Feasibility of immunocontraception for managing stoats in New Zealand

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CONTENTS

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ABSTRACT

Predation by mustelids, particularly stoats, is a significant threat to the persistence of many populations of endemic birds of New Zealand. Broadscale management of stoats is constrained by the associated risks to non-target species. Control methods that are more specific to stoats are laborious and costly. These methods can protect only a few threatened bird populations and only in small portions of habitat. Beyond these treated areas the declines of bird populations and extinctions past or imminent indicate a pressing need for more extensive management of stoats. New cost-effective methods for managing stoats over large areas are needed urgently. This study addresses that need by examining the literature to assess the potential for fertility control of stoats, especially immunocontraception, to contribute to the sustainable conservation of endemic New Zealand birds.

Key recommendations are:

- Use of current proven management techniques for stoats and other predators or pest prey should continue and be refined.
- Consideration should be given to the development of a non-disseminating immunocontraceptive genetically modified organism (GMO) as the highest priority option for fertility control.
- A robust bait attractive to stoats or mustelids and suitable for aerial delivery should be developed.
- Chemosterilants currently have low species-specificity and further research into their use should have low priority.
- The literature search for lethal biocontrol agents of stoats or mustelids did not reveal any outstanding candidates. Lethal biocontrol is a long-term research option and should have low priority.
- Further ecological research is required to:

  — determine the reduction in stoat density required to protect species threatened by predation;

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— develop strategies for integrating fertility control with existing management techniques.

TERMS OF REFERENCE

1 Assess the feasibility of applying reproductive technology to produce a species-specific immunocontraceptive agent for stoats. (Chapter 2)

2 Assess the possible methods of delivering the immunocontraceptive agent to stoats, particularly via disseminating viruses, but including bacteria, parasites and baits. (Chapter 3)

3 Comment on the feasibility of using reproductive control compounds, such as cabergoline, for stoat control. (Chapter 4)

4 Comment on the possible use of canine distemper or other diseases as a mortality agent for stoats. (Chapter 5)

5 Assess the possibility of using the same or similar stoat reproductive and lethal biocontrol methods for weasel and ferret control. (Chapter 6)

6 Suggest strategies and methods that could enhance reproductive or lethal biocontrol of stoat populations, such as using current traps, lures, baits and toxins. (Chapter 7)[1]

7 Assess the likely constraints within New Zealand and internationally relative to the use of a fertility control agent or pathogen for control of stoats in New Zealand. This should include any international agreements and New Zealand regulatory or approval processes and risk assessments for GMOs or any other new techniques. (Chapter 8)

8 Provide a 'systems' assessment of the ecological, social/political and economic consequences of carrying out no stoat control, current stoat control, innovative control techniques (excluding biocontrol), reproductive biocontrol and lethal biocontrol. (Chapter 9)

9 Provide recommendations of priority research required for modelling studies, laboratory development, field evaluation and implementation of reproductive and lethal biocontrol methods for stoats. (Appendix 2)

10 Provide a flow diagram and description of the different components and stages necessary for the consideration, development, field evaluation and implementation of reproductive or lethal biocontrol of stoats. (Appendix 3)

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[1] TOR 6 requires consideration that immunocontraception of stoats be a component of one or more of the array of potential management plans. These are reviewed in Appendix 1.
The References section (Chapter 11) covers both the body of the report and the appendices.

ABBREVIATIONS

cDNA	= complementary DNA
CDV	= canine distemper virus
CL	= corpus luteum or corpora lutea
DNA	= deoxyribonucleic acid
GMO	= genetically modified organism
IC	= immunocontraception
ISCOM	= immunostimulatory complex
LH	= luteinising hormone
LIF	= leukaemia inhibitory factor
LMO	= living modified organism
mRNA	= messenger RNA
OIE	= Office International des Epizooties
RHDV	= rabbit haemorrhagic disease virus
RNA	= ribonucleic acid
Tb	= tuberculosis
VLP	= virus-like particle
ZP	= zona pellucida

DEFINITIONS

Chemosterilant = chemical or hormone (usually synthetic), or agonist or antagonist of hormone or hormone receptor which inhibits the action of the endogenous hormones in the hypothalamic-pituitary-gonadal axis or reproductive tract.

Immunocontraception = use of the body's immune system to induce immune responses (circulating antibodies or cellular immune effector cells) against reproductive cells or proteins essential to successful gametogenesis, fertilisation or implantation, leading to infertility.

Lethal biocontrol organism = parasite, bacterium, fungus or virus that causes death of the affected animal.

Disseminating immunocontraceptive GMO = disseminating organism (virus, bacterium, parasite) which has been genetically modified to express a mammalian reproductive gene which in the infected host induces an immune response leading to infertility.

Non-disseminating immunocontraceptive GMO = as above except that the modified organism (virus, bacterium, parasite) is highly attenuated or 'crippled' such that it does not transmit between individuals.

Non-disseminating immunocontraceptive non-GMO product = reproductive protein that is produced either by conventional biochemistry or by expression from a GMO which after purification does not contain any genetically modified material. The product would be packaged in a carrier that will induce an immune response leading to infertility.

EXECUTIVE SUMMARY

The problem of managing stoats in New Zealand in an array of different habitats, in which there are various suites of predators, prey and threatened species, is complex. Conventional methods of control of predators have been effective in protecting and recovering some threatened populations. However, the cost and effort required limits the extent to which it can continue to be applied to threatened populations, and many remain unprotected and declining. If protection is to be extended to greater areas, more populations and other habitats, it will be essential to improve on the cost-effectiveness of managing predation.

Current proven management techniques for stoats and other predators or pest prey should continue and be refined. However these techniques are not likely to achieve the control or cost-effectiveness necessary for the sustainable conservation of species threatened by predation, and no single method of management will provide a universal solution.

Biocontrol, including fertility control, has potential as an additional management tool for stoats and should be considered for development as part of a longer-term strategy. However, there must be careful consideration of the essential characteristics of any biocontrol agent. Those characteristics include, for example: specificity, route of delivery, stability and efficacy, humaneness, environmental impact and public acceptance.

The cost-effectiveness of biological control depends on the means of its spread. Contagion is the most cost effective but, if contagion is not possible, broad-scale delivery is required. Biological control agents suited to these forms of delivery would require different times for development.

Both means of delivery of a lethal agent or a fertility control agent require that the agent be target-specific, or that protective immunisation of all non-target species at risk is feasible and practical. For broad-scale delivery, a bait which is robust and attractive to stoats or mustelids would need to be developed.

Five approaches to biocontrol—two of which do, and one of which may, involve the use of genetically modified organisms (GMOs)—are considered in this review. They are:

- lethal biocontrol organisms
- disseminating immunocontraceptive GMOs
- non-disseminating immunocontraceptive GMOs
- non-disseminating non-GMO immunocontraceptive products
- chemosterilants

Because no mustelid-specific mortality agents have been identified from a search of the literature, the potential for use of lethal biocontrol agents is limited unless further research is undertaken. Exploitation of pathogens for biocontrol purposes (lethal agent or vaccine vector) would require extensive research on the epidemiology, efficacy and species-specificity of existing disease agents or vectors in and on Mustelidae in New Zealand. Consideration of pathogens currently not in New Zealand would raise issues of importation and an additional delay to possible introduction.

Canine distemper virus is a possible lethal biocontrol for stoats, weasels and ferrets, but is not specific to mustelids. It is present in dogs in New Zealand and thus would not require importation. Its delivery by bait could reduce non-target impacts and a vaccine is available to protect domestic species and zoo animals. More detailed experimental assessment of the effects of infection of stoats with this virus is required.

Other parasitic organisms of stoats or Mustelidae, ecto-parasites and endo-parasites, bacteria and fungi, offer no obvious mortality agent for application to the control of stoats in New Zealand. Generally they are not highly host-specific and Mustelidae share a surprising number of parasites with members of the Felidae and Canidae. Species of *Strongyloides* often are reasonably host-specific and while they may not be useful mortality agents, they are possible candidates as vectors for immunocontraceptive vaccines.

The literature search for potential vectors of immunocontraceptives for stoats or mustelids did not reveal any clear candidates.

Viruses that affect mustelids are not well researched. Most records are case history reports of serological or histological evidence of infection rather than virus isolation.

Viruses that may be suitable vectors of an immunocontraceptive vaccine were identified, although no candidates are stoat-specific or mustelid-specific. Adenoviruses, herpesviruses and poxviruses are being used in other species but their potential for delivering an immunocontraceptive to stoats is unknown and a significant amount of research would be required to ascertain this.

Despite problems with potential vectors, the reproductive physiology of stoats appears amenable to disruption using current concepts of immuno-contraceptive fertility control. Their breeding pattern is seasonally regulated and includes a long period of embryonic diapause and delayed implantation. They produce one litter each year. Several critical steps in this reproductive cycle could be disrupted including oocyte development, function of the corpus luteum on the ovary, embryonic diapause, active pregnancy, and lactation. In addition, the mating system of stoats does not appear to have any mechanism to compensate for fertility control, for example through release of inhibition of reproduction in subordinate animals.

Research is required to identify and characterise the likely reproductive antigens and to determine their specific effects in the stoat. The specificity of these antigens for either stoats or other mustelids will need to be determined.

The reproductive cycles of ferrets and weasels are also regulated by seasonal changes in photoperiod, but these species do not show delayed implantation. They can produce two litters per season but in New Zealand this is thought to be rare. The same suite of reproductive antigens may also be effective for fertility control of ferrets and weasels but either the antigens or the delivery method would need to be mustelid-specific.

Chemosterilants currently have low specificity. Their delivery to stoats or mustelids would be similar to that of non-specific poisons, and as labour-intensive and costly. Consequently the cost-effectiveness of their use with respect to poisons is very doubtful. Research in this area would only be a low priority.

There may be some value in exploring the potential for the use of the chemical agent, cabergoline; this inhibits prolactin secretion and therefore could affect the success of pregnancy and/or lactation. The effects of this agent have been assessed in several species including dogs, cats and rodents, but not mustelids. Clearly cabergoline is not a species-specific agent and, if shown to be effective in stoats, ferrets or weasels would require a judicious program of use in the field to reduce any non-target effects. Current availability and price may curtail its immediate use.

Another chemical warranting some further investigation is the antiprogesterone compound, mifepristone or RU486. This agent binds to progesterone receptors in tissues, particularly the endometrium of the uterus, and prevents the action of endogenous progesterone. Its effects in mustelids have not been investigated. It is not species-specific although some species do not respond to this agent. Practical use of mifepristone would require a mustelid-specific delivery method, in which case its cost-effectiveness with respect to poison is questionable. Current availability and price may curtail its immediate use.

A review of the ecological, social, economic and legislative context of stoat control provides a framework for ranking the five new control options listed above. Assuming that the sustainable conservation of species threatened by predation is the primary objective, and that substantial progress towards product development is required within the timeframe of the current Stoat Research Programme, it is recommended that consideration be given to the development of a non-disseminating immunocontraceptive GMO as the highest priority. This would require parallel research into a bait matrix suitable for broad-scale fertility control of stoats.

A strategic research plan has been developed outlining possible approaches that could be taken to develop several different biocontrol agents. One of these, development of a non-disseminating immunocontraceptive GMO vaccine, has practical advantages over the other approaches at the present time.

The development of an immunocontraceptive vaccine will require a 5–10-year investment and will include the following areas of research, some of which will be conducted in parallel:

- search for a candidate organism
- assessment of the suitability of the candidate organism for genetic modification
- identification of potential reproductive antigen(s)
- construction of the non-disseminating GMO
- assessment of the effects of the non-disseminating GMO on the fertility of stoats, and/or ferrets and weasels
- assessment of species-specificity
- development of a robust bait suitable for aerial delivery
- assessment of the requirements for compliance with the regulatory process in New Zealand, including public acceptance of the technology and its benefits

The overall task is difficult, and although the pathway to achieving a product is technically feasible, there is no guarantee of success.

In conjunction with vaccine development, further ecological research, including population modelling, is required to identify the key threatening processes for native species, to determine the reduction in stoat density required to protect species threatened by predation and to develop strategies for integrating fertility control with existing management techniques.

The use of a non-disseminating immunocontraceptive GMO will require the approval of the New Zealand community, and must comply with the regulations relating to GMOs in New Zealand. This process will depend on public attitudes and perceptions regarding the safety of the product and its benefits for conservation of threatened species in New Zealand.

1. Introduction

Fertility control has been suggested as a more appropriate control strategy than enhancing mortality for species with high fecundity (Caughley *et al.* 1992; Tyndale-Biscoe 1994), high natural mortality rates and a rapid population turnover (Stenseth 1981; Bomford 1990; Hone 1992; Barlow 1994; Barlow *et al.* 1997). Also fertility control offers a more humane method of population control (Marsh & Howard 1973; Hutchins *et al.* 1982; Hutchins & Wemmer 1987) and could be of benefit for preventing or reducing population growth after other techniques have reduced numbers, particularly in long-lived species (Bomford 1990; Barlow 1994).

The objective of fertility control is to reduce population size by reducing the number of young produced and recruited into the population. Therefore the fertility control method needs to

- cause temporary or permanent sterility leading to reduced recruitment in the population,
- be deliverable in a way that allows an adequate proportion of the target population to be treated, including widespread and abundant species in areas with poor access,
- reduce the target population sufficiently to reduce damage to the desired level (Braysher 1993),
- produce no undesirable side effects to the target species (e.g. behavioural changes, interference with social structure),
- be target-specific and environmentally benign (Marsh & Howard 1973) and
- be cost-effective compared with conventional methods of control (Bomford 1990; Bomford & O'Brien 1997).

This report reviews the potential and the feasibility of immunocontraception for managing stoats in New Zealand. Will fertility control be part of management plans that will prolong the persistence of endangered bird populations in New Zealand? The terms of reference specified, among other things, five broad questions:

- Could an immunocontraceptive strategy reliably induce infertility in stoats?
- What aspects of stoat reproduction should be targeted by immunocontraception?
- What are the likely best antigens and vectors for immunocontraception?
- Is stoat population biology amenable to an immunocontraceptive approach that would cause useful reductions in stoat population abundance?
- How could immunocontraception be integrated with other management tools for likely best effect in conserving the threatened populations of native bird species?

1.1 TAXONOMY OF MUSTELIDAE

The Family Mustelidae comprises five subfamilies, one of which is the Subfamily Mustelinae. In this subfamily there are four genera of which the Genus *Mustela* contains the various species of mink, stoat, ferrets and weasels (Fig. 1). The predominant natural distribution of this family and subfamily is the Northern Hemisphere, although their range is worldwide with the exception of Australia and Malagasy. The stoat, *Mustela erminea*, the ferret, *Mustela putorius furo*[*], and the weasel, *Mustela nivalis*, all occur in New Zealand with the stoat considered the major pest and predator of native birds. These species were introduced in the 1880s (King 1990).

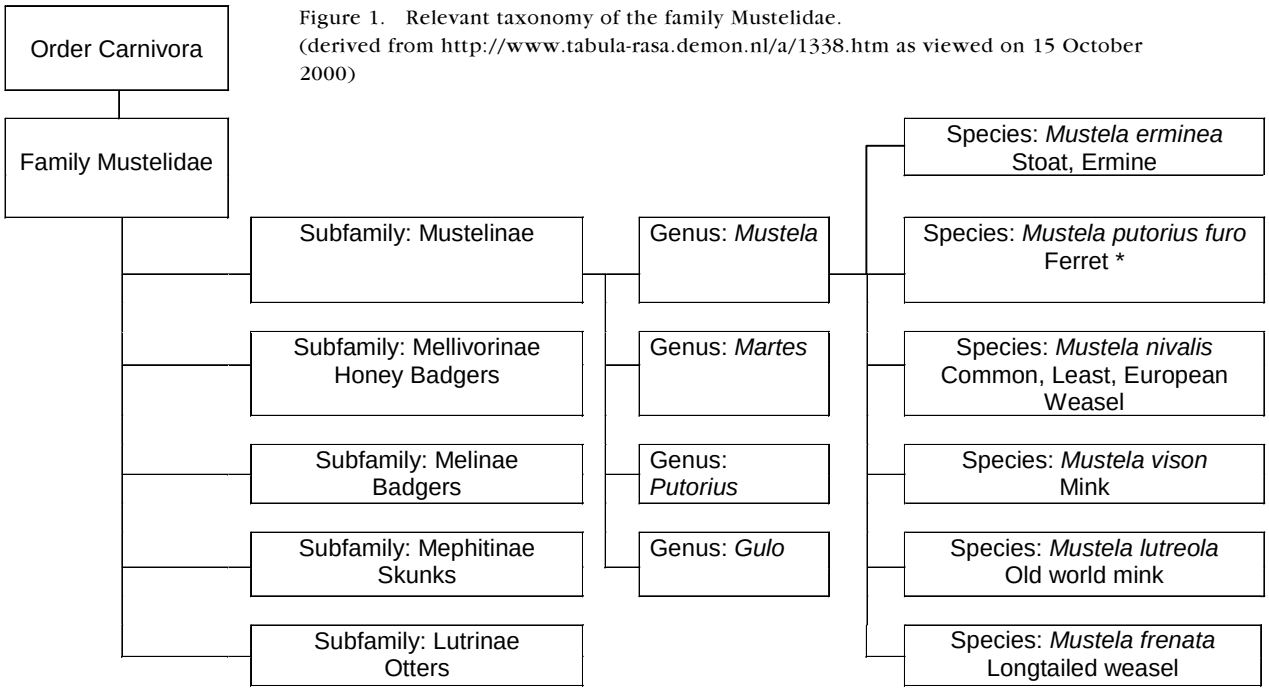

Figure 1. Relevant taxonomy of the family Mustelidae.
(derived from http://www.tabula-rasa.demon.nl/a/1338.htm as viewed on 15 October 2000)

[*]In the Handbook of New Zealand Mammals (King 1990), the ferret is referred to as *Mustela furo*

2. Applying reproductive technology to produce a stoat-specific immunocontraceptive agent

2.1 REPRODUCTIVE BIOLOGY OF STOATS

Members of the family Mustelidae show three distinct patterns of reproduction (Lamming 1984; Mead 1981, 1993a,b).

- Delayed implantation and extended embryonic diapause lasting several months. This is a feature of most mustelid species including the stoat, *Mustela erminea*.
- A short period of delayed implantation which lasts only 2–6 weeks. This pattern is seen in species such as American mink, *Mustela vison*.
- No delay in implantation. This pattern is seen in the domestic ferret (*Mustela putorius furo*) and the weasel (*Mustela nivalis*).

Figure 2 illustrates the features of the annual reproductive cycles for stoats, ferrets and weasels (adapted from Lamming 1984). However, it should be noted that in New Zealand ferrets rarely produce a second litter in a normal breeding cycle (P. Cowan, pers. comm.).

Each species breeds seasonally with births occurring in spring and summer. The general reproductive biology of many mustelids has been described (Lamming 1984). However the endocrine control of obligate delayed implantation has only

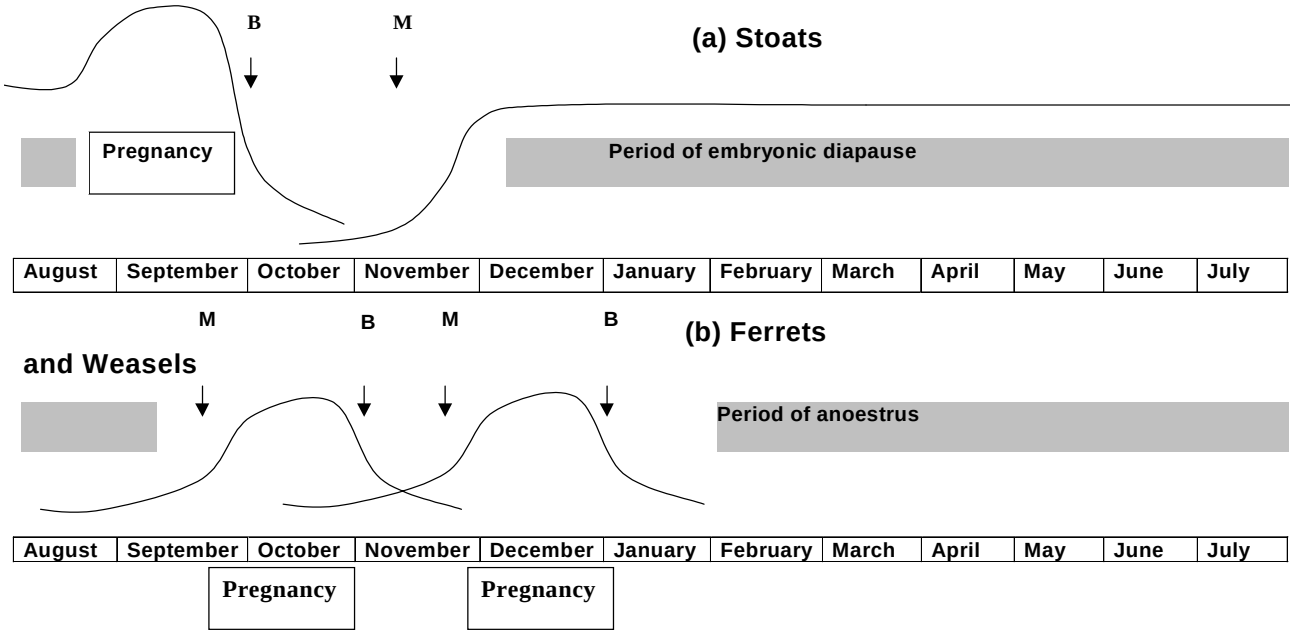

Figure 2. Diagrammatic representation of the annual reproductive cycles of (a) stoats and (b) ferrets and weasels in New Zealand. M = Mating; B = Birth. The curves represent the approximate diameter of the corpora lutea (CL) as an index of their activity. Stoats show a period of 8–9 months of embryonic diapause during which the CL are quiescent. Ferrets and weasels show a period of anoestrus for about 6 months. (Re-drawn from Lamming 1984.)

been studied extensively in three species: mink, western spotted skunk and the badger (Moller 1973; Canivenc & Bonnin 1981; Martinet *et al*. 1981a,b; Mead 1981, 1993b). The domestic ferret is used extensively for laboratory-based medical studies, and therefore the regulation of its reproductive cycle is relatively well understood. However little is known of the hormonal regulation of reproduction in the weasel (see Chapter 6).

Delayed implantation and embryonic diapause represents a period of arrested development of the corpus luteum (CL) on the ovary and of the blastocyst in the uterus. After conception the embryo develops to the blastocyst stage of several hundred cells but further development is arrested and it remains in an arrested state in the uterus for a specific period. The corpora lutea (CL) formed after ovulation do not actively secrete progesterone, but also remain in an arrested, involuted state.

Depending on the species, the period of arrested development may last for a few weeks or up to several months and is regulated by changes in daylength (Mead 1981; 1993b; Martinet *et al*. 1981b, 1983). Several studies of the regulation of embryonic diapause and the hormones involved have been undertaken and have shown that increasing daylength triggers reactivation of the diapausing corpora lutea in the ovary. In species such as the western spotted skunk, the effects of photoperiod are mediated by melatonin and prolactin (Mead 1981). Melatonin exhibits its suppression of luteal function and implantation by delaying a photoperiod-associated rise in prolactin that leads to reactivation of the corpora lutea. The secretion of progesterone from the corpora lutea induces reactivation of the blastocysts and the onset of implantation in the uteri. After reactivation of development, the luteal cells do not undergo further mitoses, but enlarge and become terminally differentiated luteal cells (Douglas *et al*. 1998a).

