Population responses to sterility imposed on female European rabbits

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Summary

1. Additional methods are needed in Australia to control the European rabbit *Oryctolagus cuniculus*, which continues to destroy valued native flora. A control option under development, immunocontraception, is intended to suppress the rabbit's high fertility. It would spread contagiously via genetically modified myxoma virus and European rabbit fleas *Spilopsyllus cuniculi*. An experiment with field populations of rabbits assessed whether suppressing fertility reduces their abundance.

2. In south-eastern Australia, four treatments in three replicates were applied to 12 subpopulations of rabbits. The treatments were surgical sterilization of 0%, 40%, 60% and 80% of the adult and juvenile females trapped before the annual breeding seasons of 1993–96.

3. The sterilized populations produced fewer young but the average adult population size remained unchanged in all treatments. Immigration was minimal in all treatments.

4. Sterilized adult female rabbits survived much better than fertile females, indicating a high cost of reproduction. Immature rabbits and unsterilized adults of both sexes also survived better in the sterilization treatments. The improved survival in all rabbit classes compensated for reduced reproductive output.

5. Fleas were fewer on both adult females and males in the sterilized populations but this did not impede transmission of myxomatosis.

6. *Synthesis and applications*. Imposing sterility on rabbit populations reduces breeding-season peaks of abundance. Improved survival compensates for the sterility of up to 80% of females and sustains populations, even in the presence of drought and myxomatosis. Immunocontraception alone has poor prospects for controlling rabbits. Cost-effective rabbit control requires multiple, integrated forms of attrition.

Key-words: compensation, cost of reproduction, European rabbit, European rabbit flea, immunocontraception, management, myxoma virus, population, sterility, survival

Introduction

The introduced European rabbit *Oryctolagus cuniculus* L. is a declared pest in Australia that damages crops and pastures and diminishes native flora and dependent fauna. Most control efforts aim to kill rabbits or destroy their warrens, burrows and other natural shelter (Williams *et al.* 1995). Both strategies have limitations: rabbits infest vast areas in Australia and human

resources are limited. Two diseases specific to rabbits, myxomatosis (introduced in 1950) and rabbit haemorrhagic disease (RHD, introduced in 1995), suppress rabbits extensively. Nevertheless, damage continues and further control is needed.

A potential addition to the current control strategies is to reduce rabbit fertility directly by biological means; virus-vectored immunocontraception (VVIC) is one proposed method (Tyndale-Biscoe 1994). Jackson *et al.* (1998) validated the concept of VVIC on mice in the laboratory. Since 1991, research has aimed to develop VVIC to constrain resurgence of rabbits after myxomatosis

epizootics using myxoma virus-vectored immunocontraception (IC myxoma) (Holland & Jackson 1994; Kerr & Jackson 1995; Kerr *et al.* 1999, 2004). The present study investigated the potential of VVIC by exploring population consequences of sterility imposed in field populations (Williams 1996).

Using myxoma virus as the vector of immunocontraception requires two important considerations. First, the spread of immunocontraception depends on the contagion of myxomatosis; this was assessed using seroprevalence to myxoma virus and virus type based on restriction fragment length polymorphisms (RFLP; Kerr 1997). Secondly, a major vector of myxoma virus is the European rabbit flea *Spilopsyllus cuniculi* Dale (Fenner & Ross 1994), which breeds in response to the rabbit's reproductive hormones in blood meals (Mead-Briggs 1964). Failure of rabbits to breed may reduce flea breeding and flea populations, and thereby limit the spread of myxoma virus and immunocontraception. This possible constraint was assessed by counting fleas on the rabbits.

The study addressed five questions. (i) How do rabbit populations respond when some females are sterilized? (ii) Does imposed sterility induce compensatory changes in rabbit population fecundity or mortality? (iii) What minimum proportion of female rabbits must be sterilized to reduce populations? (iv) Does the abundance of European rabbit fleas decline when proportions of female rabbits fail to breed and deny fleas the reproductive hormones in blood meals (Mead-Briggs 1964)? (v) Does sterility of rabbits or any population responses of the flea vector interfere with the transmission of myxomatosis?

