sterile phosphate buffered saline (pH 7.4) was used to gently wash cells from the vagina. The wash fluid was collected and the numbers of keratinised epithelial cells, non-keratinised epithelial cells, white blood cells and red blood cells were counted in a Neubauer counting chamber. Male stoats were also assessed for seasonal changes in reproductive function by measuring testis size.

Samples of blood and fresh faecal material were collected from female and male stoats for hormone analysis in the future. A non-terminal method for collecting serial blood samples from stoats was developed for future immunological and reproductive assessments. The anatomy and position of the blood vessels in the jugular groove, tail and limbs was studied in recently killed animals. The technique, whereby small volumes of blood (0.2–0.5 mL) were taken from the saphenous vein at the back of the leg of stoats under fluothane anaesthesia (2.5–4.5% fluothane in oxygen) was adapted from the method for mice described by Hem et al. (1998). It was initially tested in two anaesthetised stoats, which were then killed. Post-mortem examination showed that there was no major trauma at the sampling site. Repeat blood samples (up to five samples from an individual animal) were reliably collected on more than 50 occasions from anaesthetised stoats without complications. Other methods

were considered to be too unreliable (from the jugular or ventral tail vein) or were considered inhumane with too high a risk of complications (cardiac puncture) for repeat collections.

3.4 PHOTOPERIOD TREATMENTS TO STIMULATE OUT-OF-SEASON MATING

3.4.1 Six-month artificial photoperiod cycle

In 2002, 12 stoats (six male, six female) were exposed to a 6-month artificial photoperiod (6mAP). For the 6mAP stoats, the rate of change in day length was increased by two-fold from equinox on 22 March, so that animals experienced a cycle of the annual photoperiod pattern condensed into a 6-month period. For example, 6mAP-treated animals experienced the shortest photoperiod on 1 May (instead of 22 June) and maximum day length on 9 August (instead of 22 December) (see Fig. 3A). Stoats were kept in a light-proof shelter and artificial light was supplied by three fluorescent light strips under the control of an electronic timer. The length of light exposure was adjusted every 2–3 days, with lights turning on 30 min before theoretical sunrise and turning off 30 min after theoretical sunset (to allow for twilight). Another 12 control stoats (six male, six female) were kept in cages under an outside shelter and experienced a natural photoperiod (NP). Animals were randomly assigned to treatment groups. For continuous variables, effects were assessed by ANOVA and differences between treatment means were determined using Tukey's highly significant differences test.

3.4.2 Early summer daylength cycle

In 2003, stoats were exposed to a more extreme and abrupt light regime, because most seasonal breeding mammals respond more quickly to changes in light when the change in photoperiod is large and rapid (Karsch et al. 1988). Twenty-four wild-caught stoats were randomly assigned to treatments: early summer day length light cycle (ESD) (males: $n = 6$; females: $n = 6$) or the natural light cycle (N) (males: $n = 6$; females: $n = 6$) between February and November. ESD-treated animals experienced their shortest photoperiod on 23 May (instead of 22 June) and were abruptly exposed to long day lengths (16 h) from 23 May until 27 November (see Fig. 3B). Stoats were kept in a light-proof shelter and artificial light was supplied by three incandescent and three fluorescent light strips under the control of an electronic timer. Control animals were exposed to natural light. Maximum and minimum temperatures were recorded in each area.

Between August and November, reproductive function was assessed monthly by determining the phase of the oestrus cycle in females (vulva condition and vaginal cytology) and testis function in males (testis size). If oestrus activity was detected before 17 November, stoats were paired and allowed to mate. Males were checked in September for spermatozoa production by examining semen samples collected using a manual manipulation technique adapted from that used for dogs (*Canis familiaris*) (Christiansen 1984). This ensured that females were mated with reproductively competent males. For continuous variables, effects were assessed by ANOVA and differences between treatment means were determined using Tukey's highly significant differences test. Binomial data were assessed using Fisher's exact test.