The mink, *Mustela vison*, shows a brief period of delayed implantation. Several studies have shown that luteal activity and implantation, as well as luteal maintenance after implantation, require an intact pituitary. Subsequent studies have shown that pituitary prolactin is the major luteotrophic hormone and it is essential for the reactivation of the CL, termination of embryonic diapause and normal function of the CL after implantation (Papke *et al*.1980; Martinet *et al*. 1981a; Murphy *et al*. 1981). A role for luteinising hormone (LH) in the support of post-implantation CL function was first indicated by passive immunisation with gonadotropin releasing hormone (Murphy *et al*. 1993). Further, Douglas *et al*. (1998a) have shown that luteal cells isolated from diapausing CL of the mink can be stimulated *in vitro* by the pituitary hormones, prolactin and luteinising hormone (LH), to produce progesterone. The expression of prolactin and LH receptors in mink CL has also been determined during diapause, implantation and active gestation. The effects of treatment with bromocriptine, an inhibitor of endogenous prolactin, on the abundance of the mRNA for the prolactin receptor were also determined. Douglas *et al*. (1998b) found that prolactin receptor mRNA was low during diapause and CL activation but increased 3-fold as embryos began to implant in the uteri. The prolactin receptor mRNA abundance correlated with prolactin binding in the ovaries and with the level of circulating prolactin. LH receptor mRNA was relatively constant except for a rise at the time of termination of diapause and CL reactivation. Treatment with bromocriptine (2 mg/day) suppressed endogenous levels of prolactin and

prevented the increase in prolactin receptor mRNA. It also prevented the rise in LH receptor mRNA and reduced its abundance to below pre-treatment levels. The results indicate prolactin up-regulating its own receptor as well as influencing the LH receptor in the mink CL.

Although similar studies on the regulation of delayed implantation in stoats have not been undertaken there are likely to be close similarities in the role of prolactin in this species. Prolactin or its receptor may be a potential target for manipulating reproduction in stoats.

Termination of embryonic diapause and the process of implantation and establishment of the chorio-allantoic placenta have been described for mink (Enders 1957). Cytokines and other growth factors, which have an autocrine and paracrine action, are involved in the process of embryo implantation. One of these cytokines, leukaemia inhibitory factor (LIF) regulates proliferation, differentiation and function of many cell types during implantation. Transgenic mice expressing a null mutation in which LIF is not expressed have normal reproductive function, but embryos fail to implant (Stewart *et al*. 1992). In mink, the expression of LIF in the uterine epithelial glands occurs at the time of embryo expansion and 1–2 days post implantation, but not during diapause or later in gestation (Song *et al*. 1998). The cDNA for mink LIF has been cloned and sequenced and shows high identity (>79%) in the coding sequence and in the amino acid sequence with mouse, human, pig, cow and sheep.

Lactation lasts 4–6 weeks in Mustelids and in some species is dependent on the level of sucking stimulus for its maintenance (Lamming 1984). In general in eutherian species, from birth to just before puberty the mammary glands are quiescent, consisting only of ducts. Around the time of onset of the first oestrus there is some growth of the glands and rudimentary alveoli are formed. Cyclic changes in the mammary glands are seen in rodents and bovids, and in some species such as the dog, rabbit and ferret, quite extensive lobular-alveolar development may occur after spontaneous or induced pseudopregnancy. However the major development and differentiation of the gland and formation of true alveoli does not occur until after conception. By mid-pregnancy the connective tissue stroma has been replaced with lobulo-alveolar epithelial tissue and towards the end of pregnancy the alveoli secrete significant quantities of specific milk products (pre-colostrum). In most eutherians mammary gland growth is essentially complete at parturition although some species (rat, mouse, rabbit) show further growth (20–40%) during lactation (Knight & Peaker 1982; Knight 1984). Progesterone produced by the corpus luteum during pregnancy is essential for mammary gland development, as is the pituitary hormone prolactin. After parturition, prolactin is essential for the maintenance of milk production and oxytocin, also released from the pituitary in response to the sucking stimulus, is essential for milk let-down (Knight 1984; Maule Walker 1984). Inhibition of prolactin secretion, prolactin receptors on the mammary gland, or secretion of progesterone could lead to inadequate development of the mammary gland and failure of lactation after parturition.

In New Zealand, as in the Northern Hemisphere, the stoat is a seasonal breeder with the breeding season being regulated by the changing photoperiod. Births occur in late September and October (King & Moody 1982a). Gestation length is 9–10 months but includes 8–9 months of delayed implantation. The females of

litters born at this time are weaned at 5–6 weeks, are mated and conceive before they leave the nest. Their mothers also copulate at this time. Mating induces ovulation in the stoat and the number of ovulations indicate an ovulation rate of 10 (range 6–17) (Gulamhusein & Beck 1972). The newly formed corpora lutea and the newly conceived embryos then become arrested in their development between November and early August (King & Moody 1982a). As with mink, the CL appear inactive during delayed implantation, and the luteal cells enlarge and actively secrete progesterone just before the blastocysts implant in the uteri (Gulamhusein & Beck 1972; Gulamhusein & Thawley 1974). Ovariectomy during delayed implantation causes involution of the uterus and failure of the blastocysts to survive (Shelden 1972). The young females mature to adult body size during the early months of embryonic diapause and females can give birth to litters of about 9 young. Males are sexually mature at one year of age and are fertile between August and February in New Zealand. A high proportion of juveniles born do not survive to the end of their first year. Those that survive live, on average, about 3 years (King 1989).

Little is known of the mating system of stoats in New Zealand (Norbury 2000). The mating system can determine whether immunocontraception will cause a reduction in the population or even an increase (Caughley *et al*. 1992). The mating system in New Zealand depends also on the social organisation of which little is known although it seems to be influenced largely by the abundance of food (King 1990). Females occupy exclusive home ranges that are overlapped by those of males (King 1990; Murphy & Dowding 1994; Moller & Alterio 1999). Both sexes have a solitary habit except during mating and when females alone care for litters (King 1990). These traits suggest that it is unlikely that dominant females suppress breeding in subordinates or that sterility of dominants could release that suppression. Nevertheless, field studies of the genetics of small stoat populations would be useful to determine the mating system of stoats in years of high and low productivity.

Some possible points for disruption of fertility of stoats include:

- Interference with ovarian or oocyte development during adulthood or during puberty and prior to conception at 5–6 weeks of age in young females leading to failure to conceive. Antigens which could be disrupted include the zona pellucida proteins.
- Interference with the function of the corpus luteum during diapause which leads to failure to reactivate and produce progesterone. This failure, in turn, does not lead to implantation of the diapausing blastocyst in the next spring. Prolactin is the primary pituitary hormone involved in the reactivation of the CL in mink and spotted skunk. Prolactin release or the function of its receptor could be disrupted.
- Stimulation of prolactin release to initiate implantation out of phase with the normal season. If pregnancy did not fail, then young would be weaned at an inappropriate time to survive mid-winter conditions. If they survived they would not conceive at 5–6 weeks of age as no males would be spermatogenic at this time (King & Moody 1982a).
- Alteration of the uterine milieux such that it becomes inappropriate for the maintenance of the blastocysts which die or cannot implant. The antiprogesterone compound RU486 may have potential here.

- Disruption of the factors essential for successful implantation of the embryo in the uterus, e.g. maternal expression of LIF. The window of opportunity for this disruption may be too small to warrant further investigation.
* Interference with the development and function of the mammary gland during gestation or lactation. This could be induced through disruption of prolactin secretion or prolactin receptor expression in the developing mammary gland. Agents such as cabergoline or bromocriptine could be used to inhibit prolactin secretion. RU486, an antiprogesterone compound, could also disrupt mammary gland development during gestation.

This review has addressed only disruption of processes that have the potential to affect the fertility of an animal. Other processes in the lifespan of an individual could also be disrupted. These might include, for example, hormones affecting thermoregulation, appetite and metabolism, or pheromones that alter social or sexual behaviour. Many of these processes, if disrupted, might cause effects which are inhumane and therefore unacceptable on animal welfare grounds (Oogjes 1997; Singer 1997).

2.2 IMMUNOLOGY OF MUSTELIDS

Very limited information is available on the reproductive immunology of mustelids. The major reason is that there have been no studies directed towards fertility control which required assessment of the immune responses of, or within, the reproductive system.

As noted above, reproductive studies on mustelids have focussed on understanding the mechanisms which regulate seasonal cycles of breeding. Specifically, changes in protein and steroid hormones have been described and the effects of manipulating these hormones or other potential regulatory factors (such as photoperiod) have been assessed. To date, none of the research has focussed on understanding the immune system with respect to immune responses within the reproductive tract. Reagents such as secondary antibodies for detecting immune responses (e.g. to establish direct ELISAs) are not available for mustelids. These would have to be developed specifically for work on stoats, ferrets or weasels.

Various radioimmunoassays have been established for mink reproductive hormones (Martinet *et al*. 1981a; Murphy *et al*. 1990). These assays rely on the cross-reaction of mink hormones with heterologous proteins and antibodies raised in other species (e.g. ovine, bovine prolactin, follicle stimulating hormone, LH etc.) as key reagents. For example a heterologous radioimmunoassay using canine prolactin standards and antiserum has been validated for the Western spotted skunk (Kaplan *et al*. 1991). These assays therefore measure relative changes in hormones under different reproductive states. Such assays are likely to be effective for measuring reproductive hormone changes in stoats, ferrets or weasels if required. Steroid hormones can be measured using either radioimmunoassays or ELISAs. These assays would need to be validated for these species but this would not be difficult. They will be important when any potential fertility antigens are assessed for their effects on reproduction.

2.3 GENE SEQUENCES OF REPRODUCTIVE PROTEINS OF STOATS AND OTHER MUSTELIDS

DNA databases have been searched for cDNA and mRNA sequence data for mustelids for proteins of the reproductive tract and for related information on hormones or other factors that may be important in the regulation of fertilisation, implantation or function of the ovary, testis or mammary gland (Table 1).

No sequences for any zona pellucida antigens for mustelids were located in the databases. However, given the high degree of identity between zona proteins of many species (Harris *et al*. 1994), it would not be difficult to prepare an ovarian cDNA library for stoats and screen this library using highly conserved sequences as primers.

2.4 SPECIES-SPECIFICITY OF POTENTIAL IMMUNOCONTRACEPTIVE AGENT(S)

It is known that direct immunisation of animals with reproductive antigens derived from other species ('non-self' antigens) or from their own reproductive system ('self' antigens) can affect fertility. For example, whole sperm or components of sperm have been used to immunise females and males and have caused infertility in several species such as mice (Tung *et al*. 1979) and rabbits (Menge & Peegel 1980; Deikman & Herr 1997). Female reproductive antigens, particularly the proteins of the egg coat (zona pellucida, ZP), have also been tested. The ZP of mammalian oocytes is a unique extracellular matrix composed of several glycoproteins and plays an important role in gamete interactions such

TABLE 1. AVAILABILITY OF SEQUENCES FOR VARIOUS GENES INVOLVED IN REPRODUCTION OF MUSTELIDS.

GENE	SPECIES	SEQUENCE (NO. OF BASES)	TISSUE ORIGIN	GENBANK ACCESSION NUMBER	AUTHORS
Prolactin	*Mustela vison*	partial sequence (1–713)	Pituitary	X59785	Perelygina (1991)
Prolactin	*Mustela vison*	partial sequence (1–682)	Pituitary	X63235	Bondar *et al*. (1993)
Prolactin receptor	*Mustela putorius furo*	Partial sequence, contains ligand binding domain	Skin	AF024575	Ford *et al*. (1997)
Luteinising hormone receptor (LHr)	*Mustela vison*	Sequence encodes membrane spanning domain (1–1041)	Testis	AF029295	Douglas *et al*. (1998b)
Leukaemia Inhibitory Factor (LIF)	*Mustela vison*	Full sequence (1–758)	Uterine endometrium	AF048827	Song *et al*. (1998)
Growth hormone	*Mustela vison*	(1–573)	Pituitary	E04304 E06873	Harada *et al*. (1991)

as sperm recognition, sperm activation and prevention of polyspermy. In a wide range of species (e.g. horses, seals, deer), immunisation of females with whole oocyte proteins, such as whole pig zona proteins has been shown to affect fertility (Kirkpatrick *et al*. 1992, 1997).

In the mouse, for example, as in most species, the zona pellucida comprises three non-covalently linked glycoproteins, ZPA, ZPB and ZPC, which are expressed by the growing oocytes in the ovary. One of these proteins, ZPC (also known as ZP3), is the receptor for sperm binding at the time of fertilisation (Florman & Wassarman 1985; Rosiere & Wassarman 1992). Passive immunisation with monoclonal antibodies to ZPC inhibits fertilisation *in vivo* (East *et al*. 1984, 1985), and active immunisation with synthetic peptides which include a B-cell epitope of ZPC also induces infertility for periods from 0–8 months (Millar *et al*. 1989; Lou *et al*. 1995). The cDNAs encoding several ZP antigens from a variety of species have been cloned and sequenced (Harris *et al*. 1994). The zona pellucida proteins (and sperm proteins) show high identity between species (Harris *et al*. 1994; Bradley *et al*. 1997; Holland *et al*. 1997; Hardy *et al*. 1997; Jackson *et al*. 1998; Haines *et al.* 1999; Voyle *et al.* 1999). Therefore a key challenge is to identify or engineer the antigen to be species-specific. This may be achievable using specific peptides or epitopes of the zona proteins. The difficulty then is to determine whether such small peptides have the ability to block fertility. The use of epitopes alone or in combination with immunomodulatory molecules (such as cytokines or T-cell help epitopes) to enhance the species-specific immune responsiveness to these antigens is being investigated (Dalum *et al*. 1997; Ramsay & Ramshaw 1997).

In the Marsupial Cooperative Research Centre, scientists have been assessing the potential of various possum ZP sequence formulations for their effect on the fertility of possums (Duckworth *et al.* 1999). Further, scientists in the Pest Animal Control Cooperative Research Centre (formerly the Vertebrate Biocontrol Cooperative Research Centre) have demonstrated significant reductions in fertility of mice using recombinant mouse cytomegalovirus expressing mouse ZPC and of rabbits using recombinant myxoma virus expressing rabbit ZPB (Jackson *et al*. 1998; Chambers *et al*. 1999; Kerr *et al*.

TABLE 2. SELECTED ANTIGENS FOR IMMUNOCONTRACEPTION.

ANTIGEN	SPECIES-SPECIFICITY	COMMENTS
Zona antigens; ZPA, ZPB, ZPC	No; high identity in DNA sequence and in protein sequence	Some species-specific epitopes have been demonstrated to affect fertility
Sperm antigens	No; high identity in DNA sequences and in protein sequences	Blood-testis barrier prevents effects on spermatogenesis in the testis, immune responses in female reproductive tract difficult to induce at sufficient titre to affect fertility
Prolactin	No; ancient hormone with many functions; high identity between species	Several forms of prolactin are secreted by the pituitary
Prolactin receptor	No; but binding affinities differ between species	
Leukaemia Inhibitory Factor (LIF)	No; common growth factor/cytokine	Disrupts implantation but window of opportunity may be too narrow

1999). The results highlight that presentation in a viral vector induces an effective immune response against these 'self antigens'. Given this success for the mouse and the rabbit under laboratory conditions, the potential for the use of zona antigens for stoats should be explored, particularly if a suitable, possibly viral, vector for delivery can be identified.

Table 2 describes the species-specificity of some selected antigens with potential for immunocontraception. Small epitopes of these antigens or combinations of several epitopes could be chosen to provide species-specificity.

Research required to determine specificity of any antigens would be as follows:

1. Clone, sequence and characterise the gene of interest.
2. Express the protein or specific epitopes of the protein and determine effects on fertility using immunisation with protein alone.
3. If a suitable delivery system is available, assess potential antigen when it is delivered in this system in the species of interest and in other species.

2.5 TEMPORARY OR PERMANENT INFERTILITY THROUGH IMMUNOCONTRACEPTION?

The potential of an immunocontraceptive vaccine to provide temporary or permanent infertility will depend on its characteristics and the mechanism by which the fertility of the individual is affected.

Thus initial experiments would assess the effect of an antigen on the fertility of individual stoats compared to control animals and determine the likely mechanism of that infertility. If an immune response is generated which impairs the function of the ovary directly through disruption of the zona pellucida, and hence disruption of the development and recruitment of oocytes, then the infertility induced may be permanent for that breeding season (i.e. female does not enter oestrus and does not carry diapausing CL or blastocysts through to the next breeding season). If the female comes into oestrus in the following breeding season she may then conceive unless the immunocontraceptive vaccine is re-applied.

Experimental assessment of the duration of infertility induced by any potential antigen(s) would be required. Sufficient groups of treated and control animals would need to be assessed to determine the proportion of animals that were infertile due to the antigen.

Ideally the agent would compromise the fertility of an individual animal permanently. If the agent only caused temporary infertility then the duration of that infertility should be at least the length of one breeding season. For stoats this would be from August to February. If the infertility lasted only for the current breeding season, treatment could be re-applied at the same time in the following year and therefore reduce recruitment into the population.

Laboratory experiments will indicate the mechanism(s) of infertility, the maximum proportion of captive animals that will be infertile and the duration of that infertility. Further studies will be required to confirm the efficacy under field conditions in populations of different densities over time.

3. Possible methods of delivering the immunocontraceptive agent

3.1 DELIVERY MECHANISMS FOR IMMUNOCONTRACEPTIVE VACCINES

There are three main delivery mechanisms for immunocontraceptive vaccines under development:

- disseminating genetically modified organisms (GMOs) such as recombinant viruses or bacteria
- non-disseminating GMOs in baits
- non-disseminating, non-GMO product packaged in synthetic delivery systems

For many pests, bait delivery may be the method of choice for political, social, economic and ecological reasons.

3.1.1 Non-disseminating GMOs

Non-disseminating GMOs, such as *AroA* deletion mutants of *Salmonella*, are being developed and tested (Bradley 1994; Bradley *et al*. 1997). Selected mutant strains of *Salmonella typhimurium* have the advantage that they are apathogenic without decreasing their immunogenicity and they are not disseminating. Furthermore, the introduction of a 'suicide' plasmid into this system would have the added advantage of degrading the foreign DNA and would make it more acceptable to regulatory bodies because the bait-delivered product would be devoid of foreign genetic material (Knudsen *et al*. 1995; Tedin *et al*. 1995).

Various Gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Vibrio cholerae*, *Klebsiella pneumoniae* and *Actinobacillus* spp.) can be engineered to carry a gene (PhiX174 geneE) which, when chemically or physically induced, causes lysis and release of the cytoplasmic contents of the bacteria. This process produces a non-replicating vaccine delivery system. These bacteria can also be engineered to carry other genes (e.g. encoding reproductive proteins). After lysis, the 'ghost' bacteria contain only membrane-associated recombinant antigen. Bacterial ghosts are cheap to produce, can be stored for long periods and can contain multiple antigenic determinants that are present in a highly immunostimulatory environment (Szostak *et al*. 1996). Such features could make bacterial ghosts an attractive delivery system for immunocontraceptive antigens. However it remains to be seen whether these preparations produce immunity to reproductive antigens after oral delivery.

Other potential non-disseminating GMOs include the use of transgenic plants in which the gene of interest is either stably integrated into the plant genome, or a modified plant virus carries and expresses the gene in infected plants (Smith *et al.* 1997). Virus-like particles (VLPs) also offer an alternative adjuvant delivery system (Mor *et al.* 1998). For example, VLPs containing tobacco mosaic virus coat protein hybrids containing a 13-amino acid sequence of the murine ZP3

protein produced in tobacco, elicited immune responses but not infertility in immunised mice (Fitchen *et al.* 1995). The use of plant-derived vaccines may or may not require further bioencapsulation to prevent digestion in the stomach before the vaccine reaches the immune effector site in the intestine.

Replication-limited viruses, such as avipoxvirus and adenoviruses, are also being developed for vaccine (Hsu *et al.* 1994; Paoletti 1996; Zhu *et al.* 2000) or gene therapy purposes (Benihoud *et al.* 1999).

3.1.2 Adjuvant and carrier delivery systems

Synthetic delivery systems for antigens include ISCOMs (immunostimulatory complexes, e.g. Quil A, Cholesterol, phospholipid constructs), microspheres (polylactide-coglycolide polyphosphazenes), and liposome emulsions (Davis 1996). The current high costs of production mean these systems are only suitable for human and companion animal vaccination and not for broad-scale application to a wildlife population. Nevertheless the per-unit production cost will decrease as these systems become more popular and production technology improves.

3.1.3 Disseminating GMOs

GMOs have the greatest theoretical potential for use as vectors for immunocontraceptive agents (Jackson *et al*. 1998; Chambers *et al.* 1999; Kerr *et al*. 1999). A viral vector could potentially overcome problems associated with the distribution of an immunocontraceptive to control wild populations (Bomford 1990). The advantages of a disseminating viral vectored immunocontraceptive agent (Bomford 1990; Shellam 1994; Chambers *et al*. 1997) over a bait-delivered non-disseminating immunocontraceptive agent are:

TABLE 3. SOME ESSENTIAL AND DESIRABLE PROPERTIES OF A VIRAL VECTOR FOR AN IMMUNOCONTRACEPTIVE AGENT (AFTER SHELLAM 1994).

ESSENTIAL PROPERTIES	DESIRABLE PROPERTIES
1. Species-specific and naturally infects target species	1. Virus is already in the country
2. Readily transmitted in target species, and at low population densities	2. Virus establishes persistent and non-lethal latent infection
3. Insertion of foreign gene is stable and does not affect viral growth or transmission	3. Good local IgA response which does not interfere with transmission
4. Stimulates long-lived immune response and immunological memory	4. Mechanism for any genetically determined host resistance is known
5. Recombinant virus can be introduced and maintained in the presence of existing immunity	5. Genetically determined host resistance does not interfere with infection or transmission
6. Panel of isolates available	6. Mechanism of transmission known
7. Epidemiology of infection understood and site of viral growth known	7. Virus is sexually transmitted
8. Likely to be acceptable for approval by regulatory authorities	8. Knowledge of the epidemiology of infection and transmission of natural virus variants
	9. A DNA rather than an RNA virus (greater genetic stability)

- Replicating virus may induce a stronger immune response and greater immunological memory.
- Infective agent can potentially spread rapidly through a population and be transmissible at low densities of host population.
- Self-perpetuating, infectious agent is cheaper than baits which must be manually applied.
- Viral vector can be chosen for its species-specificity.
- Problems associated with bait aversion or bait shyness.
- Precise timing necessary for bait delivery relative to the target animal's breeding cycle is overcome.
- It reduces wastage associated with inadvertent multiple baiting of some individuals.

There are however several advantages of bait-delivered non-disseminating agents. For example a bait-delivered immunocontraceptive might be:

- More acceptable to the public than the use of a disseminating GMO.
- More easily managed for control activities because it can be readily withheld or withdrawn from use.

Clearly the selection of a viral vector requires careful consideration of its properties. Any vector of choice for delivery of an immunocontraceptive must possess a range of essential and desirable characteristics (Table 3, after Shellam 1994). Considerable research may be required to confirm the suitability of a vector.

3.2 VIRUSES (SEE ALSO CHAPTER 5)

Except for one case study on canine distemper virus (CDV) infection in weasels and stoats (Keymer & Epps 1969), there were no other references in the public databases specifically addressing viral diseases of stoats.