To address these questions, we measured population responses of rabbits and fleas and myxomatosis prevalence, by surgically sterilizing (fallopian tubal ligation) randomly chosen female rabbits in four proportions, 0%, 40%, 60% and 80%, among 12 subpopulations. This study, in the cool temperate Southern Tablelands of New South Wales (NSW), Australia, and a similar contemporaneous study in the mediterranean southwest of Western Australia (Twigg *et al.* 2000), tested the generality of responses in a range of climatic and ecological conditions. The NSW study ended just before the first local epizootic of RHD following its entry into Australian rabbit populations in 1995 (Kovaliski 1998; Fenner & Fantini 1999).

Methods

The Animal Ethics Committee of CSIRO Sustainable Ecosystems approved the handling methods and surgical techniques used in this study.

STUDY SITES

The 12 study sites were on grazing properties in the Southern Tablelands of NSW (Williams & Moore 1995). Distances between nearest-neighbour sites varied from

0·3 to *c.* 2 km. The sites contained an average of 31 rabbit warrens (range 20–46) and an expected minimum of 40 female rabbits (Parer 1982; Williams *et al.* 1995). We destroyed all warrens and burrows within 300 m beyond the warren cluster at each site. Each site warren was enclosed with a wire netting fence containing several cage traps with swing doors (smeuses) allowing the rabbits free passage. In trapping sessions, we set the doors to catch rabbits, set additional cage traps in the enclosures and spread oat grain bait. This system enabled repeated trapping and enumeration of all rabbits using the warrens.

WARREN SURVEYS

The same two people counted the active entrances (Parer 1982) and total open entrances of all warrens on the sites (Williams & Moore 1995) at about 6-weekly intervals. From May 1994, in the same area, a further five similar sites, untrapped, untreated and not modified by warren fencing or warren clearing, were monitored similarly to detect any impact of the trapping procedures on the 12 experimental populations.

EXPERIMENTAL DESIGN AND TREATMENT

In February 1993, the four levels of sterility treatment were assigned randomly in three replicates to the 12 sites. A proportion (0%, 40%, 60% or 80%) of all females (eligible weight > 500 g) on each site was sterilized by surgical ligation of fallopian tubes (see Appendix S1 in the supplementary material). Sham surgery complemented sterilization so that on all sites 80% of eligible females received surgery, assigned randomly. Annually, in February, March and July of 1993–96, new female recruits of eligible weight were sterilized randomly in the proportions initially assigned to the populations. By not sterilizing after July, young born early in the breeding season were potentially capable of breeding in the year of their birth; early birth and maturation were considered to be potential compensatory responses to sterility.

The populations were trapped and enumerated in September 1992 and annually in February, March, July and November until July 1996. The trapped rabbits were weighed, ear-tagged and assessed for status of pregnancy and lactation or testis development. Fleas congregating on their heads and loose in the weighing bag were counted. Between February 1994 and July 1996, untagged rabbits weighing ≥ 500 g and known seronegative rabbits were bled for analysis of myxoma antibodies. Throughout the study, eyes of currently infected rabbits were swabbed for analysis of myxoma virus types based on RFLP (Kerr 1997). Rabbits weighing < 1200 g when first trapped were assumed to have been born on the site. In November, any untagged rabbits weighing ≥ 1400 g were classed as immigrants, based on an assumed growth increment of 10 g day^{-1} (Myers 1964) and an annual absence of breeding before April; immigrants were excluded from estimates of production of detected young.

ESTIMATIONS

Production of detected young

The production of detected young [numbers of young (< 1200 g) trapped relative to numbers of adult females (≥ 1200 g)] was compared by ANOVA with factors of sterility treatment, study years and interaction. Fertile females were not used as the divisor, being few on the high sterility sites and causing much higher variance than in the low sterility treatments. The ratio of the number of young trapped relative to adult females was regressed on the assigned levels of sterility in order to identify any compensation for sterility treatments in the production of detected young.