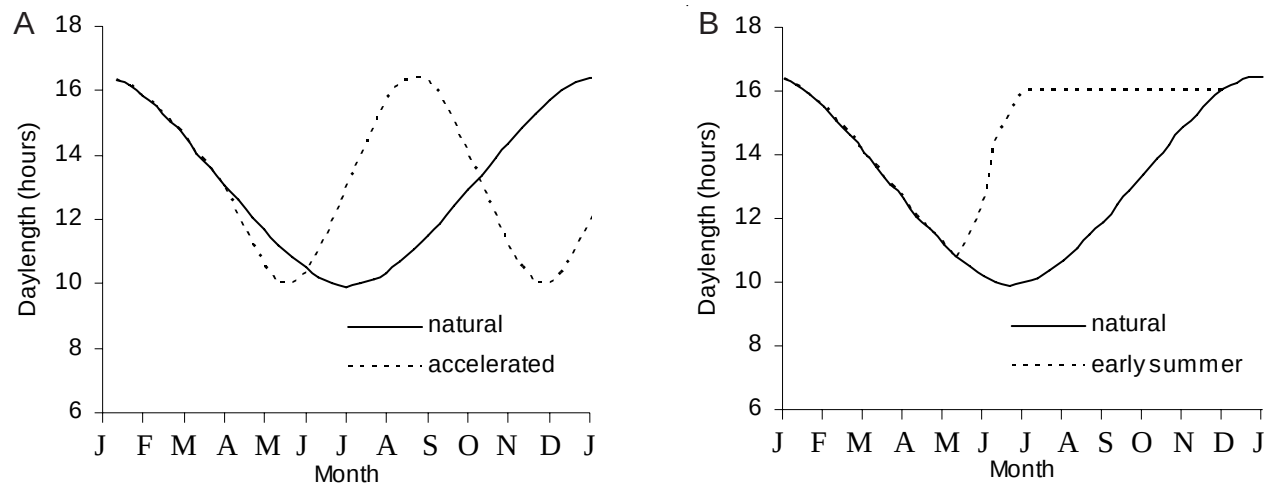

Figure 3. Photoperiod treatments of stoats (*Mustela erminea*) exposed to A. Natural or accelerated photoperiod changes in 2002; and B. Natural or early summer photoperiod changes in 2003.

Faecal samples were also collected every week for later faecal steroid analysis (oestradiol and progesterone in females, and testosterone in males). Blood samples (up to 500 µL) were collected from the saphenous vein at the back of the leg under fluothane anaesthesia in March/April, June, September and November/December for hormone analysis and validation of faecal steroid analysis. These sampling times coincide with times when the difference in light exposure between AP and NP stoats was greatest, and when animals were most likely to be reproductively active. Endocrine results will be included as part of a subsequent report.

3.5 CAPTIVE BREEDING

Twelve females were housed in the newly completed Captive Stoat Breeding Facility (Landcare Research, Lincoln) in individual pens (2 × 4 × 2 m) with natural cover and shelter. In addition, each stoat pen was supplied with two nest boxes (40 × 33 × 15 cm) containing shredded paper and feathers as nesting material. Stoats were fed a rotation of dead 1-day-old chicks, chicken pet mince and mutton/beef pet mince in the afternoon, and had free access to water.

During each breeding season (October–December), females were observed for physical signs of oestrus and breeding pairs were established by introducing an adult male into the pen of an oestrus female. Previous experience at the Landcare Research Animal Facility indicates that stoats may kill each other if care is not taken when establishing pairs of animals in the same pen. Several nest boxes with entrance and exit holes were provided for escape or as refuges. The animals were closely observed for mating and aggressive behaviour. In all cases, the female tolerated the male, and they were left together for 2–14 nights. The absence of a swollen vulva 10–14 days later indicated that mating had been successful, and that the female had ovulated and was no longer in oestrus. If a pairing was believed to have been unsuccessful, the procedure was repeated with a different male.

Females remained in individual pens until the birth of offspring (the following October/November). During the subsequent two breeding seasons, post-partum females and any juvenile females were mated 3–10 weeks post-natally using the same protocol.