Stoats belong together with weasels, ferrets and minks to the same genus *Mustela* of the subfamily Mustelinae (Fig. 1). It can therefore be assumed that they are likely to be susceptible to the same viruses that infect the other members in this subfamily. However, few experimental or epidemiological studies have been conducted on infectious diseases of non-domestic carnivores, especially regarding viral diseases of small carnivores such as mustelids, viverrids and procyonids (Williams & Thorne 1996; Gorham 1999). Extrapolations from one species to another regarding disease susceptibility or severity may therefore be incorrect. The black-footed ferret (*Mustela nigripes*), for example, is significantly affected by certain infectious diseases, some of which were expected (among them CDV), while others could not have been predicted from the generalised knowledge of diseases of the family Mustelidae (Williams & Thorne 1996).

A substantial number of virus infections are described in the literature for members of the family Mustelidae (Table 4). However, many reports are anecdotal and are based on serological evidence for the disease. Only few of the described virus infections have been studied in further detail. Ferrets are widely

TABLE 4. VIRUS AND PRION INFECTIONS OF MUSTELIDS.

used as laboratory animals for experiments on human viruses such as influenza virus (Smith & Sweet 1988; Renegar 1992) or human adenoviruses used as gene therapy vectors (Hsu *et al*. 1994).

Table 4 identifies which viruses have potential for use in mortality control or as a delivery vector. Although a variety of viruses are being developed as recombinant vaccine delivery vectors (e.g. parvoviruses, retroviruses, certain herpesviruses), very few have passed the experimental laboratory stages. At the present time only poxviruses, adenoviruses and herpesviruses have obtained practical importance as delivery vectors.

None of the viruses noted in Table 4 are specific to Mustelids. If mustelid-specific viruses were identified, they could have potential for lethal control or for delivery of vaccines. Further, mustelid-specifc viruses, even those that would not transmit between stoats, may be useful as non-transmissible GMOs. Extensive tests of species specificity would be required before they could be registered for field use.

3.3 BACTERIA AND FUNGI

The bacteria and fungi recorded from Mustelidae generally are not host-specific, frequently are pathogenic in humans and/or domestic animals, appear to have no ecological isolating mechanisms, and consequently are inappropriate, either as a direct mortality factor or as a vector for immunocontraceptive vaccines. However, research is currently being undertaken in New Zealand to develop improved vaccines against infection of cattle and possums with *Mycobacterium bovis* (the etiologic agent of bovine tuberculosis), and also *AroA* mutants of *Salmonella*. A watching brief should be maintained for possible further exploration of *M. bovis* as a delivery vector for wildlife vaccines (Buddle *et al*. 2000).

In New Zealand the mustelid-specific gastric bacterium, *Helicobacter mustelae*, is being investigated. It has the potential to be engineered as an antigen delivery vehicle to interrupt stoat fertility because it may establish as a permanent mucosal infection (DOC 2000).

3.4 PARASITES OF MUSTELIDAE

There is only a sparse literature on the parasites of stoats (Smith & Addison 1982). However, there is a substantial volume of literature on the parasites of the Mustelidae and a list involving the following host genera from which parasites are known can be made available on request.

Mustela, Martes, Gulo	weasels, ferrets, stoats, minks and wolverine
Meles, Taxidea, Melogale	badgers
Mephitis, Spilogale, Conepatus	skunks
Lutra, Enhydra	otters

This family of carnivores has a broad geographic distribution, especially in the Northern Hemisphere. They are furbearers and a number of them are farmed.

Consequently, their parasites and diseases have been studied in substantial detail, possibly more than of any other family of carnivores.

There are numerous (537) parasite records from Mustelidae representing 390 parasite species. The number of parasite species in each of the above-mentioned host genera and the parasite groups to which they belong are presented in Table 5. These include:

Rhizopoda	the amoebae
Sporozoa	the coccidian, eucoccidian and haemosporin protozoa plus *Toxoplasma* and *Sarcocystis*
Piroplasma	the babesian protozoa
Zoomastigophora	protozoa of the digestive and genital tracts (direct transmission) and of the blood stream and tissues (biological vector)
Digenea	trematodes/flukes
Cestoda	cestodes/tapeworms
Nematoda	nematodes/roundworms
Acanthocephala	thorny-headed worms
Acari	ticks and mites
Phthiraptera	lice
Siphonaptera	fleas

Two features of the macro ecto- and endo-parasites known from Mustelidae are conspicuous: most are not host-specific, especially the ecto-parasites, and many Mustelidae harbour parasites of canids and felids. Brief comments on the different parasite groups occurring on and in Mustelidae are presented below.

TABLE 5. PARASITE RECORDS FROM MUSTELIDAE.

	Rhizo	Sporozoa	Piropla	Zoomas	Digenea	Cestoda	Nematoda	Acantho	Acari	Phthira	Siphona
Conepatus	0	0	0	2	0	0	0	0	0	1	0
Enhydra	0	0	0	0	1	1	3	6	0	0	0
Gulo	0	0	0	0	1	3	4	0	0	0	0
Lutra	0	2	0	0	11	5	20	3	2	0	1
Martes	0	6	1	0	8	9	38	0	12	1	21
Meles	0	8	2	2	15	11	32	1	29	3	19
Melogale	0	0	0	0	1	0	0	0	2	0	0
Mephitis	0	3	0	1	2	0	15	0	8	1	5
Mustela	3	19	3	0	25	10	38	2	36	10	44
Spilogale	0	0	0	0	0	0	2	0	0	0	2
Taxidea	0	1	0	0	0	1	12	0	5	1	1

Rhizo = Rhizopoda; Piropla = Piroplasma; Zoomas = Zoomastigophora; Acantho = Acanthocephala; Phthira = Phthiraptera; Siphona = Siphonaptera

Rhizopoda

Only 3 species of Rhizopoda are known from Mustelidae, one each occurring in the genera *Entamoeba, Iodamoeba* and *Endolimax,* and all inhabiting the gastrointestinal tract (Appasov & Nazyrov 1977).

Sporozoa

The Sporozoa of Mustelidae include a number of species of *Sarcocystis* (intermediate hosts usually the small rodent prey of the mustelids), the cat parasite *Toxoplasma gondii*, numerous species of coccidia belonging to the genera *Eimeria* and *Isospora* and species of *Cryptosporidium*. While the coccidia may be a major problem in farming situations, there is no evidence that they are a major disease or mortality factor in the wild (Anwar *et al*. 2000).

Piroplasma

Few Piroplasma are known from Mustelidae: *Babesia missirolii, Hepatozoon mustelis* and undetermined species of *Babesia, Hepatozoon* and *Hammondia*. Nothing is known of their pathogenicity, life cycles or specificity.

Zoomastigophora

Three of the four Zoomastigophora occurring in Mustelidae are zoonoses, the animals serving as reservoir hosts in nature for the human diseases cutaneous or mucocutaneous leishmaniasis (*Leishmania tropica* and *L. brasiliensis*) and of Chagas disease (*Trypanosoma cruzi*). *Trypanosoma pestanai* appears to occur only in badgers in the UK and Europe (Peirce & Neal 1974a; Macdonald *et al*. 1999), possibly in several forms (Truc *et al*. 1991). No Zoomastigophora are known from the genital tract of Mustelidae.

Digenea

Numerous Digenea are known from Mustelidae although many are shared with other vertebrates and acquired on account of common food items, especially fish. Four are particularly worthy of comment, being associated with substantial pathological changes in the host of their own accord or in association with nematodes: *Paragonimus miyazakii, P. kellicotti* and *P.ohirai* in the lungs (Ashizawa *et al*. 1977, 1980a, b) and *Troglotrema acutum* in the frontal sinuses of the nasal cavity (Artois *et al*. 1982). A number of nematodes occur also in the lungs of Mustelidae (Anderson 1992) and a second digenean, *Mamorchipedum isostoma*, occurs in the nasal cavities (Ryzhikov *et al*. 1985) as well as a number of nematode species belonging to the genus *Skrjabingylus* (Anderson 1992).

Cestoda

Relative to the numbers of Digenea, Nematoda, Acari and Siphonaptera occurring in and on Mustelidae, few cestodes are known and pronounced pathological changes have not been associated with their presence.

Nematoda

By far the greatest number of parasites known from Mustelidae are nematodes. Although the greatest degree of pathogenicity is associated with them, no particular species is recognised as a mortality agent in its own right. As noted above, co-occurrence of metastrongyloid nematodes (so-called lungworms belonging to the genera *Aelurostrongylus, Andersonstrongylus, Crenosoma,*

Filaroides and *Skrjabingylus*) and digeneans in both the lungs and in the nasal sinuses result in substantial pathological changes (see References under Digenea). *S. nasicola* occurs in stoats in New Zealand (King 1974) although to our knowledge the obligatory gastropod intermediate hosts are not known in this country. In the Northern Hemisphere, rodents are capable of serving as paratenic hosts and King (1991) hypothesised that the feral house mouse, *Mus musculus,* may play such a role in New Zealand. In a survey of 1492 stoats in New Zealand, King & Moody (1982b) reported that there was no evidence that animals infected with *S. nasicola* were smaller or lighter than uninfected animals. However, Maldonado & Kirkland (1986) in the USA reported reduction in brain case size in skunks infected with *S. nasicola* resulting in abherent behaviour. The lungworm, *Filaroides martis,* has been reported recently in both stoats and ferrets in New Zealand although its prevalence and pathogenicity in mustelid hosts in that country is unknown (McKenna *et al*. 1996). Stockdale & Anderson (1970) described the development, route of migration in the definitive host and pathogenesis of this species in mink in Ontario, Canada, and Stockdale (1970) described pulmonary lesions in mink with a mixed lungworm infection of *F. martis* and *Aelurostrongylus (=Perostrongylus) pridhami. Gnathostoma nipponicum* causes tumours in the oesophageal mucosa of weasels and these may extend to the thoracic cavity and lungs (Ashizawa *et al*. 1978). However, when the second intermediate host, loaches, are eaten raw by humans, zoonotic infection occurs (Ando *et al*. 1988).

Could nematodes be used as disseminating vectors of vaccines?

While many of the nematodes of Mustelidae occur in a number of other vertebrate families, species of *Strongyloides* often are reasonably host-specific. While these may not cause severe pathology in the host gut they may nevertheless exhibit potential as candidates for engineering delivery mechanisms or vectors for immunocontraceptive vaccines. Their importance lies in two spheres:

- They occur deep in the intestinal mucosa in close proximity to lymphatic tissues.
- They have both parasitic and free-living generations.

The parasitic form produces only genotypically female eggs by mitotic (apomictic) parthenogenesis. Eggs embryonate in the intestine and after hatching, first-stage larvae pass in the faeces. In the external environment they develop either into infective larvae which are females (the homogonic pathway) or grow and moult four times to produce a single free-living generation consisting of both males and females (the heterogonic pathway). Females resulting from heterogonic development produce eggs by meiotic parthenogenesis but development of the eggs must be initiated by the penetration of the sperm, although the sperm and egg pronuclei do not fuse. Apparently, nuclei of the second maturation division recombine to give the diploid chromosome number. Larvae arising from these eggs develop only into infective larvae which will invade and mature as females in the vertebrate host. The prepatent period, i.e. development in the vertebrate to egg-laying, is relatively short (3–17 days); this renders these free-living and parasitic nematodes a pragmatic system for laboratory culture and experimentation. In addition, both transplacental and transmammary transmission are known in

some species. Autoinfection also is known to occur in some instances resulting in fatal disseminating strongyloidiasis in immunocompromised hosts. These comments pertain to members of the genus *Strongyloides* generally. Literature pertaining to life cycle details or specificity of the species of *Strongyloides* known to date in Mustelidae was not found.

Acanthocephala

A number of Acanthocephala reported in Mustelidae occur normally in other vertebrate hosts and are acquired by mustelids as a consequence of their fish diet. For example, the adult parasites (n=12) of sea otters, *Enhydra lutris* are derived primarily (n=9) from pinnipeds (Margolis *et al*. 1997).

Acari

A spectrum of Acari—trombiculid, sarcoptiform and demodectic mites, ixodid ticks—occur on Mustelidae but as with the Siphonaptera, generally they are not host-specific.

Phthiraptera

A small number of Mallophagan lice are known to occur on Mustelidae and appear to be relatively host-specific, although there was no evidence of their role as intermediate hosts or vectors of pathogenic organisms. Two anopluran lice, *Linognathus setosus* and *Hoplopleura acanthopus,* also have been recorded (Sergiyenko 1973; Haitlinger 1980; Hancox 1980).

Could lice be used as disseminating vectors of vaccines?

As with some nematodes, the phthirapteran lice appear to exhibit a degree of host-specificity. Although there was no evidence of any role as vectors or hosts of pathogens affecting Mustelidae, theoretically they may have a role in the engineering of a vector to produce an effective method of vaccinating populations of stoats.

Siphonaptera

The Siphonaptera occurring on Mustelidae generally occur also on a wide range of other vertebrate hosts. For example, none of the 104 fleas representing 14 species and sub-species collected from 397 mustelids in Switzerland over a 4-year period was specific to these animals, all being known as ectoparasites of moles, birds and rats (Debrot & Mermod 1982). Cox *et al*. (1999) considered that the flea *Paraceras melis* was specific to European badgers, *Meles meles*, however there is one record of this parasite on the stone marten, *Martes foina*, in Switzerland (Zimmerli 1982). Sleeman (1989) examined 189 Irish stoats and recovered 2580 arthropods. He considered the ticks and fleas to have come mainly from the nests of other hosts. The flea species did not reflect the status of their usual hosts in the diet of stoats but rather the use by stoats of the habitat of their usual hosts. The species distribution of fleas on Mustelidae suggests that they are acquired primarily from the host and runways of their prey which they have hunted through or taken over. The fleas reported from stoats in New Zealand by King and Moody (1982c) highlight this reality—*Ceratopsyllus gallinae* from fowl and other birds, *Leptopsyllus segnis* from mice, *Nosopsyllus fasciatus* from mice and rats and *Parapsyllus nestoris* from the Kea.

3.4.1 Reproduction and macro-parasites

No literature on macro-parasites inhabiting the reproductive tracts of Mustelidae or causing pathological effects in these tissues was found. Similarly, information on the role parasites may play in suppressing or reducing reproductive success in Mustelidae was not found.

3.4.2 Epidemiology of macro-parasites

While there is a reasonable volume of knowledge on the life cycles of the macro ecto- and endo-parasites of Mustelidae there are, with one exception (Anwar *et al*. 2000), no appropriate epidemiological studies of these. Literature apparently dealing with the latter subject generally pertains to surveys of the prevalence of particular parasite species in local animal hosts which are serving as reservoirs of human diseases: e.g. the trematodes *Clonorchis sinensis, Paragonimus westermani* and *Opisthorchis felineus,* and the cestode *Echinococcus granulosus.*

3.4.3 Conclusion

Macro ecto- and endo-parasites, bacteria and fungi of stoats in particular and Mustelidae in general offer no conspicuous direct mortality agent for application to the control of stoats in New Zealand. Generally they are not highly host-specific and they share a surprising number of parasites with members of the Felidae and Canidae. The potential role of these parasites as a vector for immunocontraceptive vaccines appears weak and would be placed well down a list of priorities for research.

Nevertheless, it should be noted that in New Zealand research is being conducted to genetically engineer the nematode *Parastrongyloides trichosuri* as a vector for biological control of possums. *P. trichosuri* appears to be specific to brushtail possums (Mackerras 1959; Spratt *et al.* 1991) and is highly amenable to laboratory manipulation (Heath *et al.* 1995; Stankiewicz 1996). It has a free-living stage similar to *Caenorhabditis elegans*, a nematode which has been extensively modified genetically, and hence demonstrates considerable potential as a vector for DNA expression of immunocontraceptive vaccines (Gruenberg *et al.* 1999). A controlled field release of the parasite is underway in New Zealand to assess the dynamics of parasite spread in a free-ranging, *P. trichosuri*-naive possum population (Ralston *et al.* 1999).

3.5 CANDIDATE BAIT TYPES AND DEPLOYMENT IN THE FIELD

3.5.1 Methods of disseminating of an immunocontraceptive

Contagious immunocontraceptive pathogens could be disseminated initially by injection or inoculation into trapped stoats. This method was used for the initial official spread of myxomatosis and rabbit haemorrhagic disease (RHD) to rabbits in Australia. Alternatively methods could be developed that inoculate stoats repeatedly and automatically when they enter tunnels. Such methods were devised for spreading myxomatosis in Australia, although the effectiveness

and extent of use in the field are not known. Technology for directing aerosol sprays onto stoats in traps or tunnels may need to be developed if a disseminating immunocontraceptive GMO transmits only via respiratory surfaces.

3.5.2 Types of baits and methods of delivery

Baits could be used to deliver contagious or non-contagious organisms or immunogenic products or chemosterilants that can persist undegraded, either naked or within some form of packaging, and can be absorbed from the stoat's alimentary canal into the lymph or blood circulation.

Spurr (1999) tested a variety of poison baits, lures and toxins for management of stoats and recommended the use of raw hen egg baits presented in egg tunnels in preference to day-old chicken carcasses which decay quickly. Similar baits and presentation would be needed for immunocontraceptives if they were not target-specific. If the immunocontraceptives were target-specific, it would not be necessary to confine the loaded eggs in egg tunnels, except to reduce wastage to non-target species. This would enable broad-scale delivery, including the use of bait stations and aerial dropping. Eggs or day-old chickens might not suffice for use in bait stations, and for aerial delivery a robust bait would need to be developed. Bait stations delivering immunocontraceptives may be uneconomical because of repetitive use by individuals already sterilised; poisoned baits would have killed these animals earlier. Consequently, broad-scale delivery, especially aerial, may be more cost-effective for delivering non-disseminating immunocontraceptive GMO baits than bait stations or eggs. Therefore a new bait appears necessary to deliver non-disseminating immunocontraceptive GMOs most cost-effectively.

Several factors will influence the design of a delivery system, including possible integration with other control techniques, bait uptake rates, the timing of baiting and the intensity of baiting relative to the movements of stoats, topography and vegetation. The cost-effectiveness of different control strategies could be evaluated within a modelling framework.

If the immunocontraceptive were capable of contagious dissemination, aerial delivery may be effective but might not be needed, and populations might be seeded using less intensive ground-based methods, including egg baits, bait stations, or trapping and inoculation.

3.6 TECHNICAL SPECIFICATIONS FOR POTENTIAL METHODS OF FERTILITY OR LETHAL CONTROL

The broad technical specifications required for delivery of the potentially viable methods of fertility or lethal control are shown in Table 6.

TABLE 6. BROAD TECHNICAL SPECIFICATIONS FOR POTENTIALLY VIABLE METHODS OF FERTILITY OR LETHAL CONTROL OF STOATS.

SPECIFICATION	LETHAL BIOCONTROL	DISSEMINATING IMMUNOCONTRACEPTIVE GMO	NON-DISSEMINATING IMMUNOCONTRACEPTIVE GMO	NON-DISSEMINATING IMMUNOCONTRACEPTIVE NO GMO PRODUCT
Requirement for species-specificity	High	High	Low if delivery is specific, otherwise high	Low if delivery is specific, otherwise high
Delivery route	Contagious, seeding by injection, bait or aerosol	Contagious, seeding by injection, bait or aerosol	Broad-scale bait, preferably suitable for aerial delivery, if target-specific	Broad-scale bait, preferably suitable for aerial delivery, if target-specific
Target in body	Various	Zona pellucida, prolactin receptor	Zona pellucida, prolactin receptor	Zona pellucida, prolactin receptor
Viability in environment	Long half-life useful	Long half-life useful	Long half-life in bait	Long half-life in bait
Half-life in host	Long	Long	Short	Short
Need for agent to be encapsulated	No, maybe necessary for seeding population	No, maybe necessary for seeding population	Yes	Yes
Lethality	Moderate to high	Low to moderate	Not lethal	Not lethal
Required scale of production	Small scale only	Small scale only	Mass production	Mass production
Ongoing quality assurance	Essential	Essential	Essential	Essential
Response/dose	Not applicable	Not applicable	Probably not applicable	High response needed

4. Feasibility of using reproductive control compounds

4.1 POTENTIAL OF CHEMOSTERILANTS FOR CONTROLLING REPRODUCTION IN STOATS.

Many techniques have been developed for managing or controlling the fertility of individual animals in captivity or in confined areas that are not subject to immigration. These methods include surgical sterilisation or castration, use of chemical sterilants, agonists, which block the function of natural hormones, and inhibitors of lactation (Table 7) (Marsh 1988; Bomford 1990; McIvor & Schmidt 1996). Each of these approaches is expensive and time-consuming to

TABLE 7. SUMMARY OF POTENTIAL CHEMICAL COMPOUNDS FOR FERTILITY CONTROL OF PEST POPULATIONS AND ASSESSMENT OF THEIR RELEVANCE FOR MANAGING STOATS. SOURCES: HOWARD (1967); MARSH & HOWARD (1973); MEAD (1981); MARSH (1988); VICKERY *ET AL.* (1989); BOMFORD (1990); SANKAI *ET AL.* (1991); JOCHLE & JOCHLE (1993); GAO & SHORT (1994A,B); HINDS & TYNDALE-BISCOE (1994); TYNDALE-BISCOE (1994, 1997); MARKS *ET AL.* (1996); MCIVOR & SCHMIDT (1996); BECKER & KATZ (1997); JOCHLE (1997); ECKERY *ET AL.* (1999); NORBURY (2000).

CHEMICAL COMPOUND	MAJOR ADVANTAGES	MAJOR DISADVANTAGES	EFFICACY FOR STOAT PEST POPULATIONS	
			CURRENT	FUTURE
• Agonist and antagonists of gonadotropin releasing hormone (disrupt natural hormone functions and the hypothalamic-pituitary-gonadal feedback axis); e.g. buserelin (agonist) • GnRH-toxin complex		• Costly to administer • Not permanent, may only reach a portion of the population • Side effects (dose dependent) • Not appropriate for promiscuous species • Not species-specific	Low, but untested	Low, but untested
• Steroids and synthetic steroids (progesterone, megestrol acetate, medroxyprogesterone acetate, melengesterol acteate; oestrogens, Diethylstilbestrol and related compounds, quinoestrol) • Testosterone propionate • Anti-steroids, anti-steroid receptor (e.g. RU486-mifepristone) • Alpha-chlorohydrin (Epibloc); disrupts male fertility	• Moderate to low cost • Bait or implant	• Not permanent • Side effects (dose dependent) • Must be administered regularly • Non-target effects • Taste aversion can develop • Not species-specific	Low, but untested; possible potential for RU486	Low, but untested
• Prolactin inhibitors (affect lactation and /or gestation (e.g. Bromocriptine; Cabergoline)	• Oral delivery • Moderate to low cost	• Not permanent • May not be ethically acceptable as starves young or aborts fetuses • Must be regularly administered • Not species-specific	Moderate, but untested	Moderate, but untested

apply, with several of the methods having undesirable side effects (e.g. some chemosterilants), and some affecting non-target species. Their applicability and effectiveness for free-ranging populations, however, has never been very high because effective delivery is difficult and maintenance of inhibition of reproduction over lengthy periods at the population level is low.

These sterility control agents fall into two categories: those that are anti-gametic and those that are anti-gonadal. Until recently most of the available agents have been effective due to their anti-gonadal action. These chemosterilants usually disrupt the endocrine feedback loops of the hypothalamic-pituitary-gonadal axis of males or females and induce a temporary or permanent sterility in either sex, reduce the number of offspring or alter the fertility of offspring produced. In the female, these chemicals may affect the secretion of releasing hormones from the hypothalamus, suppress the secretion of gonadotrophins from the anterior pituitary, prevent the development and maturation of follicles, disrupt the passage of oocytes in the oviduct, prevent or impair fertilisation, or alter the hormonal milieu in the uterus—thus preventing successful implantation and gestation. In the male, similar effects may occur in the pituitary and hypothalamus, with effects in the reproductive tract leading to inhibition of spermatogenesis in the testis or interference with transport or storage of the sperm in the epididymis.