Survival

Survival was analysed in three ways. First, we determined whether sterilization affected the longevity of the rabbits using three ANOVAs comparing proportions of rabbits in the sex/sterility classes (null, no operation; sham, sham operation; tubal, tubal ligation) and males, all of weight ≥ 500 g, surviving 1-year intervals from February-March 1993. The effect of sex was investigated by comparing males with null females, the effect of surgery by comparing sham with null, and the effect of sterility on individual females by comparing tubal with sham treatments. Life expectancy was calculated as 1/(minus natural log of the geometric mean of the three annual survival rates).

Secondly, three complementary tests determined whether the level of sterility affected the longevity of unsterilized individuals in the population. The proportions of unsterilized rabbits that survived annually from each February–March were compared between sterility levels by ANOVA with the 3 years treated as blocks. Evident trends were explored further by generalized linear model (GLM) regression with binomial distribution and logit link function, with factors of sterility level and year interval. The third comparison comprised isotonic regression testing, H_A: $\mu_{0\%} \leq \mu_{40\%} \leq \mu_{60\%} \leq \mu_{80\%}$, with at least one inequality (Gaines & Rice 1990).

Thirdly, we determined whether the level of sterility affected the longevity of adults (≥ 1200 g) and young (< 1200 g). Lifetime survival of all rabbits in the populations during the 3 years from September 1992 to July 1996 was determined with the Cormack–Jolly Seber (CJS) method using the computer program JOLLYAGE (J. E. Hines version 16/3/1992; Pollock *et al*. 1990). Chi-square tests in JOLLYAGE showed that separation into age classes was appropriate. The time-specific survival rates (ϕ) for the adults and young were converted to survival per month ($\phi^{1/T}$, where T = months between trapping sessions and each month comprises 30·44 days). For adults and young separately, we compared the mean survival rates per month on the 12 sites by ANOVA with factors of sterility level, season and year for the 3 years July 1993 to July 1996.

Population enumeration

The populations were trapped four times before breeding began after surgical treatments were imposed: in September 1992, during breeding; in February and March 1993, when surgical treatments were imposed; and in July 1993 before breeding began. These preliminary enumerations were used as covariates accounting for the initial size of the populations in analyses of the effects of treatments on subsequent enumerations. All enumerations were adjusted to minimum number known-alive (MNA), and reported means and variation were adjusted for the covariates.

Population abundance

Population numbers were estimated by the capture–mark–recapture CJS method using the program JOLLY (J. E. Hines version 24-1-1991; Pollock *et al*. 1990). The mean estimates were analysed by ANCOVAR, with the factors sterility level, season and year and the three covariates of initial CJS population estimations for February, March and July 1993, there being no estimate for September 1992. The mean CJS estimates were also analysed by two GLM regressions that assumed a normal distribution and logarithmic link, using the mean of the three covariate estimates to account for site variation, and the factors of season and year. The first model regressed population estimates on the mean of actual sterility levels realized in each prior July and November. The second model produced a similar outcome, regressing population estimates on the factor of the nominal sterility levels, 0%, 40%, 60% and 80%.

Flea abundance

The average number of fleas for each sex/sterility class were analysed by ANCOVAR, with covariates of pre-sterility average flea load per rabbit per site for February, March and July 1993.

Myxoma virus transmission

The effect of sterility on the efficacy of transmission of myxoma virus was assessed from the proportions of rabbits with antibodies to myxoma. The data spanned 2.5 years and were unbalanced for season. To achieve balance, the full data set was included in two ANOVAs for overlapping 2-year intervals, from February 1994 to November 1995, and from November 1994 to July 1996. The outcomes agreed and we report only the former analysis.