4. Results and discussion

4.1 EXISTING STOAT DATA RELEVANT TO BREEDING SUCCESS

Between 1998 and 2001, 11 female stoats died or were euthanased at the Landcare Research Animal Facility. These individuals were necropsied, but none were found to be pregnant. Unfortunately, most of the females had been in captivity for more than 10 months and none of the female stoats had died in the months during which blastocyst reactivation occurs or when implanted embryos are expected to be present (August–October). Blastocysts were recovered from all eight animals caught in the St Arnaud area during December and from the two females caught in July. A female that was trapped in late August was visibly pregnant and eight embryos were recovered (Fig. 4). For frozen samples, blastocysts could not be visualised through the uterine wall; however, they were recovered by flushing the tract with PBS solution. A total of 51 blastocysts were recovered from the eight stoats. All were damaged by the freeze-thaw process.

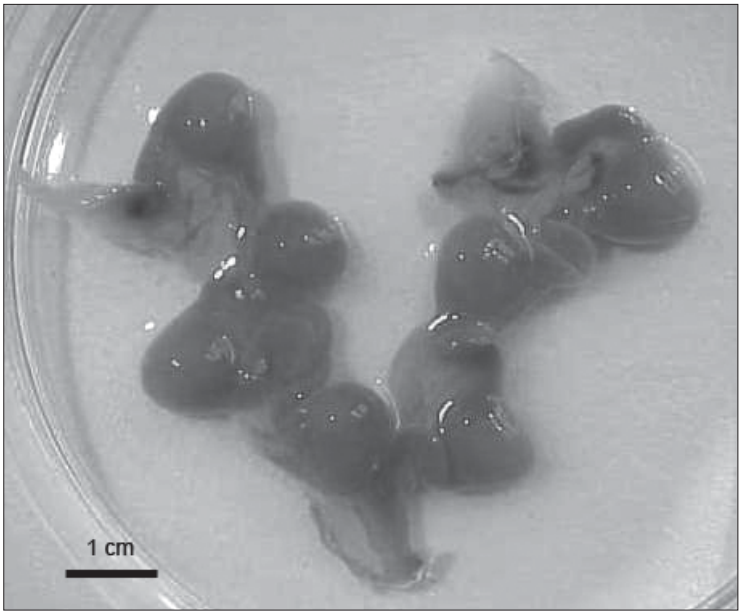

Figure 4. Developing embryos *in utero* from a female stoat (*Mustela erminea*) captured at St Arnaud in August.

4.2 ACCLIMATISATION TO CAPTIVITY

4.2.1 Immunological tests

As there are no published reference ranges for haematological parameters for stoats, baseline data were collected from 24 individuals (see Appendix 1). There were no differences between the blood parameters of freshly captured male (n = 5) and female (n = 7) stoats (unpubl. data), but values did change during acclimatisation (Appendix 1, Table 1).

In the acclimatisation study, differential counts of white blood cells from freshly captured females (Week 0) were dominated by neutrophils (85%), while lymphocyte populations were low (8%) (Table 1), which is indicative of stress. By Week 12, the proportion of neutrophils (52%) and lymphocytes (30%) (Table 1) were more like those reported for captive-born mustelids (Thornton et al. 1979; Lee at al. 1982; Quesenberry 1997), indicating that stoats had adjusted to captivity. In addition, mean corpuscular haemoglobin concentration had increased in the wild-captured females by Week 12, possibly reflecting improved nutrition.

Stoats had readily detectable proliferation responses to lymphocyte mitogens ConA (Fig. 5) and PWM (Fig. 6), but not to LPS (Fig. 7). Maximum rates of cell division were detected following the treatment of cultures with 50 μg/mL ConA and with 6 μg/mL PWM. At these concentrations, lymphocyte proliferative responses peaked at 12 weeks post-capture. These results demonstrate that it is possible to measure the cell-mediated immune response of the blood lymphocytes of stoats and that the T cell function of wild-caught stoats may be detrimentally affected for several weeks post-capture, possibly due to high levels of physiological stress (Buddle et al. 1992; Cross et al. 1999).