Many chemosterilants (for example, synthetic oestrogens and progestins, alpha chlorohydrin) have been developed and tested with various degrees of success. Several of these have been tested in different species: most require high doses to be effective and unless administered continuously have only short-term effects on fertility (Bomford 1990; Gao & Short 1993; McIvor & Schmidt 1996).

Agonists and antagonists of gonadotrophin releasing hormone, which is secreted from the hypothalamus, or direct immunisation against this non-steroidal hormone, have been investigated for fertility control. These agents suppress the feedback pathways within the hypothalamic-pituitary-gonadal axis. As with the other chemical sterilants the treatment must be sustained and there can be suppression of sexual behaviour (Vickery *et al*. 1989; Becker & Katz 1997).

Prolactin is important for successful reproduction in many species. It provides essential luteotrophic support of the corpus luteum on the ovary or support for placental function during gestation and/or for the onset and maintenance of lactation. Prolactin is the primary pituitary hormone responsible for initiating increased function of the corpus luteum and blastocyst implantation in the Western spotted skunk (Mead 1981; Berria *et al*. 1989) and mink (Murphy *et al*. 1981). Several prolactin antagonists are commercially available for both human and veterinary use. The best known is bromocriptine, an ergot alkaloid which acts as a dopamine agonist. It inhibits the release of the hormone dopamine in the hypothalamus and results in the inhibition of prolactin secretion from the pituitary. Bromocriptine has been used in the mink and Western spotted skunk to assess the role of prolactin in delayed implantation. When bromocriptine was administered as two subcutaneous injections (2 mg/day) implantation was delayed for the duration of the treatment (15 days) (Martinet *et al*. 1981a) and prolactin levels were low to undetectable in mink (Martinet *et al*. 1981a; Douglas *et al*. 1998b). Similar results were seen in Western spotted skunk treated with 1.5 mg/day for 5 days (Berria *et al*. 1989).

Cabergoline, another dopamine agonist, also inhibits prolactin release but has fewer side-effects, and a longer-lasting action (Jochle 1997) compared to bromocriptine. Cabergoline has been used with varying degrees of success in the suppression of lactation in pseudopregancy of bitches, and as an abortifacient in canids and felids (Jochle 1997). Oral delivery of cabergoline has been shown to be practical in a feral cat population (Jochle & Jochle 1993) where it reduced breeding in females. Cabergoline has not been used in the field or laboratory for any mustelids; however based on the effects of bromocriptine on prolactin secretion, this compound should be assessed in laboratory trials. Whether broad-scale use can be implemented is uncertain because cabergoline will not be target-specific.

Prolactin is also important in the hormonal regulation of reproductive function in the male mink. Sundqvist *et al*. (1988) have examined the role of prolactin in the regulation of testicular development. In comparison with untreated control animals, those male mink treated with fluphenazine decanoate, a stimulator of prolactin secretion, had elevated serum prolactin levels and also appeared to have accelerated testicular growth, while those animals treated with bromocriptine had retarded testicular growth. Furthermore, serum testosterone levels were lower in the bromocriptine-treated animals than in the fluphenazine or control groups (Sundqvist *et al*. 1988). The effects of cabergoline treatment in male stoats should be determined.

Another compound that has been used to induce chemical sterility in some species is alpha chlorohydrin (Bomford 1990; Gao & Short 1993). This compound has a dose-dependent effect; with increasing doses the effect was infertility, sterility or death in rats, sheep and pigs, but it had no effect in mice or rabbits. Alpha chlorohydrin, depending on the dose, appears to reduce sperm motility, block sperm passage by inducing lesions in the epididymis, or degenerate the epithelium (McIvor & Schmidt 1996).

All of the above chemosterilants can be readily applied to captive individuals. They were, and still are, generally inappropriate for broad-scale use in large free-ranging populations, and are unlikely to be cost-effective compared with use of poisons.

4.2 COMMERCIAL AVAILABILITY AND CONSTRAINTS OF CANDIDATE CHEMOSTERILANTS

Experimental field use of cabergoline for research purposes has been permitted in feral cat populations in North America (Jochle & Jochle 1993), and in foxes in Australia (Marks *et al*. 1996). Cabergoline is used in veterinary practice and is priced for this purpose. Bromocriptine is used for both veterinary and medical purposes, and is also similarly priced. Alternative ergot alkaloids with similar effects on prolactin secretion are held by pharmaceutical companies. These could be accessed for research purposes and if proven suitable could be more reasonably priced for field use. Mifepristone or RU486 has not been used under field conditions. It is also priced for use in human medicine.

If, after extensive laboratory trials, a chemosterilant were proven suitable, it would need to be delivered in baits that are mustelid-specific. The stability of

the compound(s) would need to be tested in this bait matrix under various field conditions. The timing and frequency of application would depend on the results from laboratory trials. It is likely that two or more applications would be required over intervals of 2-4 weeks during the early breeding season (September–October) for stoats. For ferrets and weasels, which could return to oestrus and produce a second litter (see Chapter 8), further applications through to January may be required. However it is not clear how ferrets and weasels might respond if breeding in the early part of the breeding season was inhibited. This area would require further research.

If the bait matrix is not specific for mustelids, suitable trials assessing the effects of the chemosterilant on non-target species would be required. For example, some birds such as pigeons produce crop-sac milk when feeding their nestlings. Prolactin secretion is essential for this process and can be disrupted by bromocriptine.

5. Canine distemper or other diseases as mortality agents

Mortality agents for stoats, and the feasibility of using canine distemper or other diseases as a mortality agent have been addressed in Chapter 3, Tables 4 and 5.

5.1 CANINE DISTEMPER

Canine distemper virus (CDV) infection is an acute to subacute contagious systemic disease with a high mortality rate in dogs and other carnivores. CDV is an enveloped virus with a negative sense RNA genome. It belongs to the genus *Morbillivirus* of the family Paramyxoviridae (Murphy 1995) together with measles virus (MV), Rinderpest virus (RV), peste de petits ruminants (PPRV) and other recently discovered viruses such as phocid distemper virus (PDV) or cetacean morbillivirus (CMV). Although only one serotype is presently recognised, a variety of strains exists that vary greatly in pathogenicity and tissue tropism (reviewed in Appel 1987). CDV is rapidly inactivated by solvents, heat and light and hence is comparatively labile in the environment.

All species in the family Mustelidae are known to be susceptible to CDV infection (Appel 1987). However, the susceptibility and mortality rate varies greatly between species (Appel & Summers 1995). Substantial literature is available on CDV infection of minks and ferrets bred in captivity (reviewed in Pearson & Gorham 1987b). However, there are very few studies published on the prevalence, or other aspects of epidemiology, of CDV infection of species of Mustelidae in the wild (Anderson 1995; van Moll *et al*. 1995).

Distemper in mink has a worldwide distribution. Serious outbreaks of distemper in mink occur mainly in summer and fall when highly susceptible kits are abundant (Pearson & Gorham 1987b). The disease in mink is manifested by signs of catarrhal inflammation, systemic infection and central nervous system disturbance (Pearson & Gorham 1987b). Aerosol transmission of saliva and nasal exudate is the most significant means of exposure to the virus which replicates in the respiratory tract. Transmission does not occur through the gastrointestinal tract or across the placenta (Hagen *et al*. 1970). This restricts infection to direct contact between animals. Delivery of the virus using a bait would require inclusion of a capsule that would become an aerosol when the animal chewed the bait formulation. This approach has been used successfully with the delivery of the rabies vaccine in recombinant vaccinia (Blancou *et al*. 1988; Pastoret *et al*. 1992; Artois 1997).

CDV is nominated as having potential for biocontrol as a mortality agent (Table 4). However, there would be risks associated with its use, due particularly to its broad host range: carnivores and pinnipeds (see O'Keefe 1995). For example, CDV can be transmitted from terrestrial animals to pinnipeds with fatal consequences as the death of thousands of freshwater seals (*Phoca sibirica*) in Lake Baikal in Russia has shown (Likhoshway *et al*. 1989). There are also known cases of CDV transmission from diseased seals to mink (Blixenkrone-Moller *et*

al. 1990). The mortality rate in adult mink is variable and around 90% in kits, while CDV infection is always fatal in ferrets. CDV does not persist in an infectious form following an acute infection which results in a life-long immunity in recovered hosts (Barrett 1999).

5.2 OTHER VIRUS INFECTIONS

Mink enteritis virus (MEV) and **Aleutian disease virus (ADV)** are two parvoviruses that infect mink and have a worldwide distribution (Pearson & Gorham 1987a, c). Both viruses are closely related to other parvoviruses of carnivores with nucleotide sequence homologies for example to feline and canine parvovirus of more than 98% in the capsid protein (Langeveld *et al*. 1995). Studies of feline and canine parvovirus pathogenesis have shown that only a few evolutionary nucleotide changes may radically alter the host range of parvoviruses (Truyen *et al*. 1998).

Mink enteritis virus is an acute, highly contagious disease of mink characterised by severe inflammation of the intestine with high mortality in both kits and adults. MEV entry is via the gastrointestinal tract and transmission occurs through direct contact of susceptible mink with infected animals or by indirect contact with contaminated fomites. Recovered animals develop a long-lasting immunity, but are known to function as virus carriers. MEV is stable in the environment for up to 6–9 months. Adult ferrets respond serologically to MEV, but do not develop clinical signs.

Aleutian disease virus is another parvovirus of mustelids (in particular mink and ferret) and was first described in mink with Aleutian mutation (aa). It causes a persistent infection and is characterised by plasmacytosis, immune complex arteritis and glomerulitis (Pearson & Gorham 1987a). Horizontal and vertical transmission occurs with virus found in saliva, urine and faeces. Virus entry is either perorally or by the respiratory tract (Pearson & Gorham 1987a). The pathogenicity of the virus depends almost entirely on the genotype of the mink with a mortality rate of more than 90% in Aleutian mink (Pearson & Gorham 1987a). However, strain differences between mink and ferret isolates do also occur (Saifuddin & Fox 1996).

5.3 SPECIES-SPECIFICITY AND ENHANCEMENT OF THE EFFICACY OF CANDIDATE PATHOGENS

The likelihood is high that all species in the family Mustelidae are more or less susceptible to the same viruses, although species differences in the severity of the resulting diseases have been described (Appel & Summers 1995).

Newly emerging morbilliviruses with potentially disastrous ecological consequences for marine mammals have been discovered in the past decade: phocid distemper virus in seals and the cetacean morbillivirus in dolphins, whales and porpoises (Barrett 1999). Other newly discovered morbilli-like viruses such as the equine morbillivirus (now known as Hendra virus) in Australia are of great concern as zoonotic agents for humans (O'Sullivan *et al*. 1997).

Specific strategies to enhance the efficacy of a mortality agent can only be considered once a specific infectious agent is selected. General strategies would include the release of the agent during times of high susceptibility (e.g. abundance of young after maternally derived immunity has waned or in adult populations with low or no immunity). Detailed knowledge of the epidemiology of the disease agent in the target species is necessary for such strategies. See Chapter 3, Table 3.

5.3 ADDITIONAL ACTIONS FOR THE SAFE USE OF THE MORTALITY AGENTS

If canine distemper virus were to be considered for use as a mortality agent then all domestic canid species would need to be vaccinated against distemper virus. Similarly if parvoviruses, adenoviruses, or poxviruses were considered then routine vaccination of domestic species, which can also be affected by these viruses, would be essential.

Prophylactic vaccination is available and widely used for the most common virus infections of domestic carnivores including CDV or canine parvovirus infection.

6. Using stoat reproductive and lethal biocontrol methods on weasels and ferrets

The reproductive cycles of weasels, *Mustela nivalis*, and ferrets, *Mustela putorius furo,* are very similar. Both breed during the spring and summer and can produce 2 litters of about 9 young (Lamming 1984; Fig. 2). Gestation in the weasel is 34–37 days. Unlike the stoat, the ferret and weasel do not exhibit delayed implantation. However, both species, like the stoat, are induced, reflex ovulators. The ferret displays a reflex or mating-induced ovulation with the preovulatory luteinising hormone (LH) surge occurring only after an intromission from a male (Carroll *et al*. 1985). Embryos implant 12 days after mating and parturition occurs on day 41–43. If a mating is sterile, pseudopregnancy follows with no apparent difference in the function of the corpora lutea (CL) in terms of their morphological development, life-span and progesterone secretion (Heap & Hammond 1974). Throughout pregnancy the CL requires support from the pituitary—CL do not develop or they regress if the pituitary is removed in early pregnancy (Murphy 1979a,b) or pseudopregnancy (Blatchley & Donovan 1972). Treatment with prolactin maintains progesterone secretion by the CL after hypophysectomy (Murphy 1979b) and more recent evidence indicates that LH also is part of this luteotrophic complex supporting the CL (Agu *et al*. 1986). Daily treatment with bromocriptine (2 mg/kg), from day 2 to day 16 of pseudopregnancy lowered the plasma concentrations of both prolactin and progesterone in these studies (Agu *et al*. 1986).

Ferrets can return to oestrus for a third time and mate. Although conception occurs, this pregnancy inevitably fails. The reasons for this failure are unknown (Lamming 1984) but, if it was understood, could provide a useful mechanism for induction of infertility.

Lactation in the ferret lasts 4–6 weeks depending on the number of young being suckled. The sucking stimulus is of prime importance in the maintenance of lactation since a reduction in the number of young being suckled to less than 4 usually results in the cessation of lactation and the recurrence of oestrus 7 days later.

All reproductive antigens under consideration for stoats should be tested for their effects in ferrets and weasels. Given that these species could produce 2 litters per season, the timing of the delivery of an immunocontraceptive may need to be more frequent in the breeding season to ensure efficacy. In New Zealand, however, ferrets rarely produce more than 1 litter per breeding season (P. Cowan pers. comm.), so this may not be an important concern for management of the species.

Candidate lethal biocontrols for stoats, in principle, would also be suitable for ferrets and weasels. Specificity in bait delivery for mustelids would be essential.

7. Strategies and methods that could enhance reproductive or lethal biocontrol of stoats

7.1 GENERAL ISSUES FOR IMMUNOCONTRACEPTION

7.1.1 Is there a role for immunocontraception?

The successful strategies and methods being used for adaptive management of kokako might be adapted readily to other New Zealand threatened bird populations and species with equal success (Innes *et al.* 1999). On the other hand, different suites of predators and interacting pest prey species threaten other New Zealand native bird populations, such as New Zealand pigeons (James & Clout 1996) and the northern brown kiwi (McLennan *et al.* 1996). Control of stoats may be crucial to removing the threat (McLennan *et al.* 1996; O'Donnell 1996a,b; Basse *et al.* 1999). Nevertheless, there are complex interactions among predators, between predators and prey and among pest prey species. The unpredictable outcomes of specific management ensure that multiple species must be considered, management must involve well designed experimental testing of hypotheses in the specific field situations, and management must adapt progressively to the accumulating information. Consequently, adaptive management is the appropriate milieu in which to consider a useful role for immunocontraception of stoats.

The kokako example and a study of the impact of trapping stoats on reproduction and survival of mohua (O'Donnell *et al.* 1996) show that effective conservation of native bird species in New Zealand is technically feasible, and there is little doubt that it is desired socially and politically. Nevertheless, the resources required are large and expensive and the areas that can be treated may be small, perhaps too small, as seems to be the case for the yellow-crowned parakeet (Elliott *et al*. 1996). Where predation has been shown to cause population decline, costs rather than available habitat are likely to limit the areas in which these populations can be conserved, although some populations may periodically reach carrying capacity and decline in years of severe predation (e.g. Elliott 1996b).

The conservation status of many species threatened by predation could be improved if areas and numbers of conserved populations could be increased, and if costs could be reduced so that economic feasibility was assured in an uncertain future economic climate. Immunocontraception and lethal biocontrol might play a role in making the conservation of the native bird populations cheaper by complementing or replacing the more inefficient components of current control, by replacing any current methods that may have an uncertain future, and by extending stoat control to areas where current techniques cannot be used.

The background information on conservation of threatened bird species in New Zealand, and the context for immunocontraception are summarised in Appendix 1.

7.1.2 What would be required of immunocontraception?

The absolute density of stoats in New Zealand has been estimated only once (Griffiths *unpublished*). Alterio *et al*. (1999) used capture-mark-recapture in South Island beech forest with Edgar traps placed at 150 m intervals in circular patterns. Stoat density was estimated to be only about 4.2 km^{-2} (95% confidence interval 2.9–7.7 km^{-2}) in summer and 2.5 km^{-2} (95% confidence interval 2.1–3.5 km^{-2}) in winter. The average maximum density of stoats required to conserve northern brown kiwi (*Apteryx mantelli*) in lowland mixed forest has been estimated at less than 2 km^{-2} for about 9 months while juvenile kiwi are vulnerable (McLennan *et al.* 1996; Basse *et al.* 1999). In most years, densities of stoats probably are higher in podocarp and mixed forests than those estimated for beech forests (see Wilson *et al.* 1998). Therefore, to conserve northern brown kiwi it would be necessary firstly to reduce stoat numbers and then prevent increase due to influx of juvenile stoats. The required conditions could be achieved if the resident adults were reduced by lethal means to less than 2 km^{-2}, were incapable of reproducing and were able to deter immigration of dispersing juveniles. The requirements for maintaining a low density of stoats to conserve localised populations of prey species include: an initial lethal reduction of stoat density, imposition of sterility at some effective rate, deterrence of immigration at some effective rate, and repetition of the sterility control. The sterility control would need to be repeated at some interval that accounts for inefficiency of the sterility control, the remaining immigration of fertile stoats, and natural increase from both groups, possibly with compensatory survival.

The abundances of stoats and prey species in mixed broadleaf-podocarp forest vary seasonally (King *et al.* 1996a), and vary markedly in beech forest in association with mast years and mouse plagues (King 1983; Alterio *et al.* 1999). The dynamics of stoat populations and prey such as rats, mice and birds, are likely to be complex with the impact of predation on bird populations varying in time. If possible, interactive population models should be constructed to predict the likely effectiveness of various control strategies, including immunocontraception. (See Pech & Hood (1998) for an example of the use of an interactive population model to predict the consequences of a new control method for rabbits in Australia.)

7.1.3 Could sterilised stoats exclude fertile animals from core conservation areas?

Could sterile stoats act as a barrier to migrating fertile stoats or dispersing young stoats, say in a buffer zone or habitat periphery? In Europe stoats are known to be territorial seasonally (King 1990). Stoat populations are in low densities in New Zealand (King & Edgar 1977; King 1990), occupying large home ranges (100–200 ha in most years (King 1990; Murphy & Dowding 1994; Moller & Alterio 1999). The home ranges may shift over time, and usually overlap, at least for males, without evidence of territorial exclusion (Murphy & Dowding 1994; Moller & Alterio 1999; Griffiths *unpublished*). In the breeding season males tend to wander seeking females (King 1990) and juvenile stoats disperse widely during summer (King 1983; 1990). Individuals move large distances in short times, and commonly may traverse fast-flowing rivers (Murphy & Dowding 1994). Consequently, there can be little expectation of

sterilised stoats excluding fertile stoats from core conservation areas, or of reducing their rate of incursion.

Exclusion or deterrence of immigration into core conservation areas could be tested by experimentation in field situations with tubally ligated females and vasectomised males, or with animals sterilised by some other means that is appropriate for any candidate form of sterility or contraception. Dispersing juveniles would present less of a problem for maintaining low densities of stoats if immunocontraception were widespread, implying that it would be spread contagiously or by extensive aerial baiting.

The density of sterilised stoats, over time, depends on the lifespan of the residents (about 80% of juveniles are lost in their first year, the rate of loss is very much lower for years 2–8: King 1989), the rate of immigration, and the rate of sterilisation (which would vary according to whether delivery is by a bait or pathogen). Models would be needed to cope with these levels of complexity (Norbury 2000; Griffiths *unpublished)*, and the models would need to be tailored to the ecological situation under consideration for the species being conserved.

What would be the impact of the sterilised stoat residents on bird populations? Sterilised female stoats would probably have lower dietary requirements for maintenance than would fertile stoats because of the lack of pregnancy, lactation and the need to feed young. Nevertheless, they would consume prey at similar rates outside the breeding season, and sterile males are likely to consume prey at similar rates to fertile males throughout the year. Consequently, the gain for conservation would result from the absence or reduction of breeding peaks of abundance of stoats. Later, conservation in adjacent areas would benefit from the reduced immigration of dispersing young.

7.1.4 Compensatory responses in fecundity and/or survival and size of required population reductions

Populations of stoats comprise mainly juveniles during the spring-summer breeding season of most bird species (King 1989). Juvenile stoats have a different pattern of mortality to adults and their survival is related to availability of prey (King 1990). Consequently, artificial reduction of stoat density by any method, especially in summer when juveniles are present, is likely to result in significant compensation in survival of juvenile stoats. Fecundity (but not fertility) of females also is affected by availability of prey (King 1983). Models of reproductive control of stoats must take such compensation into account (Norbury 2000). The effect of any such compensation would be a tendency to nullify the impact of control measures and thereby increase the required levels of effectiveness of lethal control or sterility in terms of the proportion of the stoat population affected. This has been shown experimentally for rabbits (Williams & Twigg 1996; Twigg & Williams 1999). Experimentation could estimate this increment in stoats, although the ecology and dynamics of stoats would seem to pose difficulties for such a study. Unless such a study were undertaken, approximate values could be assumed for modelling purposes from measured rates of decline of stoats, especially juveniles, and related variables, in past field studies where populations have been poisoned. Norbury (2000)

indicates the need for separate models of reproductive control for different ecosystems where suites of pest species, predators and threatened species differ.

7.1.5 Immunocontraception improving the low cost-efficiency of conventional control?

Could immunocontraception of stoats or mustelids assist conventional control and management for conservation of threatened bird populations by reducing stoat densities to required levels (e.g. McLennan *et al.* 1996; Basse *et al.* 1999) at appropriate times (e.g. Elliott 1996a,b; Elliott *et al*. 1996)?

TABLE 8. OPTIONS FOR THE ORDER OF USING A DISSEMINATING IMMUNOCONTRACEPTIVE GMO AND LETHAL CONTROL METHODS.

OPTION	CONDITIONS	BENEFITS	DETRIMENTS
• Lethal control and reproductive control together; i.e. a single agent kills some predators and survivors are sterilised	• Lethal vector needed • Effectiveness depends on rate and extent of transmission	• Potentially the most economical strategy • Specificity needed for only one form of control	• Requires repetition or persistence to sustain sterility in the population • Immunocontraception would need to spread contagiously at reduced densities or low densities would not be achievable
• Lethal control first, followed by reproductive control	• Specificity needed for two forms of control	• Two types of control likely to achieve lower densities than one type	• Requires the immuno-contraceptive to spread contagiously at low densities, or delivered via baits to low-density populations
• Reproductive control, followed by lethal control	• Specificity needed for two forms of control	• Could enable transmission of immunocontraception if higher densities of host needed for transmission • Could enable persistent or pulsed lethal control to achieve and sustain low target densities • Two types of control likely to achieve lower densities than one type	• Likely low cost-efficiency, wastage of transmission of immunocontraception to sterilised animals that are soon killed
• Lethal control first, reproductive control second, lethal control third (e.g. 1080 baits, immunocontraception, trapping)	• Specificity needed for three forms of control	• Three types of control likely to achieve lower densities than one or two types	• Requires immuno-contraception to transmit contagiously at low densities, or delivered via baits to low-density populations • Wastage of transmission of immunocontraceptive to some sterilised animals that are soon killed • Requires more effort and resources

Adding one or more methods to an existing control program might improve effectiveness. However, cost-effectiveness, a critical issue once the choice has been made to manage stoat predation of threatened species, will be improved if the added method relative to the original method is cheaper and about equally effective, or more effective and costing about the same, or both cheaper and more effective. Immunocontraception is not likely to be more effective than proven lethal methods, but it might be cheaper if it can be delivered aerially or if a contagious vector can deliver it. Each of these solutions has a caveat. Aerial delivery requires the immunocontraceptive to be species-specific. At present no bait matrix can be defended on grounds of being specific for stoats or mustelids or pest predator species, and chemosterilants are not species-specific. A contagious vector must be capable of transmission at quite low densities (Table 3), in view of, for example, the low densities required for conservation of northern brown kiwi (McLennan *et al.* 1996; Basse *et al.* 1999).