Results

RAINFALL

Rainfall and growth of green feed strongly influence rabbit breeding in Australia (Poole 1960; Myers &

Poole 1962). The annual rainfall during the study was highly variable, ranging between 383 mm and 800 mm. It varied from above normal in 1992 (average of 12 monthly deciles 6·9, pre-sterility) to normal in 1993 (5·7 deciles, sterility imposed February), very deficient in 1994 (3·7 deciles), low and variable then high in 1995 (5·8 deciles) and above normal in 1996 (6·1 deciles, study ended August). Summarizing, rainfall was high in the year preceding treatment, normal in the first year of treatment, very deficient in the second year and high early in the third year before tending to normal.

COUNTS OF ACTIVE ENTRANCES

The 6-weekly warren surveys (1992–96), including the five additional untrapped sites (1994–96), showed a progressive decline in the percentage of active entrances. While declining, warren activity matched trends seen in population trapping, declining during the drought of 1994–95, increasing in late 1995, and declining after the summer of 1995–96. The untrapped sites behaved identically; the progressive decline was a regional trend independent of our actions.

STERILITY LEVELS ATTAINED

The levels of sterility attained over time (see Table S1 in the supplementary material) varied from the initial assigned levels through losses and recruitment. The average sterility attained exceeded assigned levels, particularly on the 40% sites, being 0%, 51·8%, 64·2% and 82·9%. Nevertheless, sterility levels on all sites reflected the intended design.

STERILITY AND RABBIT ABUNDANCE

Rabbit abundance estimated by MNA closely matched estimates by the CJS model ($r^2 = 0.98$, $n = 144$); on average MNA, estimates were 87% of CJS estimates. We used CJS estimates in our analyses to avoid the negative bias of MNA, but all comparisons were similar for both estimators.

Adjusting for the substantially different initial sizes of the rabbit populations on the 12 sites, Fig. 1 shows the adjusted mean abundance estimates for the three sterility treatments in relation to the control populations. One conclusion is clear from this graph; apart from temporary seasonal effects, no sterility treatment reduced the size of the rabbit population by the end of the experiment in 1996.

Rabbit abundance in relation to sterility level, season and year showed highly significant first-order interactions between sterility level and season (ANCOVAR, $P < 0.001$) and season and year ($P < 0.01$) but no interaction between sterility level and year ($P = 0.651$) and none between sterility level, season and year ($P = 0.951$) (Fig. 1). Seasonal variation, the contrast of the spring breeding peaks and summer–autumn depressions, was particularly reduced in the 80% sterility treatment and
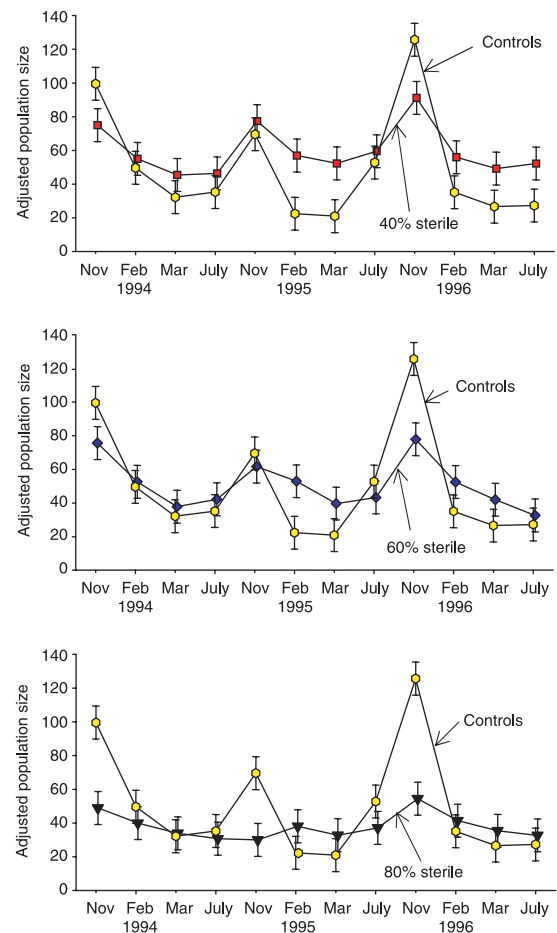

Fig. 1. Effect of sterility on rabbit abundance. Adjusted mean estimate of rabbit abundance (CJS) in populations with a proportion of adult females sterilized in relation to unsterilized populations. Error bars ± 1 SE.

less muted in the 60% and 40% treatments. Seasonal trends differed among years as a result of the drought in 1994–95.