TABLE 1. CHANGES IN DIFFERENTIAL COUNTS OF WHITE BLOOD CELLS AND CORPUSCULAR HAEMOGLOBIN CONCENTRATION OVER TIME IN FEMALE STOATS (*Mustela erminea*) IN THE ACCLIMATISATION STUDY.

Mean (± SEM) values are shown for neutrophils (neut), lymphocytes (lymph) and corpuscular haemoglobin concentration (CHC). ANOVA results are presented in the footnotes; values that are assigned different letters within a column are significantly different (Tukey's test; $P < 0.05$).

WEEKS IN CAPTIVITY	NUMBER OF STOATS	NEUT (%)*	LYMPH (%)†	CHC (g/L)‡
0	7	85.6 ± 3.3 a	8 ± 1.5 a	144 ± 11.3 a
4	4	57.3 ± 7.8 b	27 ± 6.1 ab	160 ± 8.4 ab
12	5	52.3 ± 4.2 b	30 ± 5.4 ab	171 ± 4.2 b
> 24	2	43.5 ± 12.5 b	37 ± 7.5 b	180 ± 3.5 b

* F = 7.5, df = 1, 13, P = 0.006.

† F = 3.7, df = 1, 13, P = 0.05.

‡ F = 3.6, df = 1, 13, P = 0.05.