Nevertheless, if immunocontraception is neither cheaper nor more effective than existing control, the addition of immunocontraception still could improve the cost-effectiveness of controlling stoats to conserve threatened species. This would depend on achieving an interaction of immunocontraception with the existing control methods, so that the impacts comprising main effects plus interactions substantially exceed the additive impacts of the components (e.g. Cooke 1981; Williams & Moore 1995). For example, to conserve kokako (Innes *et al.* 1999), the experimental design of controlling possums and rats by poisoning and stoats by trapping may have resulted in secondary poisoning of stoats (Murphy *et al.* 1999), a serendipitous interaction that may have enabled the economy of abandoning labour-intensive trapping of stoats. However, interactions can be positive, helping to achieve the conservation objective, or they may be negative and counterproductive (Williams & Moore 1995). A possible example of a negative interaction in cost-effectiveness could result from lethal control of stoats by trapping after an operation to spread immunocontraception in the same stoat population. Such a design could be highly effective but wasteful of effort and thereby costly. The reverse order seems to have more potential for additive effects or positive interaction in both cost-effectiveness and efficacy, although this could be untrue if a contagious immunocontraceptive required high densities of stoats for transmission. Table 8 examines conditions, benefits and detriments of such options. Williams & Moore (1995) provide a model definition for quantifiable cost-effectiveness of control of the threatening population and it could be adapted to conservation of the threatened population.

7.2 BENEFITS AND DETRIMENTS OF IMMUNOCONTRACEPTION

7.2.1 Potential benefits: Direct reduction of predation

The main effect of immunocontraception is likely to be reduced pulses of births and juveniles. This was the main impact on surgically sterilised rabbit populations (Williams & Twigg 1996; Twigg & Williams 1999). Rabbits, like stoats, are opportunistic r-strategists and, in the absence of specific information, seem to be a suitable model for immunocontraception in stoats.

This benefit of reduced pulses of juvenile stoats would be obtained mainly during bird breeding seasons when it is most needed. Consequential benefits would be reduced predation because of lower nutritional requirements of females during the breeding season and for feeding litters. Also, immigration of juvenile stoats to adjacent areas is likely to be reduced.

7.2.2 Potential benefits: Mop-up function

Contagious immunocontraceptives could sterilise animals that would not encounter traps or baits if placement or density of traps or baits were unsuitable. They could also sterilise those animals that may be reluctant to enter traps or will not accept baits. However, these benefits depend on the agent spreading contagiously.

Non-contagious contraceptives in baits, such as chemosterilants or agents that may infect stoats taking baits without spreading to other stoats, may sterilise animals that receive a sub-lethal and aversive dose of poison in baits, possibly countering selection of poison aversion, bait phobia or poison tolerance. This effect would depend on relative dose responses of the poison and the sterilising agent; infective agents may require only very small doses to be effective in individuals. It would require the sterilising agent to remain viable in the presence of the toxin. Another possible strategy for baits is to pair either a non-contagious infective immunocontraceptive pathogen or a chemosterilant, with a lethal pathogen; this may sterilise stoats that survive infection by the lethal pathogen. However, the feasibility, utility and effectiveness, of this strategy are untested and doubtful.

7.2.3 Potential benefits: Immigration function

As mentioned above, current information suggests that sterilised stoats seem unlikely to present a territorial barrier to dispersal of juvenile stoats into the controlled areas. Reduction of immigration into core conservation areas is more likely to result from widespread control or sterilisation rather than from a peripheral barrier imposed by territorial sterilised stoats. These conjectures would require experimentation for further enlightenment.

7.2.4 Potential detriments

Research and development of fertility control of stoats could absorb substantial funds that could be used to improve existing methods (Griffiths *unpublished*). Nevertheless, some aspects of fertility control may be worth pursuing (Chapter 9).

Pursuit of immunocontraception presents risks if there is failure for all involved staff, resource and land managers, administrators and politicians to understand clearly the intended role for fertility control in relation to other forms of control. New methods will complement but not replace existing costly or laborious methods. They will be intended to complement or interact positively with current methods to enable the existing level of effort and funding to be spread further or achieve greater effect. Nevertheless, effort or funding for trapping and poisoning may be reduced if this is not understood. In Australia, in spite of substantial publicity, the introduction of myxomatosis and rabbit haemorrhagic disease resulted in extensive reduction of effort and spending on

conventional control, and this is seen as a wasted opportunity to achieve cost-effective gains in the longer term.

Interest/pressure groups also may make a similar error. They may see the introduction of fertility control as an opportunity to phase out the use of poisons and traps in New Zealand, irrespective of a need to combine fertility control with conventional methods to achieve targeted levels of conservation security.

A decision to include fertility control in the management of predation for conservation has implications for monitoring its effectiveness. Monitoring the effectiveness of conventional control currently involves estimation of indices of abundance of the threatened and threatening populations. Monitoring may become more complicated when some of the control effort includes sterility of some individuals, requiring assessment of the serological or histological status of stoat populations to determine the proportions sterilised. The costs and effort of monitoring ongoing effectiveness would increase and may involve significant delay. Age structure of populations just after the breeding season of stoats would give a retrospective indication of levels of sterility in the critical season. However, this assessment would require statistical comparison with experimental control populations, with replications, and it would be too late for remedial action if outcomes showed it was needed.

7.3 DELIVERY TECHNIQUES FOR IMMUNOCONTRACEPTIVE VACCINES

Baiting is likely to be the main method of delivering immunocontraceptive agents, especially non-disseminating types. Species-specificity is highly relevant to the choice of method of delivering baits to stoats. The following briefly considers current methodology of baiting and the implications for delivering immunocontraceptives.

Aerial baiting Cost-effective coverage of large areas by aerial baiting cannot be achieved with the baits presently available for stoats because of problems with species-specificity. At present species-specificity seems to restrict the control of mustelids via aerial baiting to secondary poisoning from baits targeting pest prey species. An effective species-specific or mustelid-specific immunocontraceptive or lethal pathogen or toxin would make aerial baiting of stoats or mustelids feasible. However, it would be necessary to develop simultaneously a robust and attractive bait medium; this would be a valuable development.

Bait stations Compared to aerial baiting, bait stations decrease the risk to non-target species from non-specific lethal agents or sterilants, but the required effort and cost is greater (Innes *et al.* 1995), limiting the areas that can be treated. Bait stations containing immunocontraceptive bait may limit access to the bait for young or subordinate animals and may result in overuse and wastage by dominants. Therefore, any use of immunocontraceptives in baits at bait stations might best be combined with toxins so that target individuals die, or those receiving a sublethal dose may be sterilised; this would depend on the dose-response relationships unless the immunocontraceptive is infective.

Egg tunnels Spurr (1999) tested a variety of poison baits, lures and toxins for stoats. He recommended for management: raw hen egg baits containing 1080 for rapid effect, or diphacinone where 1080 is problematic, and further research on cholecalciferol baits. No mustelid-specific poison is available at present although one candidate seems possible and might eventually become available (Wickstrom & Eason 1999). Placement of egg baits containing non-specific toxins in egg tunnels attains species-specificity, but it limits the area that can be treated. Nevertheless, McDonald & Murphy (*in press*) consider that this method may be a cost-effective way of controlling stoats over large areas.

In principle, eggs probably would be a suitable medium for delivering immunocontraceptive agents, although some packaging may be needed to enhance longevity. However the use of an immunocontraceptive agent in eggs probably offers no advantage over poisons delivered in the same way.

Controlling prey populations Reducing the food of stoats by controlling populations of prey such as rats and mice is a potentially valuable strategy for reducing predation on threatened bird populations in the long term (see Appendix A1.6.3). However, issues such as the ability of the bird populations to withstand increased predation when rodents or other prey decline, must be examined carefully. Murphy et al. (1998b) considered this issue but the conclusions were equivocal for reasons indicated in Appendix A1.6.3. Research is needed to evaluate the benefits and detriments of strategies for reducing populations of rodents and other prey. Research should examine also the effect of combining reductions in these prey with secondary poisoning of stoats because these processes may be used separately or together in management actions.

Secondary poisoning Exploitation of secondary poisoning has great potential for reducing numbers of stoats over large areas, although more research is needed to ensure that the risks can be constrained within acceptable limits. The method needs to be combined with others that target the stoats remaining or immigrating after the initial kill of the pest prey and predators. This is because, after the initial impact, insufficient poisoned pest-prey or survivors containing toxin remain to further reduce the numbers of stoats (McDonald & Murphy *in press*).

There are four possible combinations for aerial baiting, bait stations and egg tunnels. Assuming that sterilised stoats do not deter immigration of fertile stoats into core conservation areas, most benefit seems to be in combining secondary poisoning by aerial baiting of pest prey over large areas, with later delivery of toxin and immunocontraceptive in eggs in egg tunnels in the core conservation areas. (However, as noted above, there may be little or doubtful advantage in delivering immunocontraceptive in this way.) This assumes that the risks of secondary poisoning can be constrained within acceptable limits, effective doses of the immunocontraceptive are smaller than those of the associated toxin, and the immunocontraceptive can remain active in the presence of the toxin in bait and in the alimentary canal.

Enhancement of delivery of baits depends on the specificity of the baits, and largely on the ecology of the species being conserved and the specific situation. These include the area treated, for example, large areas need to be treated for the yellow-crowned parakeet (Elliott *et al*. 1996), and smaller areas are possible

for mohua (O'Donnell *et al*. 1996). Spacing of the bait source is important to prevent saturation of stations or individual overuse (King & Edgar 1977) or sequestering by dominants. Spacing depends on the target sex, necessitating finer-grained spacing for females based on home ranges (King 1990). Placement of traps affects the sex ratios obtained (King & Edgar 1977; Murphy & Dowding 1995a,b) and this is an indicator for placement of baits, depending on the target for immunocontraception: males or females or both.

Immunocontraception also affects decisions on optimal timing of baiting. Optimal timing depends on the target within stoats for immunocontraception, male or female, oestrus, embryonic diapause, implantation, or lactation. For example, females are hard to trap in the breeding season and pregnant females are rarely caught (King 1990). These considerations would need to be integrated with the variable requirements for different specific situations. Examples of specific situations are: when stoat abundance is low for yellow-crowned parakeet (Elliott *et al*. 1996); just before breeding of kokako (Innes & Barker 1999); or in the year following stoat irruptions for mohua (O'Donnell *et al*. 1996).

Other aspects that need to be considered include frequency of replenishment, bait composition, material, size (also egg size), and its longevity, lures and attractants involving smell (including ferret lure), taste, colour, and sound (Spurr 1999; Spurr & O'Connor 1999) and free-feeding. The current research and specific situation will determine how these factors would be manipulated to integrate immunocontraception into management programs.

Free-feed baits are used to train individuals of target species to ingest sufficient quantities of bait so that they obtain a lethal dose and do not survive with aversion to lethal baits offered subsequently. Prior free-feeding may be unnecessary for baits containing some, though perhaps not all, live pathogens, whether lethal, immunocontraceptive or both. However, prior free-feeding may be useful if the bait contains non-contagious poorly-replicating pathogens or immunogenic immunocontraceptive products or chemosterilants, depending on the dose-response relationship.

7.4 INTEGRATION OF IMMUNOCONTRACEPTION WITH OTHER TECHNIQUES

In Chapters 3–6 above, the candidate methods for fertility control are as shown in Table 9. At present the lethal biocontrol pathogen with most potential is Canine Distemper Virus (CDV).

Table 9 shows that candidates exist for all classes of fertility control. Integration of the following generic categories of lethal control or fertility control, or both, with conventional management of stoats is discussed below:

- Lethal biocontrol organism (contagious pathogen)
- Disseminating immunocontraceptive GMO (may be lethal, viruses, bacteria, macroparasites)
- Non-disseminating immunocontraceptive GMO (viruses, bacteria, macroparasites)

- Non-disseminating immunocontraceptive non-GMO product (product of gene expression, may include protective packaging)
- Chemosterilants

7.4.1 Lethal biocontrol pathogen (contagious pathogen)

Rationale Lethal reduction of stoat densities by a contagious lethal biocontrol pathogen would reduce costs and increase effectiveness of conventional control of stoats.

Species-specificity The lethal biocontrol would need to be specific to stoats, or mustelids, or mustelids plus cats and dogs provided valued dogs and cats could be immunised conveniently, effectively and cheaply.

Vector Transmission could be by contact, aerosol or insect vector, depending on the pathogen.

Delivery By injection of trapped individuals, or by baits, or by aerosol device in tunnels, depending on the pathogen. Injection or inoculation was used for the initial official spread of myxomatosis and rabbit haemorrhagic disease to rabbits in Australia. For rabbit haemorrhagic disease an easier and quicker bait method was investigated and is being reviewed for official registration for use. Alternatively methods could be developed that inoculate stoats repeatedly and automatically when they enter tunnels. Such methods were devised for spreading myxomatosis in Australia, although the effectiveness and extent of use in the field are not known. Technology for directing aerosol sprays onto stoats in traps or tunnels may need to be developed if the live immunocontraceptive pathogen transmits only via respiratory surfaces. Aerosol delivery is being investigated for control of tuberculosis in possums in New Zealand (P. Cowan, pers. comm.).

Potential combination Genetic manipulation of the lethal biocontrol could enable sterilisation of stoats that survive the infection. However, such manipulation may reduce the lethality of the organism. Lethal conventional control may be most cost-effective if implemented after annual epizootics, not before unless necessary. The conventional control may be necessary to reduce stoat densities to target densities, and it would impede selection for attenuation of the pathogen and resistance of stoats to the pathogen.

TABLE 9. CANDIDATE METHODS FOR CONTROLLING FERTILITY OF STOATS.

SELECTED TARGET	CHEMOSTERILANT	NON-DISSEMINATING IMMUNOCONTRACEPTIVE NON-GMO PRODUCT	NON-DISSEMINATING IMMUNOCONTRACEPTIVE GMO	DISSEMINATING IMMUNOCONTRACEPTIVE GMO
Zona pellucida (female)		✓	✓	✓
Progesterone receptor (female)	RU486			
Prolactin receptor (male & female)		✓	✓	✓
Prolactin	Cabergoline (?male and female)			

Enhancement Natural transmission could be enhanced by increasing the intensity of the appropriate above methods of delivery. Monitoring of the disease impact would be needed if it were desired to identify the most effective or cost-effective density pattern of delivery via traps or baits. Also, lethal biocontrol pathogens potentially could be genetically engineered to make them more lethal.

7.4.2 Disseminating immunocontraceptive GMO (genetically modified viruses, bacteria, macroparasites)

Rationale Some lethality for stoats may be inherent in the pathogen, although this is expected to be considerably less than for a lethal biocontrol. An effective immunocontraceptive pathogen of relatively low lethality would reduce the regular increases in density of stoats during and soon after the breeding season. It is unlikely to reduce the base density of stoats unless nearly all susceptible stoats are sterilised annually. If nearly all susceptible stoats are sterilised annually, population increase in irregular years of high production (e.g. beech mast years) may be constrained. Widespread immunocontraception would reduce immigration of juvenile stoats into core conservation areas. Conventional control during breeding seasons and possibly in high production years would be cheaper and possibly more effective in localised areas.

Species-specificity The contagious immunocontraceptive would need to be specific to stoats, or mustelids, or mustelids plus cats and dogs provided cats and dogs could be immunised conveniently, effectively and cheaply.

Vector Contagion could be by contact, ingestion, aerosol or insect vector, depending on the pathogen.

Delivery By injection of trapped individuals, or by baits, or by aerosol device in tunnels, depending on the pathogen.

Potential combination Optimal timing of lethal conventional control, whether before or after annual or quasi-annual disease epidemics of the immunocontraceptive pathogen, would depend in part on a 2 × 2 (low : high) matrix of lethality and transmission characteristics of the genetically modified pathogen. It also would require monitoring to detect epidemics. If lethality is high, conventional control may be most cost-effective after epidemics. If transmission requires high densities of stoats, conventional control may be most cost-effective after epidemics. Options for timing from the 2 × 2 matrix would need to be integrated with the seasonal threat of predation on the threatened species and other ecological aspects. Where and when the opportunity exists, poisoning of pest prey after disease epidemics of the immunocontraceptive pathogen may reduce stoats by secondary poisoning very cost-effectively.

Enhancement Natural transmission could be enhanced by increasing the intensity of the appropriate above methods of delivery. The intensity should be appropriate to the situation and to the sex targeted. Other enhancements to be considered for baiting include some appropriate technical improvements recommended for implementation or further research by Spurr (1999) and Spurr & O'Connor (1999).

7.4.3 Non-disseminating immunocontraceptive GMO (viruses, bacteria, macroparasites)

Rationale The main benefit of such an agent would be in its target-specificity. The potential for specificity may be greater than that for poisons, and in some situations this may favour its use. Depending on political considerations (Chapter 9), there would be limited benefit in use of the agent if an effective target-specific toxin became available at an acceptable cost.

It is likely that an infectious non-contagious agent would cause only a local infection, for example in the lymph node draining the site of entry or inoculation, and probably would not kill the target host. The infective non-contagious nature of this immunocontraceptive requires that the agent would be delivered to stoats individually, but very small quantities may cause a sterilising infection. Where the agent is included in baits, either toxic or not, it may sterilise stoats that would ingest insufficient bait to receive a lethal dose of poison and possibly become averse to baits thereafter. This rationale depends on a highly probable greater dose response to the immunocontraceptive than to candidate toxins.

Assuming that the agent would be delivered in baits, the benefit of its use in management relies mainly on achieving greater target specificity than those of poisons, obviating the need for costly target-specific delivery, such as in egg tunnels. The agent could be incorporated into robust baits (that would need to be developed) that could be delivered aerially. Its widespread delivery and uptake would reduce peaks of juvenile stoats during and after the breeding seasons, and possibly constrain stoat population increase during irregular years of high productivity. Reduction of these peaks of abundance may reduce costs of conventional control during breeding seasons and in years when stoat populations normally irrupt.

Species-specificity The need for species-specificity may be less than the absolute requirement for specificity of contagious immunocontraceptives. However, most benefit over poisons in baits is lost if the agent is not target-specific, and aerial delivery would not be an option; consequently there would be limited benefit to reducing costs of conventional control.

Vector Baits. Development of a robust bait for aerial delivery would be needed. The nature of the pathogen and the dose response would determine whether vectors other than baits, say aerosols or scratch inoculations, would be feasible or useful. Delivery in baits may be assisted by some form of packaging that may protect the agent from degradation until absorbed from the stoat's alimentary canal into the lymph or blood circulation.

Delivery If specific to stoats, or mustelids, or mustelids and cats, it could be delivered aerially in robust baits that would need to be developed. If not target-specific, the means of delivery would need to be specific, such as by egg tunnel, and the agent would be of doubtful utility.

Potential combination Aerial delivery of the agent in baits, implying target-specificity, would seem to be most cost-effective before the breeding season when there would be fewer stoats per bait and per individual of the threatened populations, and before conventional control were undertaken. Where and when the opportunity exists, poisoning of pest prey by prior or simultaneous

aerial delivery of toxic baits with the stoat/mustelid-specific immunocontraceptive baits may reduce stoat abundance cost-effectively as well as sterilise those that accept baits but may not succumb to secondary poisoning.

If the agent were not target specific, its inclusion with poisons in baits may sterilise mustelids that ingest insufficient poison for a lethal dose, although small improvement in cost-effectiveness could be expected. Nevertheless, the strategy might impede selection for toxin aversion, bait phobia or resistance to poisons.

Enhancement If the agent is target-specific, cost-efficiency should be enhanced by best practice aerial delivery of baits at density patterns that target the appropriate sex in the specific situation. If the agent is not target-specific, baiting could benefit from the refinements recommended by Spurr (1999) and Spurr & O'Connor (1999).

7.4.4 Non-disseminating immunocontraceptive non-GMO product (product of gene expression, may include protective packaging)

Rationale Any benefit in such agents would derive mainly from greater target-specificity than that of candidate poisons, enabling delivery by air or by methods less costly than in eggs in egg tunnels. Assuming target-specificity, the dose response relative to that of poisons would determine whether there would be any benefit in sterilising stoats/mustelids that would consume insufficient bait to obtain a lethal dose and become bait-averse subsequently. While the agents are not infective, it is likely that greater quantities are required than infective immunocontraceptive agents to promote an immunocontraceptive response. It is possible that insufficient bait may be consumed to cause contraception or sterility, although subsequent bait aversion is much less likely than if an insufficient quantity of poison were ingested. Aerial delivery of target-specific agents could improve cost-effectiveness of subsequent conventional control. Target-specificity and aerial delivery causing sterility would reduce peaks of abundance of stoats during and after the breeding seasons and possibly during irregular years of high productivity, improving the cost-effectiveness of conventional control. If the agent were not target-specific, there seems little and insufficient benefit in using such agents in place of poisons. Depending on political considerations (Chapter 9), there would be insufficient benefit in use of the agent if an effective target-specific toxin became available at an acceptable cost.

Species-specificity The need for species-specificity is less than the absolute requirement for specificity of contagious immunocontraceptives. However, most benefit over poisons in baits is lost if the agent is not target-specific, and aerial delivery would not be an option; consequently there would be little benefit to reducing costs of conventional control.

Vector Baits only. Development of a robust bait for aerial delivery would be needed.

Delivery If specific to stoats, or mustelids, or mustelids and cats, it could be delivered aerially in robust baits that would need to be developed. If not target-specific, the means of delivery would need to be specific, such as by egg tunnel, and the agent would be of doubtful utility.

Potential combination As above for non-disseminating immunocontraceptive GMOs, aerial delivery of a target-specific agent in baits would seem to be most cost-effective before the breeding season when there would be fewer stoats per bait and per individual of the threatened populations, and before conventional control were undertaken. Where and when opportune, poisoning of pest prey by prior or simultaneous aerial delivery of toxic baits with the stoat/mustelid-specific immunocontraceptive baits may reduce stoat abundance cost-effectively and sterilise stoats that accept baits but may not die of secondary poisoning.

Similarly, as for non-disseminating immunocontraceptive GMOs, if the agent were not target-specific, depending on the dose responses of agent and poison, its inclusion with poisons in baits could sterilise mustelids that ingest insufficient poison for a lethal dose. While cost-effectiveness might not improve much, this strategy might impede selection for toxin aversion, bait phobia or resistance to poisons.

Enhancement If the agent is target-specific, cost-efficiency should be enhanced by best practice aerial delivery of baits at density patterns that target the appropriate sex in the specific situation. If the agent is not target-specific, baiting could benefit from the refinements recommended by Spurr (1999) and Spurr & O'Connor (1999).