Every year female sterility affected rabbit abundance differently between the main spring breeding season and the remainder of the year when few rabbits bred (Fig. 2). Spring rabbit abundance regressed linearly and negatively on sterility level in all 3 years ($P < 0.001$), with a further apparent curvilinear component only in the 1994 drought, and spring populations were smaller at higher sterility levels. In contrast, sterility had little effect on rabbit abundance in summer, autumn and winter, although populations in the 40% treatment unexpectedly remained high, as also observed in Western Australia (Twigg *et al.* 2000). During summer, abundance declined dramatically in the unsterilized populations but only slightly in the sterilized populations. The drought in 1994 caused low rabbit numbers in the spring breeding season, particularly in the unsterilized populations.

Sterility stabilized rabbit abundance among the seasons of the annual cycle and among years, irrespective of the wide variation in rainfall quantity and temporal pattern. In contrast to unsterilized populations, rabbit
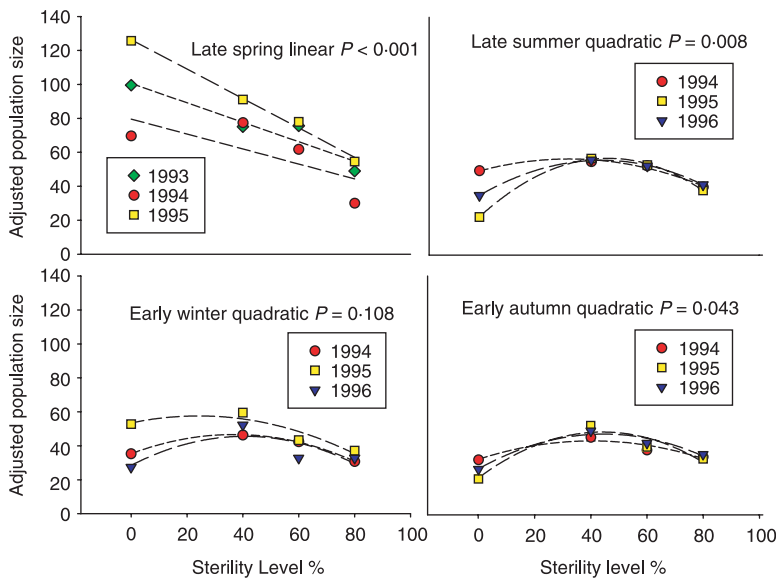
Fig. 2. Seasonal cycle of rabbit abundance. Seasonal cycle of adjusted estimates of abundance (CJS) of rabbits in populations with a proportion of females sterilized. The seasons cycle clockwise from spring 1993 to winter 1996 in three rotations. The probability levels apply to linear or quadratic parabolic regressions.

Fig. 3. Number of young per adult female. Numbers of young trapped per adult female relative to sterility levels during breeding seasons of 1993, 1994 and 1995. The regressions were not forced through the point (100% sterility/zero production of young).

abundance in sterilized populations varied relatively less among seasons (Fig. 2) and did not vary significantly among years ($P \geq 0.305$).

IMMIGRATION

The proportion of immigrants did not differ among sterility treatments ($P = 0.211$) or years ($P = 0.380$) without interaction ($P = 0.893$). The mean proportions were, respectively, 0.045, 0.035, 0.040, and 0.071 for the sterility levels 0%, 40%, 60% and 80%. The numbers of immigrants trapped per site each November were similar among treatments ($P = 0.324$) and years ($P = 0.298$) without interaction ($P = 0.995$). The respective mean numbers were 3.4, 1.4, 4.3 and 1.9; warren-clearing around each site probably contributed to these low rates. Thus immigration was minimal on all sites, and did not cause the observed compensation in abundance.