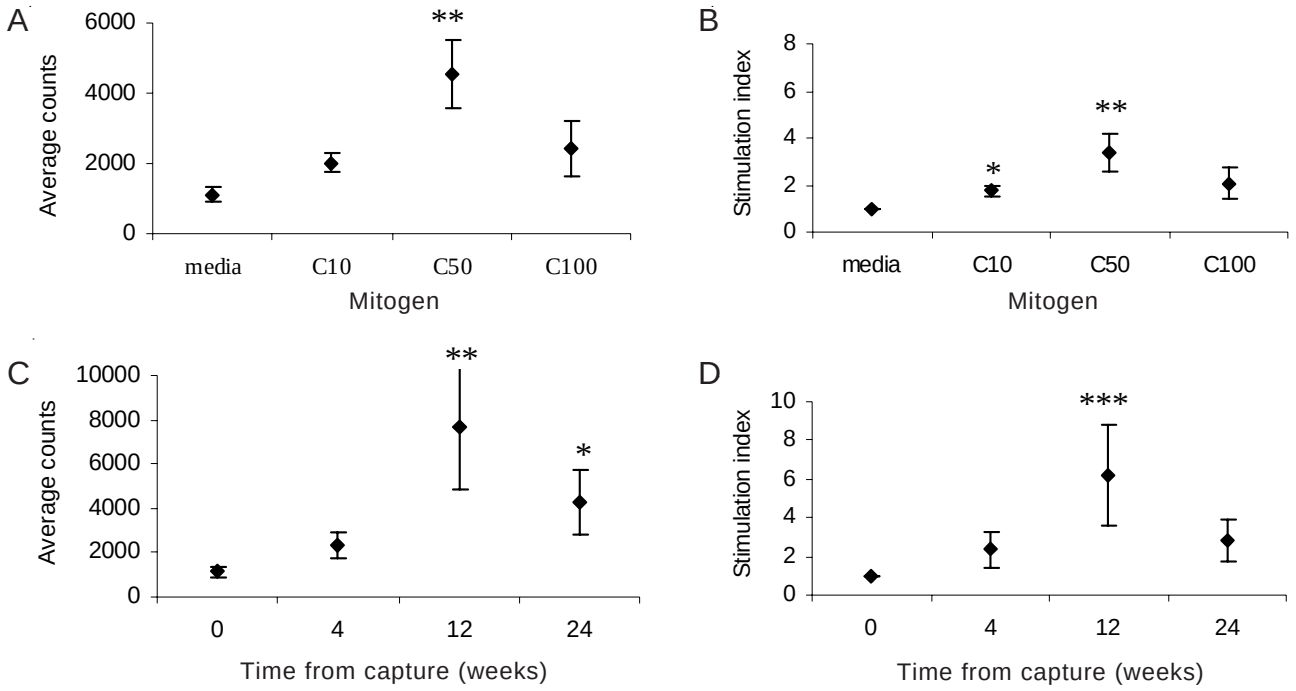

Figure 5. Stoat (*Mustela erminea*) peripheral blood lymphocyte proliferative responses to Concanavalin A, showing the effect of the addition of media, 10 (C10), 50 (C50) and 100 (C100) µg/mL Con A mitogen on thymidine incorporation (counts per minute) (A), and stimulation index (i.e. thymidine incorporation with mitogen/thymidine incorporation with media) (B); and the effect of time since capture on thymidine incorporation (C), and stimulation index (D). Values are presented as mean ± SEM. Asterisks indicate values that are significantly different from the media control or week 0 (Tukey's test, $P < 0.05$; $\log_{10}$ transformed data).