7.4.5 Chemosterilants

Rationale At present chemosterilants are not target-specific. Consequently they would need to be delivered in a target-specific manner, such as for toxins in eggs in egg tunnels. At present there seems to be no particular advantage in sterilising stoats or mustelids by the same methods used to deliver poisons. Conservation objectives are more likely to be achieved by killing the stoats rather than allowing them to continue predation during their own lifetime. If poisons become unacceptable, kill-trapping in tunnels would be a better alternative. If that also were not an option, live-trapping and euthanasia may be more costly, but cost-effectiveness may still be better than for use of chemosterilants delivered as for poisons. Also, chemosterilants may be needed annually to keep individuals from returning to fertility in the next year.

Species-specificity Limited.

Vector Baits.

Delivery As for poisons (Spurr 1999).

Potential combination Depending on the relative dose responses of chemosterilants and poisons, inclusion of chemosterilants in toxic baits may temporarily sterilise individuals that ingest insufficient bait for a toxic dose. This may have little effect on cost-effectiveness of the necessary conventional control of stoats, although it may help to impede selection for stoats that are toxin averse, phobic to baits, or tolerant of poisons. Aerial baiting of pest prey, where possible and opportune, would be compatible with such use of chemosterilants within conventional control and is likely to improve its cost-effectiveness.

Enhancement The recommendations of Spurr (1999) and Spurr & O'Connor (1999) would improve the cost-efficiency of baiting that includes chemosterilants.

7.5 SUMMARY

For all methods of control, including fertility control:

- Stoat control is one component of managing complex systems.
- Conjectures on the threatening process should be tested.
- Opportunities for adaptive management should be used.
- Research is required into levels of stoat control to meet conservation and other objectives.
- Predator-proof fencing may be viable for some highly valued/threatened prey species and may create a realistic option for local eradication of stoats (as, for example, on islands).
- Manipulation of habitat may assist conservation in core areas under some circumstances.

For fertility control:

- Addition of fertility control to management programs would help achieve conservation objectives if it improves cost-effectiveness.
- To improve cost-effectiveness, fertility control would need to be cheaper than lethal control or interact with it in a positive way; fertility control is unlikely to be more effective than lethal control applied within the same area.
- The potential for specificity of some forms of fertility control may enable aerial delivery, provided a suitable bait medium is developed, and this would reduce costs and improve cost-effectiveness.
- Some preliminary ecological research into issues such as mating systems and territoriality, especially of sterilised animals, would be useful (but probably not essential).
- Fertility control may be useful in buffer zones to reduce dispersal of juvenile stoats into core conservation areas, provided fertility control is more cost-effective than lethal control.
- Current lethal methods of stoat control (apart from secondary poisoning) are expensive and labour-intensive because of the reliance on traps or eggs as bait.
- Delivery of a non-disseminating fertility control agent in eggs confers little or no advantage over poisoning.

8. Likely constraints relative to the use of a fertility control agent or pathogen

Constraints on the development of biocontrol agents are both technical and socio-political. This chapter covers aspects of the socio-political dimension. It addresses international agreements as well as New Zealand regulatory or approval processes and risk assessments for GMOs or any other new techniques.

8.1 BIOCONTROL AGENTS AND VERTEBRATE PEST POPULATIONS

There are only a few examples of the deliberate use of biocontrol agents directed at vertebrate pest populations. The release of myxoma virus into rabbit populations in Australia in 1950 (Fenner & Ratcliffe 1965: Fenner & Ross 1994) is probably the best-known example of the use of a naturally occurring infectious biocontrol agent. Others are the use of feline panleukopaenia virus to control cats on Marion Island (Van Rensburg *et al*. 1987) and the introduction of rabbit haemorrhagic disease virus to reduce rabbit numbers in Australia and New Zealand (O'Keefe *et al*. 1999; Fenner & Fantini 1999). Ectromelia virus has been released in China in an attempt to control mice (Zuwang *et al*. 1975) as has the nematode *Capillaria hepatica* (Singleton & Chambers 1996) in Australia. There are no examples of the use of a genetically modified organism as a biocontrol agent in vertebrates although a genetically modified vaccinia virus has been released in Europe and the USA to control rabies in foxes (Pastoret *et al*. 1992) and raccoons, respectively (Hanlon *et al.* 1998).

8.2 EVOLVING PUBLIC ATTITUDES

Since the release of myxoma virus in Australia in 1950 the regulations governing the importation of biocontrol agents and the release of those agents into the environment in most countries has become markedly more stringent. The process has moved from mainly considering direct immediate impacts on human health and animal production to considering impacts on the environment, including potential direct and indirect and short- and long-term effects. Social effects are also considered. The final decision to allow the importation and release of biocontrol agents is still the preserve of bureaucrats and politicians but a more direct involvement of the public in these decisions is now standard practice.

Public attitudes may be emotive and often fickle but are influential with politicians. Valid scientific argument can be effectively countered by emotive and sometimes irrational arguments from public lobby groups. Proposals to use

biocontrol agents on pest species are understandably highly emotive topics and are bound to be of interest to the public. Unlike vaccines where the intention is to reduce the impact of disease and suffering, the use of biocontrol agents often involves advocating lethal agents where individual animal suffering is often plainly evident. Evidence of suffering is often more easily understood than more subtle arguments regarding environmental damage. The arguments for the deployment of a biocontrol agent need to be not only scientifically valid in terms of safety and efficacy but also plainly and cogently argued in terms of the trade-off between animal suffering and environmental benefits.

The use of biological control, including fertility control, is acceptable to the majority of the public as evidenced by a recent survey of public opinion in Australia (Johnston & Marks 1997) and New Zealand (Sheppard & Urquhart 1991; Fitzgerald *et al.* 1996, 2000). However, genetic manipulation adds a further dimension of concern to the development of such agents. The debate over the field release of GMOs has a high public profile, mainly related to GMO foods but no doubt will extend to other releases.

Public opinion on these issues is already being reflected in legislation and international agreements pertinent to biocontrol. It is possible that these constraints will become even more stringent as the debates on genetic manipulation, biodiversity, and animal welfare develop. Nevertheless, recent changes to legislation in New Zealand are likely to satisfy the New Zealand public, at least in the short to medium term, and the regulatory pathways to the development of a stoat biocontrol agent are reasonably clear at the moment. This has been reinforced by experience gained in Australia and New Zealand leading up to the importation of rabbit haemorrhagic disease virus (RHDV) and the subsequent releases in those countries.

8.3 AUSTRALIAN EXPERIENCE

The importation and unintentional release of rabbit haemorrhagic disease virus (also called rabbit calicivirus) into Australia tested the Australian Commonwealth legislative framework (viewed at http://austlii.law.uts.edu.au/au/legis/cth/consol_act/toc.html) under which future biocontrol agents might be released. If another agent was proposed for importation then the process would involve the Quarantine Act 1908 and the Australian Wildlife Protection (Regulation of Exports and Imports) Act 1982. Release would require approval under the Biological Control Act 1984 (BCA), the Environment Protection (Impact of Proposals) Act 1974 (EPIPA)(soon to be replaced by the Environment Protection and Biodiversity Conservation Act, 1999; EPBCA) and the Agricultural and Veterinary Chemicals Code Act 1994 (AVCCA). Equivalent mirror legislation where appropriate would need to be invoked in each State where the agent was to be released. Also relevant would be the various State and Territory Acts to regulate the use of animals for experimentation.

If the agent was genetically manipulated within Australia the process would be under the scrutiny of the Genetic Manipulation Advisory Committee which has no statutory powers, although it is the policy of Government research organisations to seek their advice. This situation will change when legislation establishing the Office of the Gene Technology Regulator (OGTR) is enacted.

Legislation will make it mandatory for any individual or organisation to seek the advice of the OGTR. The organism or product would still need approval under BCA, EPIPA (or EPBCA) and AVCCA. The environmental legislation is currently under review so this situation may also change.

Although the suite of legislation in New Zealand is different (there is no equivalent of the BCA, for instance), the legislative processes involved in the importation, construction and release of a biological control agent would be similar in principle.

8.4 NEW ZEALAND LEGISLATION

New Zealand statutes (viewed at http://rangi.knowledge-basket.co.nz/gpacts/actlists.html) that would most likely impinge on the introduction of an immunocontraceptive agent for stoats are:

- Hazardous Substances and New Organisms Act 1996
- Biosecurity Act 1993
- Agricultural Compounds and Veterinary Medicines Act 1997
- Pesticides Act 1979
- Environment Act 1986
- Animal Welfare Act 1999

The invoking of any particular Act will depend on the nature of the agent and on interpretations of those Acts by the Government Departments or Ministries that administer them. The Acts to be considered will depend on whether the agent or elements of the agent are imported or constructed entirely within New Zealand and whether the agent involves an infectious agent, either disseminating or in a bait, or a non-infectious bait delivered product. It will also depend on whether or not genetic manipulation is involved, which it most probably will be. If the agent relies on the importation of a viral vector currently not in New Zealand then the Biosecurity Act 1993 would be involved at an early stage and the importation may not succeed. The Biosecurity Act 1993 may also be involved for the importation of genetic elements although that may depend on whether elements such as plasmids containing a foreign gene are considered as capable of replication.

If the agent is to be genetically engineered within New Zealand using an organism already present in New Zealand, or imported under the provisions of the Biosecurity Act 1993, then this would require approvals from the Environmental Risk Management Authority (ERMA) established under the Hazardous Substances and New Organisms Act 1996 (HASNOA).

Recently, there has been a move in New Zealand for a moratorium on the field release of genetically modified organisms to allow a Royal Commission of Enquiry to investigate the issue. The outcome of such an enquiry may alter the feasibility of releasing a genetically manipulated biocontrol agent in the long term. If the agent is not infectious and does not involve the use of genetic manipulation then currently the Pesticides Act 1979 would need to be considered; but the provisions under this Act, and under the Toxic Substances

Act 1979, are due to be replaced some time in the year 2000 by provisions under HASNOA.

In all instances it is a likely that any biocontrol agent prepared or manufactured for use in New Zealand would need to be registered under the Agricultural Compounds and Veterinary Medicines Act 1997 (ACVMA). Although this Act is primarily used to regulate products for sale, it also covers the gift or use of such a product. In Australia the equivalent legislation, the AVCCA, became crucial to the process of allowing the release of RHDV to pest managers. The reason was that the risk assessment process under this Act was the most rigorous of all the processes being applied at the time. Approval under BCA and EPIPA became contingent upon approval under AVCCA. In New Zealand, approval under HASNOA may be sufficient to allow approval under ACVMA.

Another issue to consider would be the role of the Ministry for the Environment and the Commissioner for the Environment, both established under the Environment Act 1986. They advise the Minister for the Environment and the House of Representatives, respectively, on environmental issues and their advice could be crucial to the success of an application for release.

Finally, all work with biocontrol agents in stoats would need to be approved under the provisions of the Animal Welfare Act 1999.

8.5 INTERNATIONAL AGREEMENTS AND ATTITUDES

New Zealand is a signatory to a number of international agreements such as the Convention on Biodiversity, the World Trade Organisation, and the World Health Organisation. Under the Convention on Biodiversity, a Protocol to regulate the transboundary movements of what have been called Living Modified Organisms (LMOs) is being established. Article 1 (Objective) in the Draft Cartagena Protocol on Biodiversity presented at the Conference of the Parties to the Convention on Biological Diversity, Montreal, 24–28 January 2000 (viewed at http://www.biodiv.org/biosafe/BIOSAFETY-PROTOCOL.htm), and modified* at that meeting states:

> 'In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms [~~resulting from modern biotechnology~~]* that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.'

This can be interpreted to mean that any Party to the Convention on Biodiversity needs to take into account potential consequences for other countries of the development of LMOs in that Party's country. The implications for New Zealand of the development of a biocontrol for stoats that involved an LMO would need to be considered.

The World Trade Organisation established an Agreement on the Application of Sanitary and Phytosanitary measures (viewed at http://www.wto.org/wto/goods/spsagr.htm). In article 5 of the Sanitary and Phytosanitary measures, Assessment of Risk and the Appropriate Level of Sanitary and Phytosanitary Protection, they recommend using relevant international organisations for such assessments and the relevant organisation for stoats would be the Office International des Epizooties, OIE (viewed at http://www.oie.int/a_html.htm).

The OIE Working Group on Wildlife Diseases has voiced concerns about the use of disseminating immunocontraceptive agents for canids (Office International des Epizooties 1994) and has concerns about the use of such agents for wild animals generally. At the 65th meeting of the International Committee of the OIE the Working Group adopted the recommendations of the American Association of Wildlife Veterinarians which in 1994 recommended that fertility control only be considered an acceptable means of population regulation in free ranging animals when certain criteria are met (Appendix X)(Office International des Epizooties 1996).

Another organisation that may have an interest in fertility control of stoats is the World Health Organisation. They developed recommendations for the use of wildlife rabies vaccines and have considered the implications of immunocontraceptive vaccines for wildlife (Stohr & Meslin 1997). A World Health Organisation consultation in 1993 was the first to recommend that any vector used for free-ranging carnivores be non-transmissible (World Health Organisation, 1993) and it was this recommendation that was taken up by the OIE Working Group on Wildlife Diseases.

9. A 'systems' assessment of ecological, social/political and economic consequences of control

9.1 PURPOSE

The purpose of systems analysis is to place the issues associated with stoat control into a social and ecological context and to assess the opportunities and constraints that may flow from new research directions. Normally a full analysis would be based on expert advice as well as published and unpublished material. Expert opinion, including anecdotal information, can be invaluable in assessing the historical development of management practices and is necessary to identify the full range of future options. A workshop with representation of all the relevant interests is the most appropriate way to synthesise this information but has not been possible for this report. Therefore the analysis is restricted to information in published material and some unpublished manuscripts and documents. The aim is to assess future options for research in a framework relevant to the Stoat Research Programme and within likely development times for prospective types of control technology.

Current stoat management is based on a history of development of two main methods of control: poisoning and trapping, and a rapidly changing awareness of the importance of predation as a threatening process for native fauna. Control techniques have been adapted from those used in the areas of the Northern Hemisphere where mustelids are endemic, although the use of 1080 has been discontinued in many countries with the notable exceptions of New Zealand and Australia. Poisoning and trapping are likely to continue as the main control techniques, at least in the short to medium term, with some potential for continued refinement. However both techniques are likely to come under increasing scrutiny by public interest groups concerned with animal welfare, the environmental consequences of pesticides, and the use of control techniques that are not absolutely specific to stoats, or mustelids. In addition, low-cost techniques suitable for broadscale application are required for the sustainable management of stoats in the long term. New options for control include the use of chemosterilants (Chapter 4), and biocontrol with pathogens that decrease survival rates or fecundity of stoats (Chapter 3 and Chapter 5). In addition, recent advances in molecular biology have raised the possibility of genetically engineering fertility control agents that could be delivered either through non-toxic baits or via a disseminating parasite (Chapter 3). The new options need to be considered in the context of existing stoat control, the cost of their development, direct and indirect environmental consequences and public attitudes to issues such as biological control and the use of GMOs.

9.2 EXPECTATIONS

Based on the review of the current status of stoat control in New Zealand (Chapter 7) and the opportunities for the development of new control (Chapter 2–6), the following expectations and constraints apply to stoat management in New Zealand in the short term (within 5 years).

- Although the simultaneous control of several pest species may be desirable (Chapter 7), control effort will be required specifically for stoats.
- Poisoned eggs are an effective, but labour-intensive, method of stoat control.
- There are indirect benefits via secondary poisoning when poison baits are used for pest species that are preyed upon by stoats.
- Rates and distances of dispersal by stoats are high.
- Stoats could be an important vector for the long-range spread of tuberculosis (Tb).
- Eradication of stoats is not feasible except, perhaps, for small areas where there is no immigration (e.g. islands).
- The pre-release research for a stoat- or mustelid-specific fertility control agent, based on a non-disseminating immunocontraceptive non-GMO product, or a non-disseminating immunocontraceptive GMO, or a chemosterilant, could be completed.
- A bait matrix suitable for broadscale (e.g. aerial) delivery could be developed.
- A disseminating immunocontraceptive GMO could not be developed to the final product stage.
- Lethal biocontrol could not be developed to the final product stage within this timeframe.

In addition in the medium term (5–10 years):

- A non-disseminating immunocontraceptive non-GMO product, or a non-disseminating immunocontraceptive GMO, or a chemosterilant, suitable for use in a bait-delivery system could be developed through to the final product.
- A micro- or macro-parasite suitable for use as a lethal biocontrol organism could be identified and tested to the pre-release stage.
- The research phase could be completed for immunocontraceptive agents suitable for use in a genetically modified micro- or macro-parasite.
- A micro- or macro-parasite suitable for use as a disseminating vector for a fertility control agent could be identified and tested to the pre-release stage.

In the long term (>10 years):

- For some threatened species and some ecosystems, there will be more information on the level of predator control required to meet conservation objectives.
- At least some control effort will be required specifically for stoats and additional control effort will be required to manage other threatening processes.
- There will be increased pressure to find low-cost, economically-sustainable methods of stoat control.
- There may be some development of resistance to poisons by stoats, either as a result of direct poisoning operations or via secondary poisoning.

- There will be a high level of public and scientific scrutiny of any proposals to import new biocontrol agents for a vertebrate pest (given the experience in New Zealand with myxomatosis and rabbit haemorrhagic disease).

9.3 CONCLUSIONS

An assessment of the ecological, social and economic consequences of six generic research options is presented in Table 10. Several conclusions can be drawn from this assessment and from the discussion in Chapter 7. Selection of any of the active research options runs the risk of stoat control being confined to one pathway of development, although this is more likely for high-cost research such as genetic engineering or biocontrol. All techniques may decline in effectiveness due to selection for non-response in stoats, and in the case of lethal biocontrol there is likely to be selection for less virulent strains of the pathogen. Self-disseminating control techniques are likely to attract the most social and political, as well as scientific, attention. Increasing public awareness of environmental and animal welfare issues indicates that education programs will be necessary regardless of which research option is chosen.

For the short term, poisoning and trapping will be the most effective means of controlling stoats in localised areas that are accessible—provided the use of these techniques remains socially acceptable. There may be opportunities to improve the efficiency of current techniques, for example through the use of attractants. Recent research suggests that stoat control will always be essential to protect some prey species, and important for protecting others. Therefore reliance on labour-intensive high-cost methods of stoat control is not a preferred conservation strategy in the long-term.

In the short- to medium term, the use of poisoning and trapping should continue because these are proven techniques and because lethal control produces a more rapid reduction in a localised stoat population than fertility control. Fertility control with a non-disseminating agent would be a useful option where poisons cannot be used (e.g. due to cost, logistics or to the presence of valued non-target species) provided it is stoat- or mustelid-specific and can be applied over large areas to reduce recolonisation of intensively controlled core areas. This type of application would depend on the development of a bait matrix attractive to stoats and suitable for broadscale distribution (e.g. from aircraft). A decision to proceed with non-disseminating fertility control requires that either the sterility agent or the bait (or both) is stoat- or mustelid-specific. If it is possible to produce a new bait matrix that will not be eaten by valued species, then it should be used with a poison, rather than a sterility agent, to achieve maximum effect (i.e. rapid reduction in the density of stoats over large areas). If it is not feasible to develop this type of bait matrix then species-specificity is a requirement of the sterility agent. Since the available chemosterilants do not have this property at present, research would be required to develop a new sterility agent—for example a chemosterilant or a non-disseminating immunocontraceptive GMO—with parallel research into a suitable bait matrix.

In the long term, low-cost methods for the control of stoats are required for the sustainable conservation of native fauna and the sustainable management of diseases such as Tb. Reliance solely on current techniques would require a level of public and financial support in perpetuity that is probably unsustainable. Therefore high priority should be given to the development of at least one method of low-cost control suitable for broad-scale use. However integration with trapping and poisoning may still be required in localised areas.

The choice of a research option depends on the cost as well as the factors summarised in Table 10 (pp. 69–71, caption on p. 71). Refinement of current techniques relies on incremental research that can be conducted, if necessary, at relatively low cost. If the objective of a species-specific chemosterilant, non-disseminating immunocontraceptive non-GMO product or non-disseminating immunocontraceptive GMO is selected, then a major research cost for this form of control is the development of a bait matrix suitable for low-cost delivery. The application of genetic technology requires specialised expertise and equipment, which can be contracted to external research institutions if necessary, but the costs are likely to diminish in the future. Tests of a final product based on a non-disseminating immunocontraceptive GMO or disseminating immunocontraceptive GMO will require expensive trials with animals in a high-security facility. High-security laboratory research is also required for new lethal biocontrol organisms. Options using GMOs or imported organisms may require expensive field trials with isolated populations of stoats (for example on off-shore islands), as well as an extensive process of public consultation.

Biological control of vertebrate pests using non-modified organisms has been successful in New Zealand and elsewhere. However the public and political response to proposals for the use of myxomatosis and rabbit haemorrhagic disease to control rabbits in New Zealand indicates that significant resources would be required for a public education program to back up any future research into biocontrol. A similar effort in education would be required for any technique using a GMO. In the case of stoat control, public support may be enhanced by the recognition of predation as a threatening process for highly valued native fauna.

Fertility control using a GMO has the greatest potential to be stoat- or mustelid-specific, and for the latter it may be possible to use an endemic organism as a vector. A bait-delivered non-disseminating immunocontraceptive GMO could be used as an interim stage in the development of a disseminating system: it could proceed to the product stage more rapidly and at lower cost than a disseminating immunocontraceptive GMO, it would provide a means of testing the efficiency of fertility control and would provide managers with a technique that could be recalled if necessary. The rapid rate of progress in genetic technology indicates that the time for development of these products is likely to decrease, and that there may be additional advantages for New Zealand in maintaining scientific expertise in this area. Ultimately, the choice of a research strategy will depend on society's objectives, with priority based on political, not scientific, judgement. The preferred options for some alternative objectives are listed in Table 11 (p.72).