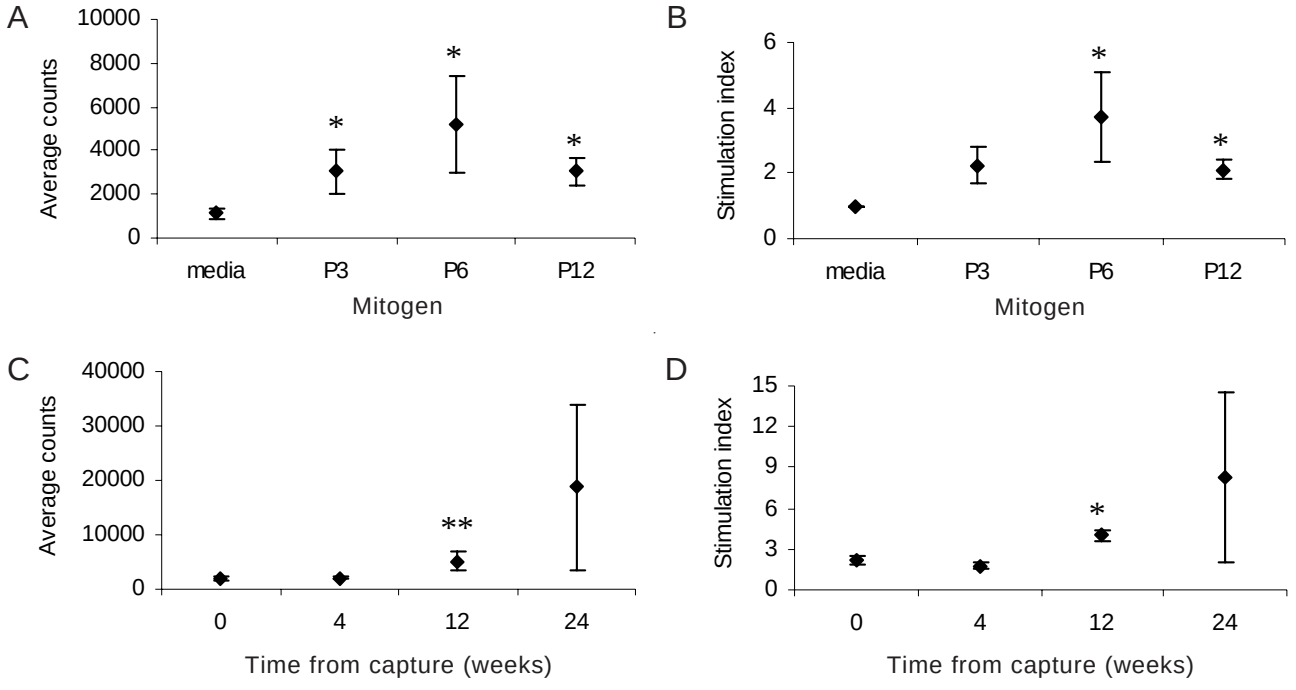

Figure 6. Stoat (*Mustela erminea*) peripheral blood lymphocyte proliferative responses to Pokeweed mitogen (PWM), showing the effect of the addition of media, 3 (P3), 6 (P6) and 12 (P12) µg/mL PWM on thymidine incorporation (counts per minute) (A), and stimulation index (i.e. thymidine incorporation with mitogen/thymidine incorporation with media) (B); and the effect of time since capture on thymidine incorporation (C), and stimulation index (D). Values are presented as mean ± SEM. Asterisks indicate values that are significantly different from the media control or week 0 (Tukey's test, $P < 0.05$; $\log_{10}$ transformed data).

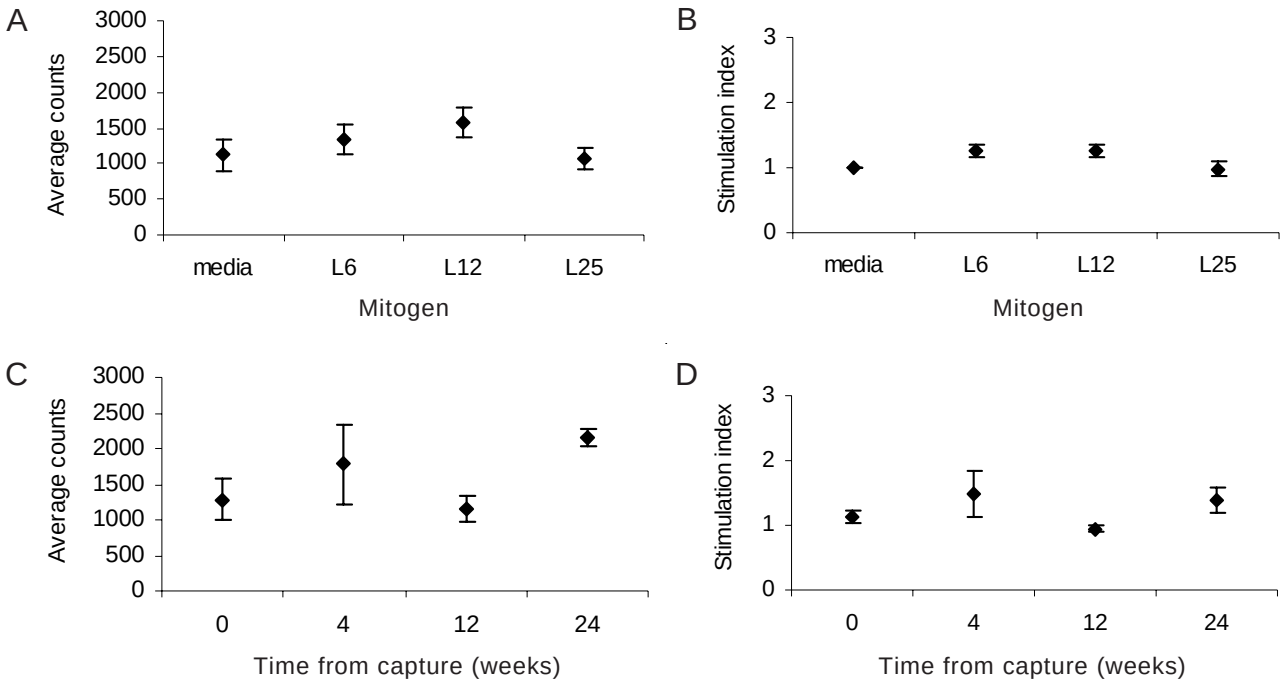

Figure 7. Stoat (*Mustela erminea*) peripheral blood lymphocyte proliferative responses to Lipopolysaccharide (LPS), showing the effect of the addition of media, 6.25 (L6), 12.5 (L12) and 25 (L25) μg/mL LPS mitogen on thymidine incorporation (counts per minute) (A), and stimulation index (i.e. thymidine incorporation with mitogen/thymidine incorporation with media) (B); and the effect of time since capture on thymidine incorporation (C), and stimulation index (D). Values are presented as mean ± SEM. No values were significantly different from the media control or week 0 (Tukey's test, $P > 0.05$; $\log_{10}$ transformed data).

4.2.2 Behavioural responses

There were no clear patterns over time for most of the immediate behavioural responses tested. The behaviour shown by the greatest number of individuals was looking at observer (mean: 36% of animals). The next most common responses were hide behaviours (16%) and showing no response (16%), and 12% of stoats vocalised. However, from 8 weeks post-capture there was a decrease in the percentage of animals that stayed concealed where they were, and a marked increase in movement behaviour (Fig. 8). It was not until after 6 weeks in captivity that at least 40% of stoats had taken the chick (eaten or cached) from the food bowl within the first hour of the food being offered. Together these observations suggest that behavioural adaptation to captivity may take 6–8 weeks.

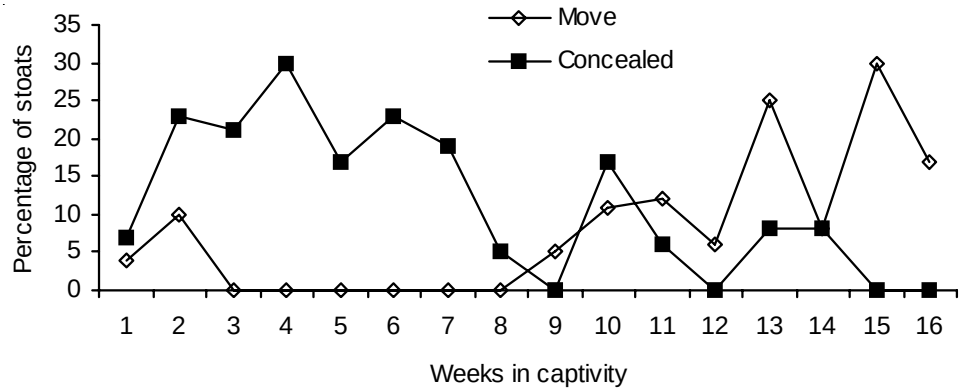

Figure 8. Proportion of stoats (*Mustela erminea*) displaying a move or concealed behavioural response over time.

4.2.3 Fate of blastocysts

Between Weeks 0 and 24, 20 of the 21 females had blastocysts present at the time of sampling. The one exception was a stoat that was sampled at Week 12. There was no effect of time from capture on blastocyst retention (F = 0.79, df = 3, 17, P = 0.515). Both animals that were sampled at Week 24 had blastocysts (n = 4 and n = 8); however, it should be noted that the small sample size (n = 2) at Week 24 makes it difficult to draw definitive conclusions about blastocyst survival after Week 12 (Table 2). Because blastocysts were still present in the two animals that were sampled 24 weeks post-capture, four remaining females were held until Week 36 to determine whether blastocysts would successfully implant and result in the birth of live young. However, no live births were recorded and, when two of these animals were euthanased at 36 weeks, no implantation sites or blastocysts were detected. Although blastocysts were present in the uteri of captive animals for at least 24 weeks post-capture, it was not possible to determine whether these blastocysts were still viable. Successful implantation is a complex process requiring live embryos, functional luteal structures and appropriate signals from the central nervous system to coordinate reactivation, implantation and pregnancy in this unique species. More research on these key physiological pathways is required.

TABLE 2. MEAN BLASTOCYST SURVIVAL OVER TIME IN STOATS (*Mustela erminea*) IN CAPTIVITY.