RESEARCH OPTIONS	ECOLOGICAL CONSEQUENCES	SOCIAL/POLITICAL CONSEQUENCES	ECONOMIC CONSEQUENCES
(a) No further research into control techniques	• Continued protection of a few key species in local, accessible areas • Continued broadscale decline of native fauna • Continued selection for increased resistance to poisons	• Funds available for alternative related research, e.g. into pest ecology and threatening processes other than predation • Locked into current control techniques • Risk of no stoat control if use of conventional techniques is blocked for social or political reasons • Education program required to maintain support for high-cost stoat control in perpetuity • No incentive to develop scientific expertise in genetic engineering or biocontrol	• High cost of control not sustainable in the long term • Continued difficulty in containing Tb • Locked into current, or rising, cost of stoat control
(b) Refinement of current techniques (excluding biocontrol)	• If poisons remain non-specific: more efficient, cost-effective protection of a few key species in local, accessible areas • If poisons remain non-specific: continued broadscale decline of native fauna • Alternatively a stoat- or mustelid-specific poison would open new management opportunities and significantly improve conservation of native fauna • Continued selection for increased resistance to poisons	• Stoat control limited to poisoning and trapping • Risk of no stoat control if use of poisons or traps is blocked for social or political reasons • Education program required to maintain support for poisoning and trapping • Risk of international pressure to limit the use of poisons such as 1080	• If poisons remain non-specific: cost of control may not be sustainable in the long term • If poisons remain non-specific: continued difficulty in containing Tb at a regional scale • If poisons remain non-specific: cheaper stoat control in restricted areas • Alternatively a stoat- or mustelid-specific poison would enable more cost effective control over large areas • Research funds focussed on improvements in current techniques
(c) Development of a bait-delivered stoat- or mustelid-specific chemosterilant *or* non-disseminating immuno-contraceptive non-GMO product	• Broadscale stoat control feasible, including areas with valued non-target species • At best, a slow decline in stoat abundance (relative to management with lethal control) in areas where fertility control is the only option • Some additional control of ferrets and weasels, unless the chemosterilant or immunocontraceptive product or the bait matrix is stoat-specific	• Pressure to increase reliance on fertility control even though integration with lethal techniques may provide the best management solution • Strong requirement to demonstrate no entry of the chemosterilant or immuno-contraceptive product into the (human) food chain • Education program for the best use of integrated pest management and to maintain support for complementary lethal control methods	• Cost of control may not be sustainable in the long term, particularly if stoats or mustelids require repeated treatment within one breeding season • No improvement on regional control of stoats as vectors of Tb • Requires investment to develop a bait matrix suitable for broadscale stoat control • May require some investment in delivery technology (e.g. aerial baiting)

RESEARCH OPTIONS	ECOLOGICAL CONSEQUENCES	SOCIAL/POLITICAL CONSEQUENCES	ECONOMIC CONSEQUENCES
(b) *Continued*	• Integration with current lethal techniques would be required for local, intensive, rapid stoat control. The timing and pattern of delivery will depend on the duration of induced infertility and/or any requirement for more than one dose • The timing and number of applications may limit the proportion of stoats made infertile • Selection in favour of animals that do not take baits or that take sub-optimal doses		• A focus of research funds on development of chemosterilant or immuno-contraceptive product will generate a product in the short to medium term • Limited funds to develop alternative control techniques or for research into pest ecology and Tb epidemiology
(d) Development of bait-delivered fertility control using a mustelid-specific non-disseminating immuno-contraceptive GMO	• Broadscale stoat control feasible, including areas with valued non-target species • At best a slow decline in stoat abundance (relative to management with lethal control) in areas where fertility control is the only option • Some additional control of ferrets and weasels, unless the immunocontraceptive GMO or the bait matrix is stoat-specific • Integration with current lethal techniques would be required for local, intensive, rapid stoat control • Selection for bait-shy animals • Selection for non-responders	• Long time frame to public acceptance • Public pressure to increase reliance on fertility control even though integration with lethal techniques may provide the best management solution • Strong requirement to demonstrate no entry of the immunocontraceptive GMO into the (human) food chain • Risk of no improvement on current stoat control if use of GMOs is blocked for social or political reasons • Risk of international pressure to block the use of an immunocontraceptive GMO • Education program required for an informed public response to the use of an immunocontraceptive GMO	• Cost of control may not be sustainable in the long term, especially given new expectations of effective control at a regional scale • Requires investment to develop a bait matrix suitable for broadscale stoat control • May require some investment in delivery technology (e.g. aerial baiting) • Insufficient funds to develop alternative control techniques or for research into pest ecology and Tb epidemiology
(e) Initiation of research into fertility control using a disseminating immuno-contraceptive GMO	• No change in control options in the short to medium term • Continued broadscale decline of native fauna, in the short term • In the long term, potential for improved stoat (mustelid) control at the national scale	• Alternative pathways of development for stoat control constrained by high cost of research • Risk of no improvement on current stoat control if use of GMOs, or disseminating GMOs specifically, is blocked for social or political reasons within NZ or in response to international pressure	• Potential for a substantial reduction in cost of stoat control in the long term • Insufficient funds to develop alternative control techniques or for research into pest ecology and Tb epidemiology, in the short term • Risk of impact on trade if there is international opposition to the use of a disseminating immuno-contraceptive GMO

RESEARCH OPTIONS	ECOLOGICAL CONSEQUENCES	SOCIAL/POLITICAL CONSEQUENCES	ECONOMIC CONSEQUENCES
(e) *Continued*	• Broadscale control may inhibit dispersal of stoats and open options for local eradication of stoats to protect native species highly sensitive to predation • Selection for non-responders • Lethal control techniques (trapping and poisoning) may be required for local, intensive, rapid stoat control	• Increased expertise in rapidly expanding research in molecular biology • In the long term, potential for improved conservation benefits at the national scale, provided a sufficient reduction in stoat abundance is achieved • Requirement to increase quarantine and trade security to counter international concerns over use of a disseminating immunocontraceptive GMO • Risk of long development time to final product • Education program for an informed public response to the use of a disseminating immunocontraceptive GMO • Potential for unrealistic expectations of 'release and forget' stoat control, with risk of a reduction in funding for research and management	• Potential need for long-term funding of research to maintain effective control because of co-evolution of a disseminating immuno-contraceptive GMO and the target species • Increased cost of quarantine
(f) Initiation of research into lethal mustelid-specific biocontrol organism	• No change in control options in the short term • Continued broadscale decline of native fauna, in the short term • In the long term, potential for improved stoat control and conservation at the national scale • Broadscale control may inhibit dispersal of stoats and open options for local eradication of stoats to protect native species sensitive to predation • Integration with current lethal techniques may be required for local, intensive, rapid stoat control • Selection for host resistance and for less virulent strains of biocontrol organism	• Alternative pathways of development constrained by high cost of biocontrol research • Risk of no improvement on current stoat control if use of biocontrol organism is blocked for social or political reasons within NZ • Risk of long development time to final product • Education program for an informed public response to the use of biocontrol organism • Potential for unrealistic expectations of 'release and forget' stoat control, with risk of a reduction in funding for research and management	• Potential for a substantial reduction in the cost of stoat control in the long term • Insufficient funds to develop alternative control techniques or for research into pest ecology and Tb epidemiology, in the short term • Potential need for long-term funding of research to maintain effective control because of co-evolution of biocontrol organism and host

TABLE 10 (PP. 69–71). OPTIONS FOR FUTURE RESEARCH ON THE CONTROL OF STOATS IN NEW ZEALAND. THE OPTIONS FOR FERTILITY CONTROL ARE CLASSIFIED INTO FOUR TYPES: CHEMOSTERILANTS, NON-DISSEMINATING IMMUNOCONTRACEPTIVE NON-GMO PRODUCT, NON-DISSEMINATING IMMUNOCONTRACEPTIVE GMO, AND DISSEMINATING IMMUNOCONTRACEPTIVE GMO. THE FIRST THREE WOULD BE DELIVERED IN A NON-TOXIC BAIT.

TABLE 11. MATCH BETWEEN CONSERVATION, OR BUDGETARY, OBJECTIVES AND OPTIONS FOR RESEARCH.

OBJECTIVE	PREFERRED OPTION
Low short-term cost of research (<5 years)	Models and management strategies for the best scale/timing/frequency of stoat control *and* Improved current techniques (better efficiency of baiting, trapping and monitoring methods)
Lowest cost of research with new product in the medium term (5–10 years)	Mustelid-specific non-disseminating immunocontraceptive GMO and bait matrix suitable for broadscale delivery
Lowest cost of research with new product in the long term (>10 years)	Mustelid-specific lethal biocontrol
Shortest time to new product development	Mustelid-specific non-disseminating immunocontraceptive GMO and bait matrix suitable for broadscale delivery
Sustainable conservation of native fauna	Mustelid-specific disseminating immunocontraceptive GMO, integrated with localised conventional control *or* Mustelid-specific lethal biocontrol organism, integrated with localised conventional control
Sustainable control of Tb	Mustelid-specific disseminating immunocontraceptive GMO, integrated with localised conventional control *or* Mustelid-specific lethal biocontrol organism, integrated with localised conventional control
Maximum conservation benefits with lowest research risk in the short term	Improved current techniques

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Appendices

Appendix 1. Background and context for the application of fertility control

Integration of immunocontraception with other management for likely best effect (Chapter 7) extends beyond immunocontraception to other undefined and unspecified aspects of the array of potential strategies and methods for conserving the threatened populations of native bird species. Chapter 7 requires consideration that immunocontraception of stoats be a component of one or more of the array of potential management plans.

A1.1 INTERACTIONS AMONG SUITES OF PREDATORS AND PREY

A1.1.1 Predator guilds

A growing body of evidence suggests that stoats are significant predators of numerous threatened native birds (King 1984, 1990; McLennan *et al.* 1996; O'Donnell 1996a,b; Wilson *et al.* 1998; McDonald & Murphy in press), building on indirect or correlative evidence, e.g. observations that high mortalities in threatened bird populations correlate with high abundances of stoats (reviewed in Griffiths *unpublished*). Other field studies have shown that stoats are not the only predators of threatened bird populations, others being possums, cats, ferrets, weasels and rats, as well as three native raptorial birds (King *et al.* 1996b; Innes *et al.* 1999) and perhaps mice. Periodically, e.g. in heavy beech mast years and during equivalent events in other forest types, increases in the abundance of bird species, insects and especially mice, may contribute to increases in stoat populations (Murphy & Dowding 1995a). The abundance of irruptive pest prey species, particularly mice, but also rabbits and rats, affects the abundance of the higher predators (King 1983, 1989, 1990; Murphy 1992; Murphy *et al.* 1998b).

A1.1.2 Interactions among suites of pest prey

Pest prey also may affect the abundance of other pest prey species (Clout *et al.* 1995; Innes *et al.* 1995), or they may interact in ways that influence their detection and estimation of their density, or they may interact in both ways (Brown *et al.* 1996). Nevertheless, the type and abundance of pest prey can influence levels of predation on the threatened bird populations (King 1983; Murphy & Dowding 1995a,b). Stoat predation of birds depends largely on hunting strategies and defensive strategies of the prey (King 1983) although, in principle, habitat attributes can affect a prey species' ability to avoid predation. Similarly, Murphy *et al.* (1998b) suggest that stoats are semi-generalist predators and therefore are unlikely to control irruptions of mice, and Murphy & Dowding (1995a,b) show that stoats select birds as substantial prey irrespective of high abundance of mice. Control of prey species can reduce the abundance of

predators by reducing their primary food supply (see for example Pech & Hood (1998) in the case of rabbits as primary prey for foxes in Australia), although the starving predators temporarily may pose a greater threat to birds (Murphy *et al*. 1998b). Therefore, stoat predation of birds is thought to depend more significantly on stoat numerical responses rather than functional responses to irruptions of pest prey, although the product of the two responses determines the 'bird predation index' (King 1983; Murphy *et al*. 1998b).

A1.1.3 Interactions among suites of predators

Several predator species may consume or compete for the same prey species (e.g. Alterio & Moller 1997) and may respond numerically and differently to the changes in prey abundances. As an example of herbivorous predation, King (1983) observed different population responses in *Mus musculus*, *Rattus rattus* and *R. exulans* to increases in available beech seed. The abundance of one predator may affect the abundance of others (King 1990), through either competition for resources, interference competition, or predation (Alterio & Moller 1997). Consequently, control of stoats or other predator species can be expected to induce population responses in both prey and other predators in the ecosystem, increasing uncertainty for predictions of predation levels and impacts on the threatened populations of native birds.

A1.2 MANAGING A COMPLEX OF PREDATORS AND PREY

The potential interactions among stoats and other predators and prey show the complexity of the problem of choosing which predators to control. King *et al.* (1996b) stated: 'The uncertainty about which predator is more damaging, and the possibilities of diet switching and/or rodent [mesopredator] release, demand that pest control operations to protect threatened birds at Pureora should include all mustelids, rodents, feral cats and possums together, until further research suggests otherwise.' This cautionary statement would seem to apply generally to problems of conserving native birds in New Zealand, with different predators and prey assuming greater or lesser importance according to the location, habitat, season, prevailing conditions, and species of threatened native bird. The predator management regime adds a further level of complexity.

Managers have options of using a broad strategy of controlling multiple species of predators and/or prey (Morgan 1993), or, for specific cases, undertaking the necessary research to determine the specific sources of threat to the persistence of the native bird populations (e.g. O'Donnell *et al.* 1996). Innes *et al.* (1999), after initially planning a research and management framework to quantify the separate effects of controlling predators and browsers, chose to control all introduced mammalian pests whether they were recognised as predators or not. The outcome was salutary for conservation of the North Island Kokako *Callaeas cinerea wilsoni*. The adaptive management research program also provided some limited understanding, though perhaps confounded (Murphy *et al.* 1998b), of the separate impacts of the different pest species. One of the controlled pest species, initially thought not to prey on kokako, was

identified as one of its main predators. The possum *Trichosurus vulpecula* initially was considered to be a competitor for resources but later was recognised as primarily a predator and secondarily a resource competitor of kokako (Innes *et al.* 1999). This example shows the potential value of treating all pest species together and, most importantly, the need to design research or management to test conjectures about threatening processes (Caughley & Gunn 1996).

The alternative would be to undertake research to identify, measure and compare the relative impacts on the threatened species of all of the predators and competitors, creating for each species an index of impact (e.g. bird predation index: King 1983; Murphy *et al.* 1998b) which could be set in order. However, comparisons of the relative impacts of each of the predators and competitors on a threatened population would be specific to the threatened population, situation and temporal circumstances, and current management regime. The intended changes to the management regime to control the worst predator(s) and/or competitor(s) would modify the impacts of the remaining predators and competitors, and the situation need not necessarily improve (see King *et al.* 1996a,b) and further examination of the problem would be needed. At the present time confident prediction of the outcomes of planned management regimes for threatened populations of New Zealand birds needs considerable prior ecological knowledge. Ideally, information is needed on the numerical and functional responses with respect to bird impact (King 1983), of all potential predators and competitors *for each intended management regime*. This information would be needed for years of high and low production of biotic drivers of animal abundance in the various habitat types (e.g. beech mast: King 1983; Murphy & Dowding 1995a), or for contrasting years where the drivers are primarily edaphic (e.g. Bell 1981; Brockie *et al.* 1981). Where local information does not exist, modelling could assist in partial syntheses, but information would be insufficient in most cases until continuing research yields much more information (see Innes & Barker 1999). A valuable resource, where available information is limited, is the intuition of skilled people with long or extensive experience in the target ecosystems. Even then, experimentation is needed to test the hypotheses derived from their expert knowledge. Where experienced people are not available it is necessary to manage experimentally on the basis of limited information for the appropriate habitat, and to measure responses in the threatened species as well as the predators and pest prey. All situations point to a need for management to respond to continual experimental regimes and monitoring.

A1.3 THE NEED FOR ADAPTIVE MANAGEMENT OF BIRD POPULATIONS

Urgency, capability and resources may be the dominant factors in the choice of which species to control (e.g. O'Donnell 1996a,b; O'Donnell *et al.* 1996). If protective management is needed urgently, and if resources are limiting, a suitable compromise in the short term may be to concentrate on controlling one or a few suspected species initially (e.g. O'Donnell 1996b) within a framework of adaptive management, until outcomes show the need for

including more or fewer or different species. Alternatively, depending on the situation, resources, if adequate, may be better used to control all mammalian pests in a framework of adaptive management for the desired conservation outcome, as did Innes *et al.* (1999).

It is noteworthy that Innes *et al.* (1999) dropped labour-intensive control of stoats from the management regime after several years because of lack of direct evidence of predation on kokako nests, but secondary poisoning of stoats may have effected some useful control (Murphy *et al.* 1999). This experience has further defined questions on the relative levels of predation by possums, rats and stoats that should be tested in kokako populations that have recovered sufficiently. In this way management of predation on kokako may be refined and future management effort economised.

The adaptive management approach seems to be the best way to attend to the urgency of protecting most New Zealand threatened bird populations from predation, acquiring more and better knowledge as experimental management and monitoring progress. Irrespective of the initial strategy, in the long term, adaptive management will be needed; suitable monitoring inevitably will be needed to ensure that management always is responsive to its own impacts. Innes *et al.* (1999) present persuasive argument for its general use.

A strategy and methods for conserving threatened native birds are available already. The problem is to extend this to greater areas and more populations with the limited resources available. Essentially this is a problem of cost-efficiency and economics. What are the best available strategies and methods that would extend the conservation effort over more populations and greater areas within the constraints of resources? Initially priorities need to be set on:

- which species
- which populations
- which locations
- how extensive an area to manage in these locations
- location/population versus size of area to be managed. These priorities are influenced by societal values, characteristics of the natural assets, and population processes

GIS analyses (e.g. LUPIS: Ive & Cocks 1999) may assist in establishing these lists of priorities and resolving conflict where values differ. Examples may be conflict over timber harvesting and conservation of threatened (say, tree hole-nesting) populations (e.g. Elliott *et al*. 1996), or conserving more ecologically important species compared to more iconic species, or conserving fewer larger populations of a threatened species in preference to more of smaller populations. Choices for management, where the line is drawn within the priority list, will depend on the political allocation of available resources and on the cost-efficiency of the adopted combination of strategy and methods. Therefore, beyond political involvement, scientists need to consider strategies and the cost-efficiency of combinations of current methods. The need for such research is recognised and well advanced for some situations. For example, O'Donnell & Phillipson (1996) attempted to predict years of high stoat abundance from beech seedfall as a means of economising control effort to the period critical for conserving mohua. Now we need to consider the cost-

efficiency of combinations of current methods in relation to combinations that include immunocontraception of stoats, within a strategic framework of adaptive management.

We assume that core conservation areas will be managed to supply and maintain the habitat requirements of the threatened species, and a program instituted to control predators there. Other choices are whether to deter immigration of stoats using predator-proof fences, and buffer zones within which may be instituted any combinations of habitat management to deter re-invasion by predators, control of pest prey, and control of predators.

A1.4 FENCING?

Predator-proof fencing (Griffiths *unpublished*) will vary in cost-efficiency over time and this option should be reviewed periodically. Costs of construction and maintenance may be offset partly by the reduced ongoing costs of control, or even of eradication, within the fence. Periodic review would be beneficial because of changing circumstances in control technology, conservation status, and economies of materials and labour for fence construction and maintenance and checking compared to other labour-intensive control practises. In some circumstances, the economics also may change if predator-proof fencing were to be combined with well-planned, highly controlled and ecologically sustainable ecotourism from which *all* income were returned to management of the threatened population. (Partial return of income could create distortions that may compromise the conservation objective.) This option also should be considered periodically.

A1.5 MANIPULATING HABITAT IN BUFFER ZONES?

For very large areas, apart from direct predator control, unfenced buffer zones may be the only option for deterring re-invasion by all mammalian pest species. However, Murphy & Dowding (1995a,b) warn that buffer zones may be useful only while trapping (or other control) of stoats continues because of the extensive and protracted dispersal and movement of young stoats, especially males.

Different species of predators and pest prey have different habitat preferences (King *et al.* 1996a; Murphy *et al.* 1998b). It may be possible to expoit these differences to meet management objectives. Alterio *et al*. (1998) found that a particular type of vegetation barrier intended to protect colonies of yellow-eyed penguins *Megadyptes antipodes* from predation seemed to increase the threat of predation. This finding could be used to refine strategies for including buffer zones for conservation of threatened bird species rather than used simply to preclude habitat types or other barriers as an option. Nevertheless, this example demonstrates that buffer zones should be considered and developed only in an adaptive management framework that tests the assumption that a buffer zone is advantageous. Initially the manipulation of habitat in the buffer zones should be readily reversible or of limited extent in case outcomes are detrimental.

A1.6 EXISTING EFFECTIVE METHODS OF MANAGING PREDATORS

A1.6.1 Trapping

Mustelids are difficult to control by trapping (King & Edgar 1977; King 1989). Trapping is labour-intensive, laborious, and restricted to localised areas (Spurr 1999) and thereby is expensive. Not all stoats are trappable or will enter tracking tunnels (Clapperton *et al.* 1999) or tunnels where traps or baits are laid (reviewed in Griffiths *unpublished*). Consequently alternative methods are sought, especially poisoning with minimal risk to non-target species. Nevertheless, trapping can have a substantial impact on stoat populations (e.g. King 1983), it can help substantially for recovery of threatened bird populations (O'Donnell *et al*.1996), and it is a useful monitoring tool (King & Edgar 1977). Consequently, trapping in various forms is likely to remain a potentially useful component of management plans, and improvements in technique would be very valuable.

A1.6.2 Poisoning

Griffiths (*unpublished*) reviewed recent research on poisoning stoats. To poison mustelids, Spurr (1999) recommended use of special tunnel bait stations (egg tunnels) with hen eggs. The eggs should be poisoned with 1 mg of 1080 for rapid reduction of stoats or, where predator reduction is less urgent, the eggs should be injected with 5 mg of diphacinone (detailed instructions see Spurr & Hough 1997). Outcomes of poisoning operations should be monitored using other methods in addition to monitoring the take of the eggs/bait in the bait stations (Spurr 1999). Dilks & Lawrence (2000) recorded behaviour of stoats towards egg tunnels and poisoned eggs. These observations are valuable in assessing impacts of egg tunnels and improving their effectiveness. Suggested areas of research may improve the attractiveness and efficacy of egg tunnels for poisoning mustelids (Spurr 1999), including the addition of a meat lure (*op. cit*.) or stoat scent lures (Clapperton *et al.* 1999), or particular sounds (Spurr & O'Connor 1999). This research should be pursued further. If the ongoing searches for taxa-specific toxins (Wickstrom & Eason 1999) should succeed, the array of possible options for management strategies would be increased considerably.

A1.6.3 Controlling prey populations

Controlling prey species such as mice or rats as a means of managing predation of native birds by stoats may decrease population densities of stoats by reducing their food supply, and perhaps by less direct processes such as secondary poisoning. However, reducing the abundance of prey for stoats, such as rats and mice, may result in the short-term problem of greater predation by stoats on valued bird populations, followed by the long-term benefit of reduced numbers of stoats and stoat predation. Threatened populations of birds would need to survive the initial pulse of increased predation before they could benefit from the lower abundance of stoats. Murphy *et al.* (1998b) examined these processes within the constraints of rat poisoning programs in two podocarp-hardwood forests in the North Island. However there are some problems with the methods

and analyses. Data for a numerical response, in the form used by the authors, should be collected when predator and prey populations are quasi-stationary. This condition did not apply and was further compromised by using kill-trapping to estimate the abundance of stoats. The series of rat control programmes, which used varying poisons, application rates and delivery techniques, also exacerbated problems with transient responses by populations of rats, stoats and other species. The percentage frequency of occurrence was used as an index of the functional response but appeared not to include data from empty stomachs nor provide a good measure of the number (or biomass) of prey killed per predator per unit time. In addition, linear regressions were inappropriately applied to catch rate data transformed to percentage frequency of occurrence. The studies lacked experimental controls, and thereby failed to correct the post-poisoning estimates of the Bird Predation Index for their consistent confounding coincidence with the season of recruitment of young stoats. Finally, the impact of stoats on birds will be determined by a functional response that measures the predation rate as a function of the availability of birds, not rats.

Consequently protecting threatened populations from stoat predation by reducing the abundance of rodent prey remains a possible valuable option. Further research is needed to examine the short-term and longer effects of controlling prey on the impact of stoat predation on bird populations. Secondary poisoning of stoats from rodent poisoning operations is likely to be involved when poisoning is the method chosen for controlling prey and may prove beneficial to conservation of threatened bird populations (Alterio 1996; Moller *et al.* 1996; Alterio *et al.* 1997; Heyward & Norbury 1998; Murphy *et al.* 1998 a,b; Gillies & Pierce 1999; Murphy *et al.* 1999; Robertson *et al.* 1999). Future management decisions would be improved by greater understanding of the processes by which management actions may affect stoat abundance and the level of predation on threatened species. Therefore it would be beneficial if research were to examine the functional and numerical responses of stoats to management-induced changes in prey density, separately and in combination with the responses when secondary poisoning of stoats is included with the induced reduction in prey density.