WEEKS IN CAPTIVITY	NUMBER OF STOATS		NUMBER OF BLASTOCYSTS (MEAN ± SEM
	SAMPLED	WITH BLASTOCYSTS	
0	7	7	8.6 ± 0.8
4	6	6	8.3 ± 1.2
12	6	5	5.8 ± 1.9
24	2	2	6.0 ± 2.0

4.3 ASSESSMENT OF REPRODUCTIVE FUNCTION

Techniques now exist to accurately assess oestrus activity in stoats. For females, the vulva is enlarged and swollen with a slight discharge during oestrus. However, the signs in female stoats were much more subtle than those reported for mink, ferrets and canids (Christiansen 1984; Andrews & Illman 1987; European Mink Biology and Conservation 1995) or observed in ferrets and canids (JT, pers. obs.). A total of 47 vaginal wash samples were collected from non-oestrus (n = 34), possibly oestrus (n = 4) and oestrus (n = 9) females, and cell types were tested against reproductive state (Table 3). When compared with stoats that had been classified visually as non-oestrus or possibly oestrus, oestrus females had significantly higher numbers of keratinised epithelial cells present in the vaginal wash (F = 26.1, df = 2, 44, P < 0.001) and the ratio of keratinised to non-keratinised epithelial cells increased (F = 14.4, df = 2, 44, P < 0.001). For subsequent trials, an animal was described as being in oestrus if

the ratio of keratinised to non-keratinised epithelial cells exceeded 7.0 (the lower value of the 95% confidence interval of this ratio in female stoats displaying visible physical changes associated with oestrus).

In wild male stoats, as the breeding period approaches there is a rapid growth in the size of the testes, and testis weight at least doubles between June and September. The increase in testis weight is associated with increases in the quantity and quality of sperm present in the epididymides (King & Moody 1982). Similar increases in testis size were found in captive stoats (Table 4).

TABLE 3. CONCENTRATIONS OF KERATINISED AND NON-KERATINISED EPITHELIAL CELLS AND THEIR RATIO, AND NUMBERS OF RED AND WHITE BLOOD CELLS IN VAGINAL WASHES FROM FEMALE STOATS (*Mustela erminea*) THAT HAD BEEN CLASSIFIED VISUALLY AS IN OESTRUS, POSSIBLY IN OESTRUS OR NON-OESTRUS.

Values are expressed as mean ± 95% confidence interval. ANOVA results are shown; values that are assigned different letters within a row are significantly different (Tukey's test; $P < 0.05$).

CELL TYPE	NUMBER OF CELLS ($\times 10^6$ CELLS/mL)			*F*	df	*P*
	NON-OESTRUS ($n = 34$)	POSSIBLY OESTRUS ($n = 4$)	OESTRUS ($n = 9$)			
Keratinised cells	15.7 ± 8.1 a	23.0 ± 22.2 a	97.2 ± 39.8 b	26.1	2, 44	< 0.001
Non-keratinised cells	9.8 ± 2.4 a	13.0 ± 8.3 a	3.9 ± 3.5 a	2.1	2, 44	0.132
Ratio (keratinised:non-keratinised)	2.56 ± 0.71 a	1.95 ± 1.27 a	57.5 ± 50.3 b	14.4	2, 44	< 0.001
White blood cells	55.6 ± 23.1 a	51 ± 61.9 a	36.8 ± 48.8 a	0.15	2, 44	0.857
Red blood cells	27.6 ± 35.2 a	14.2 ± 45.3 a	29.8 ± 51.6 a	0.04	2, 43	0.957

TABLE 4. TESTIS LENGTH AND WIDTH (BOTH TESTES) IN CAPTIVE MALE STOATS (*Mustela erminea*) UNDER NATURAL PHOTOPERIOD.

Values are means ± SEM. ANOVA results are shown in the footnotes; values that are assigned different letters within a column are significantly different (Tukey's test; $P < 0.05$).

DATE	TESTIS LENGTH (mm)*	TESTIS WIDTH (mm)†
18 March	15.4 ± 1.1 a	14.7 ± 1.2 a
30 April	16.7 ± 097 a	13.9 ± 0.74 a
25 June	17.4 ± 1.06 ab	15.5 ± 0.79 ab
26 July	15.6 ± 0.73 a	18.2 ± 0.82 b
26 August	20.7 ± 0.62 b	18.7 ± 0.57 b

* $F = 5.67$, df = 1, 52, $P = 0.002$.

† $F = 5.41$, df = 1, 52, $P = 0.001$.

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