A1.6.4 Secondary poisoning

Conservation of some bird populations has benefited significantly from secondary poisoning of predators (Robertson *et al.* 1999a,b). This has been observed for 1080 or anticoagulants applied in aerial or ground operations targeting prey species, including rabbits, possums and rats (Alterio 1996; Moller *et al.* 1996; Alterio *et al*. 1997; Heyward & Norbury 1998; Murphy *et al.* 1998a,b; Gillies & Pierce 1999; Murphy *et al.* 1999; Robertson *et al.* 1999a,b). Restoration of kokako populations (Innes *et al.* 1999) may have been assisted inadvertently by secondary poisoning of mustelid predators (Murphy *et al*. 1998b). Nevertheless, in some instances secondary poisoning has not reduced predators sufficiently to benefit threatened bird populations (Gillies & Pierce 1999), and evident prey-switching may increase the threat to bird populations (Murphy *et al*. 1998b). The risks of selection for resistance to the toxins through intake of sublethal doses in the prey should be considered. Secondary poisoning needs to be combined with other methods to achieve the desired

objectives (Gillies & Pierce 1999). Further research is needed to understand the processes of secondary poisoning to minimise detriments and maximise the benefits of this potentially very promising technique (Griffiths *unpublished*).

Substantial risks accompany the benefits of secondary poisoning by both anticoagulants and 1080 to threatened bird populations (Innes *et al.* 1999; Robertson *et al.* 1999a,b). The second-generation anticoagulant brodifacoum, now in common use, is extremely persistent and may present risks of secondary or tertiary poisoning that could be unacceptable in many circumstances (Murphy *et al.* 1998a; Dowding *et al.* 1999; Eason *et al.* 1999; Robertson *et al.* 1999a; Stephenson *et al.* 1999). The risks of using brodifacoum sometimes have been reduced by particular strategies that minimise the quantity deployed, e.g. bait stations in preference to aerial drops, and as a maintenance control after an initial 1080 poison control operation (Robertson *et al.* 1999a). In some circumstances, alternative poisons, e.g. cholecalciferol or cyanide, are used in particular ways and circumstances (Robertson *et al.* 1999a). Further research is needed, particularly for brodifacoum, on baiting, delivery, timing, intensity and frequency, to improve efficacy and safety for its use on multiple species (Alterio & Moller 1997).

1080 poisoning operations directed at prey pests also benefit bird populations through more acute secondary poisoning of predators (Innes & Barker 1999; Murphy *et al.* 1999; Robertson *et al.* 1999b) with apparently lower non-target risks because of its much lower persistence than that of brodifacoum (e.g. Eason *et al.* 1999). On present information, 1080 should be used, where possible, rather than persistent anticoagulants. Anticoagulants should be considered cautiously as an alternative or intermittent complement where it may be advantageous, e.g. in countering 1080 aversion, or targeting female stoats (Murphy *et al.* 1998b).

The most effective and safe strategies for combining methods for multi-species poisoning operations are likely to be specific to situations and circumstances. Innes & Barker (1999) showed the importance of timing pulses of poisoning possums and rats to just before the breeding season of kokako. Murphy *et al.* (1998b) showed that secondary poisoning by brodifacoum reduces stoats for 2–3 months (Oct–Dec), sufficient for species with short breeding seasons, e.g. kokako. However, it would be ineffective for those birds with extended breeding seasons such as kiwi (*Apteryx* spp.), kereru (*Hemiphaga novaeselandiae*) and kaka (*Nestor meridionalis*) because of dispersal of juvenile stoats from January onwards (*op. cit.*). Mohua (*Mohoua ochrocephala*) also have an extended breeding season (Elliott 1996a). Also, in some circumstances, it may be inefficient or detrimental to use pulses of poisoning against prey populations that are at low density (Gillies & Pierce 1999; Griffiths *unpublished*). Consequently optimisation of efficacy and safety of multi-species poisoning operations require that they be carefully planned on the basis of the species present, local conditions, and current circumstances. The methods and strategies need to adapt progressively on the basis of careful monitoring of outcomes and processes (see Innes *et al.* 1999; Innes & Barker 1999). Further directed concurrent research to enable the planning, monitoring and adaptation of management is essential and of high priority (viz. Innes & Barker 1999). Reliable techniques for monitoring are essential to this process.

A1.6.5 Monitoring methods

Responsiveness of strategies and methods to outcomes of management require rapid unbiased assessments of pest prey, predators, and threatened prey (King & Edgar 1977; O'Donnell 1996b). Some recent and current research is addressing this need (Griffith *unpublished*; Brown *et al.* 1996; Clapperton *et al.* 1999; Thomas 1999) and efforts should continue to seek cost-efficient methods that can be standardised for most situations and seasons (Innes *et al.* 1995). Most monitoring methods, e.g. trapping, tracking and bait take, depend on effort, e.g. trap spatial pattern and density, and tend to provide relative abundance indices only, although these are often sufficient. However, their selectivity requires several methods to be used concurrently, and effort may be expensive or limiting. Existing methods need to attract the animals to the detection/ recording device. This causes attendant biases, seasonal and temporal changes in susceptibility, and the concern that an unknown proportion of the population is not detectable by these means (cf. infra-red triggered cameras (Lawrence & Loh 1997, cited in Griffiths *unpublished*). Calibrations of methods may or may not reduce or remove some of these problems, but this requires repeated further effort.

Progression of time during the monitoring of management operations reduces confidence in the comparability of samples, so contemporaneous monitoring of experimental controls is extremely important. Recognition by managers and administrators of the need for experimental controls, usually areas receiving partial or null treatment, is most important to prevent confounding of even simple designs for adaptive management and to minimise equivocal outcomes of tests of hypotheses on causation or management or policy. Changes to management policy or plans can be a substantial threat to the security of these areas (Murphy *et al.* 1998b, 1999) with considerable cost to outcomes of research and adaptive management. Timing or conditions of surrender of experimental control areas to potential alternative management treatment can be defined in formal Memoranda of Understanding.

Appendix 2. Recommendations of priority research required for modelling studies, laboratory development, field evaluation and implementation of reproductive and lethal biocontrol methods for stoats

The development of new technologies for the management of stoats and other mustelids in New Zealand will require strong commitment by scientists, managers and government to enable the completion of an extensive and highly integrated research project over a minimum period of 7–10 years. It requires the interaction and input of several scientific disciplines (ecology, reproductive physiology, microbiology, molecular biology, mathematical modelling) as well as a communication program to interact with the public and other interested parties regarding the use of new technologies which may involve GMOs (Tyndale-Biscoe 1994, 1995; Williams 1997)

Tables A2.1–A2.4 provide an outline of the four major projects which could be undertaken to develop various types of biocontrol agents for the management of stoats. The need to refine current management techniques is considered to be high priority and is included in each project. Projects 1–4 are presented as alternative research strategies. A revision of the time lines would be required if a mixed strategy were selected.

The recommended strategy is to develop a non-disseminating immunocontraceptive GMO for stoats (Table A2.1). The minimum time for the development of such a product would be 5 years, at which stage it would be available for testing in large-scale field studies. The time-line assumes that all registration and regulatory processes can be completed within this time. A second pre-requisite to ensure that laboratory studies are successful is the development, in New Zealand, of a capacity to adequately maintain and breed stoats in captivity. This is essential for the progress of the many laboratory-based phases of the research.

A2.1 PROJECTS

The four projects with the overview of the tasks to be completed within each of these are as follows:

Project 1 Non-disseminating immunocontraceptive GMO for stoats

Sub-Project 1.1: Refine current management techniques

Sub-Project 1.2: Select potential antigens

Sub-Project 1.3: Identify candidate vectors for oral delivery

Sub-Project 1.4: Construct and test recombinant vector for oral delivery

Sub-Project 1.5: Develop bait formulation attractive to stoats

Sub-Project 1.6: Registration of bait matrix and incorporated biocontrol agent

Sub-Project 1.7: Ecological studies: pre-release

Sub-Project 1.8: Integrated pest management

Sub-Project 1.9: Release strategy

Project 2 Disseminating immunocontraceptive GMO for stoats

Sub-project 2.1: Refine current management

Sub-project 2.2: Select potential antigens

Sub-project 2.3: Identify candidate disseminating vectors

Sub-project 2.4: Construct and test recombinant disseminating vector

Sub-project 2.5: Develop bait formulation attractive to stoats, if required

Sub-project 2.6: Registration of disseminating GMO, with the bait matrix if required

Sub-project 2.7: Ecological studies: pre-release

Sub-project 2.8: Integrated pest management

Sub-Project 2.9: Release strategy

Project 3 Use of chemosterilants for stoat management

Sub-Project 3.1: Refine current management techniques

Sub-Project 3.2: Assessment of cabergoline

Sub-Project 3.3: Assessment of RU486, Mifepristone

Sub-Project 3.4: Cost/benefit analysis of use of chemosterilants versus lethal techniques

Sub-Project 3.5: Develop bait formulation attractive to stoats

Sub-project 3.6: Registration of bait matrix and incorporated chemosterilant

Sub-project 3.7: Ecological studies: pre-release

Sub-project 3.8: Integrated pest management

Sub-Project 3.9: Release strategy

Project 4 Development of a lethal biocontrol agent

Sub-Project 4.1: Refine current management techniques

Sub-Project 4.2: Assess potential of canine distemper virus as a candidate lethal biocontrol agent

Sub-Project 4.3: Assess potential of other endemic candidate lethal biocontrol agents

Sub-Project 4.4: Develop bait formulation attractive to stoats

Sub-Project 4.5: Registration of bait matrix and incorporated lethal biocontrol agent

Sub-project 4.6: Ecological studies: pre-release

Sub-project 4.7: Integrated pest management

Sub-project 4.8: Release strategy

A2.2 OBJECTIVES

As noted above, one of the assumptions for each strategy is that there will be continued refinements of existing management techniques for stoats. Future and ongoing strategies should include the following objectives:

Conservation choices If it does not exist already, there is a need to develop the organisational infrastructure, systems and methodologies for deciding on priorities for populations, sites and size of areas to manage for conservation of threatened native species. This would include also the adoption of methodologies for resolving conflict over these choices, such as described in Appendix 1.

Targets for conservation management If not already available, there is a need to develop methodology for identifying the numbers of sites and respective areas that are required to be protected, for conservation of threatened bird species, relative to resource requirements.

Prediction of stoat densities There is a need to further develop methods for prediction of high stoat numbers, or indices of abundance in beech forest (O'Donnell & Phillipson 1996). The absolute abundance of stoats in other forest types, particularly mixed forest, needs to be assessed by capture-mark-recapture or other suitable means (Alterio *et al*. 1999, also cited in Griffiths *unpublished*). These data could be used to estimate stoat numbers in productive and unproductive years from relative trap rates for these forest types. These estimations would allow assessment of whether stoat densities are low enough and variable enough for pulsed control, or multi-year spacing of control operations, to protect the respective threatened bird populations. If such control operations were feasible it would be very useful to determine whether stoat numbers can be predicted for those situations by methods similar to those developed for beech forest.

Target densities for stoats There is an urgent need to estimate maximum target densities of stoats for specified sites and areas for conservation of target threatened bird populations.

Improve efficiency and target specificity of secondary poisoning This should concentrate on initial control using 1080, and maintenance control using 1080 or less persistent anticoagulants. However there is a concern that the occurrence of sub-lethal doses through secondary poisoning may increase the selection pressure for resistance in stoats. The search for species-specific toxins should be continued because this technique would greatly increase cost-efficiency through enabling aerial baiting over very large areas.

Models for adaptive management of target threatened native populations It is important to identify the relative threats of the range of predator species to threatened native species under the applied management regimes. Positive interactions in alternative strategies (such as in secondary poisoning) should be sought wherever possible. The impact of secondary poisoning on predators is a valuable tool that needs to be developed, together with the use of its role in the identification of relative threats of the various predators. Programs of adaptive management need flexibility of design and security to carry out agreed management plans according to defined and clearly

TABLE A2.1: STRATEGIC PLAN AND PROPOSED PROJECT TIMELINES FOR DEVELOPING A NON-DISSEMINATING IMMUNCONTRACEPTIVE GMO FOR STOATS. THIS IS THE RECOMMENDED STRATEGY AND THE *MINIMUM* TIMELINE. DARK SHADING INDICATES MAIN FOCUS, LIGHT SHADING INDICATES REDUCED RESOURCES AND EFFORT; *IC = IMMUNOCONTRACEPTIVE.

TABLE A2.2: STRATEGIC PLAN AND PROPOSED *OPTIMISTIC* PROJECT TIMELINES FOR DEVELOPING A DISSEMINATING IMMUNOCONTRACEPTIVE GMO FOR STOATS.

TABLE A2.3: STRATEGIC PLAN AND PROPOSED *OPTIMISTIC* PROJECT TIMELINES FOR DEVELOPING THE USE OF CHEMOSTERILANTS FOR STOAT MANAGEMENT.

TABLE A2.4: STRATEGIC PLAN AND PROPOSED *OPTIMISTIC* PROJECT TIMELINES FOR DEVELOPING LETHAL BIOCONTROL FOR STOAT MANAGEMENT.

transparent decision criteria. This can be achieved through developing a system of Memoranda of Understanding on management plans and conditions for surrender of experimental controls to alternative management.

Develop monitoring methodology The considerable development of monitoring methodology needs to be extended by experiments in the field to determine the proportions of stoat populations that are not detectable by the various methods of monitoring—trapping, tracking, bait take. Development of new methods, such as videography and infra-red photography, might enable rapid assessment of stoat abundance with low labour input.

Appendix 3. Components and stages necessary for reproductive or lethal biocontrol

A3.1 COMPONENTS IN THE DEVELOPMENT OF FERTILITY CONTROL

The development of a non-disseminating immunocontraceptive GMO for stoats, the strategy recommended in TOR 9, requires consideration of an extensive laboratory-based research programme and relevant, focussed ecological studies to collect appropriate data for models which will assist the prediction of the efficacy of fertility control for stoats.

As stated above and outlined in the flow diagram below (Figure A3.1), the key steps (some of which will be concurrent) for fertility control include:

- Identifying suitable reproductive antigens which can be demonstrated to disrupt reproductive success via immune responses (Years 1–3).
- Identifying a suitable vector which can be genetically engineered (Years 1–4).
- Constructing a recombinant vector which can be demonstrated to affect the fertility of captive stoats (by the end of Year 5).
- Demonstrating species-specificity of the recombinant vector (by end of Year 5).
- Developing and registering a bait attractive to stoats for delivery of the non-disseminating immunocontraceptive GMO (by the end of Year 4).
- Ecological studies of stoat populations (ongoing).
- Approval to use a non-disseminating immunocontraceptive GMO in field trials (by the end of Year 5).
- An integrated large-scale management trial, including monitoring and evaluation of the non-disseminating immunocontraceptive GMO (Years 6–8).

Completion of the above steps within the suggested minimum time-frames provides the essential criteria for determining whether progress is sufficient and appropriate. If progress is slow, but on course, then a re-direction of activity may be unnecessary. However if little or no progress is being made against the first two key steps and within 2-3 years, reconsideration of directions will be essential.

Times for major reviews and associated decision points are indicated in Figure A3.1, with the minimum resources and effort required outlined in Table A3.1.

It must be noted that the rate of progress, as specified in Figure A3.1, cannot be guaranteed and that reviews to assess progress and, if necessary modify, the flow chart and timelines will be required at all times during the period of development of a new biocontrol agent for stoats.

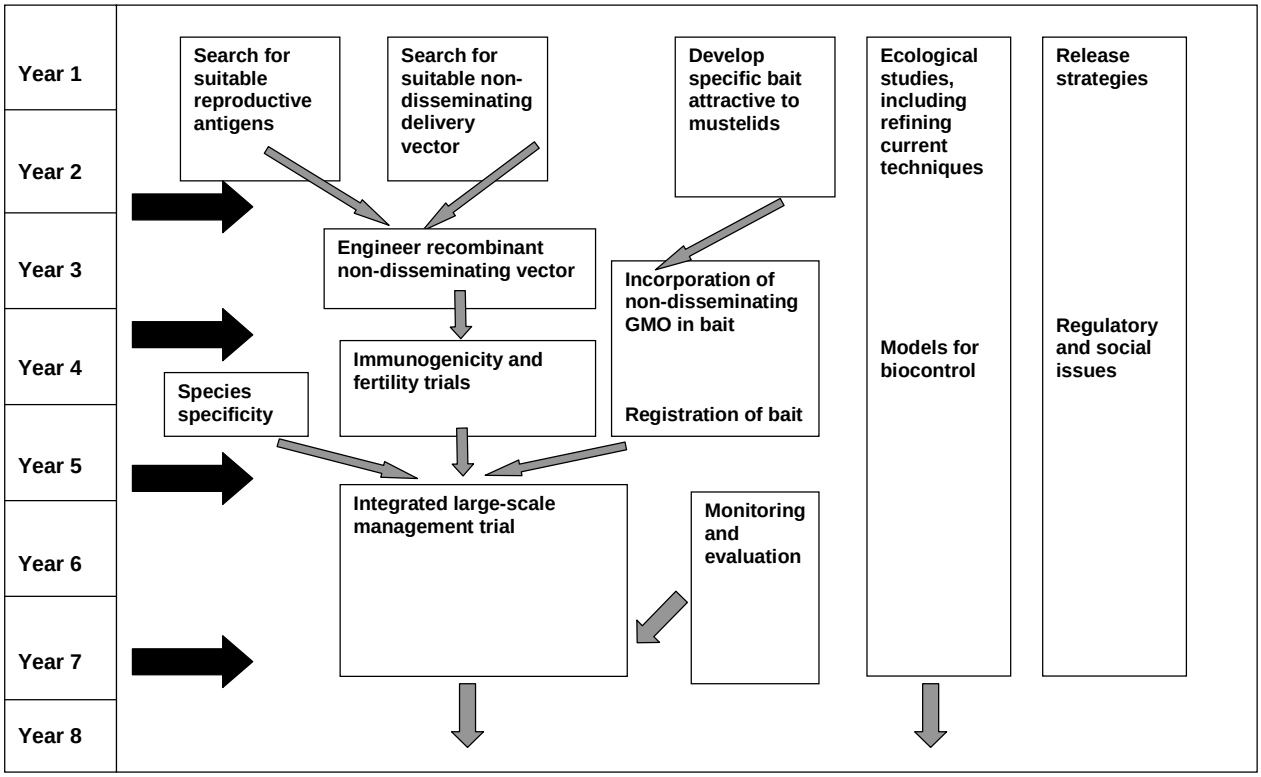

Figure A3.1. Development of a non-disseminating immuncontraceptive GMO for stoats. Large arrows indicate key review and decision points for the program.

TABLE A3.1. STAFF RESOURCES AND FACILITIES REQUIRED FOR THE DEVELOPMENT OF A NON-DISSEMINATING GMO.

TASK	MINIMUM STAFF RESOURCES	FACILITIES REQUIRED
Search for antigen	Scientist plus technician	High-quality molecular biology labs; captive stoats for lab-based animal experiments
Search for vector	Scientist plus technician	Field surveys and high-quality analytical labs for assessment of different potential vectors
Construction of recombinant vector	Scientist plus technician	High-quality molecular biology labs; captive stoats for lab-based animal experiments, including access to containment facilities
Species specificity	Scientist plus technician	Access to pre-determined array of species for testing; suitable containment facilities; suitable labs for the conduct of analyses
Bait development	Scientist plus technicians	Appropriate facilities and access to pilot manufacturing plant; field site for testing baits
Ecological studies, including modelling	3 scientists, plus technicians	Field sites and support facilities

A3.2 COMPONENTS IN THE DEVELOPMENT OF LETHAL BIOCONTROL

A similar flow diagram and points for review and decision for the alternative research strategy involving the development of lethal biocontrol can be developed from Table A2.4. The key time points for review would be at the end of Years 2–3 if either canine distemper virus was found to be unsuitable or if another potential lethal agent had not been identified. At year 3, it may be appropriate to consider a search for a lethal biocontrol agent overseas. This would require a substantial re-assessment of the research strategy and associated timelines.

A3.3 FIELD EVALUATION

Registration After development of a lethal or reproductive biocontrol it will be essential to register the product for the purposes of experimental evaluation in the field. It is relevant to consider whether or not it would be possible to recall the biocontrol from field evaluation; this would be possible for some biocontrols, such as a non-disseminating immunocontraceptive GMO delivered by bait. Registration of this type of biocontrol is most likely to be a provisional 'off-label' registration that allows experiments to determine efficacy under field conditions without registering the product for general use. Full registration would be necessary before implementation, after efficacy had been proven under field conditions. Where the biocontrol could not be recalled, such as a disseminating immunocontraceptive GMO, full registration would be required before it could be evaluated in the field. The requirements for 'off-label' registration are likely to be almost as stringent as that for full registration. This means that preparation of a registration package should be started as soon as an efficacious product is identified under contained conditions. The following refers to field evaluation of a non-disseminating immuno-contraceptive GMO bait.

Field evaluation pre-release The first step would be to determine whether the bait without the active component is accepted readily by stoats under field conditions. This could be done in field trials using rhodamine biomarkers and assessing bait take in animals trapped preferably alive, or trapped lethally if necessary. The rate of bait acceptance (%) would be compared with some pre-set level. Failure to achieve the required level of bait acceptance would require analysis of the rates of bait encounters, including delivery method, site, pattern and density of placement, or bait sequestering by stoats or non-target species. Failure may also require re-examination of bait characteristics, including stability under field conditions, and composition including toughness, flavours and attractants. A satisfactory level of bait acceptance would allow progression to field evaluation of the active bait.

Field evaluation with release Prior to the stoat breeding season, the active bait including a permanent-type biomarker, such as tetracycline, could be delivered in the proposed way to the field populations of stoats. The protocol for delivery would be designed using results from population models (see

Appendix 2) as well as field experience. Soon after the stoat breeding season, the stoats could be live-trapped and examined for age, permanent biomarking, and prevalence of delayed blastocysts. The critical test of the efficacy of the immunocontraceptive would apply to the adult age class, comparing the proportions that were biomarked and not carrying delayed blastocysts with those biomarked that had blastocysts. Experimental controls for this type of trial could include populations that were given only non-active baits with permanent biomarkers and monitored similarly, and replication would be necessary. Repetition in the following year would enable effects on demography to be compared between treated and untreated populations; this would not be possible if lethal trapping were used to monitor the outcome in the first year.

A3.4 IMPLEMENTATION

The manner in which a developed and field-proven method of immunocontraception could be implemented would depend on the intended roles of the various relevant New Zealand organisations. The organisational aspect of implementation will not be commented upon, and only broad categories of implementation processes can be given.

Implementation would require full registration of the immunocontraceptive bait, as mentioned above. The manner of implementation would depend on the specific requirements for conservation of the various native species and the particular threatening processes. Therefore it would be necessary to plan, for individual cases, how to integrate immunocontraception with existing or potential conventional controls, preferably using experimental adaptive management. The threatened populations chosen initially for experimental treatments and experimental controls should be representatives for which failure to achieve objectives is not catastrophic. The numbers of sites and size of impacts that would be needed to achieve a given probability of detecting an effect of the treatments should be determined.

The relevant parties should agree in formal management plans or memoranda of understanding on the management regimes and criteria for relinquishing management treatments and especially experimental controls; this would define criteria for failure and success that are acceptable to all agreeing parties. An important part of implementation is education and training of staff, including field staff and managers, and education of administrators and politicians at all levels, in the concepts of integration, adaptive management, implementation of the techniques, monitoring, reporting and review of progress.

Appropriate arrangements would be needed for manufacture of the immunocontraceptive bait and quality control, and for its distribution and deployment.