PRODUCTION OF DETECTED YOUNG

The production of detected young (number of surviving young trapped per adult female) declined from unsterilized populations to a plateau at 40%, 60% and 80% sterility (Fig. 3; see Table S2 in the supplementary material). The regressions suggested that detected production partially compensated for sterility; plots and regression of production of detected young on actual autumn sterility level concurred. The linear regression ($P < 0.001$) accounted for 84.4% of the variance attributable to the level of sterility; the quadratic and cubic components improved the fit by 9.0% ($P = 0.022$) and 6.6% ($P = 0.046$), respectively. Cubic regression was consistent with the logical concept that no young would be produced if all females were sterilized (regression
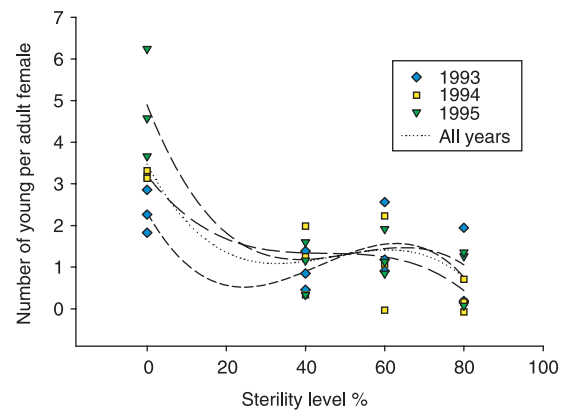
not forced through the point 100% sterility/zero production). The plateau of values in the range of 40–80% of females sterilized suggested possible compensation. A minimum of 15.6% of the variance attributed to the level of sterility was consistent with possible compensation in recruitment to the trappable population. Compensation was minimal in the 1994 drought year.

High sterility also tended to reduce the yearly variation in detected production. The detected production tended to differ among years ($P = 0.058$), increasing progressively over time (see Table S2 in the supplementary material) and relatively more in the unsterilized populations (sterility level × years, $P = 0.092$, divergent slopes of the linear regressions, $P = 0.026$). Therefore 40–80% sterility tended to stabilize the production of detected young among years, including the drought year.

SURVIVAL OF STERILIZED FEMALES

Sterility increased female survival rates, indicating a cost of reproduction. Sterilized females (tubal) had increased individual longevity ($P = 0.005$, see Table S3 in the supplementary material). Male survival equalled that of null females ($P = 0.522$). Survival was similar for sham and null females ($P = 0.779$), showing that surgery did not influence survival. Adult females, when sterilized, had a further life expectation of 1.5 years, compared with 0.8 years for males and 0.9 years for the null and sham operated females (see Table S3 in the supplementary material), a benefit of a 67% longer life span. This comparative benefit increased to a *c.* 95% longer life span when accounting for the collateral survival benefit of female sterility to all adult rabbits (see below). Reproduction is expensive.

SURVIVAL OF COHABITING RABBITS

Isotonic regression indicated that sterility caused a near-significant increase in the proportions of unsterilized adults surviving 1-year intervals ($P = 0.063$). The mean

proportional annual survival rates for 0%, 40%, 60% and 80% sterility were 0·339, 0·348, 0·421 and 0·434, respectively. Further analysis by GLM regression showed that survival was similar at 0% and 40% sterility ($P = 0.909$) and higher at 60% sterility ($P = 0.002$) and 80% sterility ($P = 0.009$). Therefore, the higher levels of sterility, 60–80% of females, conferred a collateral survival benefit of up to 28% to unsterilized adult rabbits living with the sterilized females.

Do young rabbits benefit similarly? Isotonic regression assessed the effect of sterility level on the proportion of young (< 1200 g, unsterilized and sterilized combined) surviving from their breeding season of birth (July–November trapping) over their first summer (to the next February–March trapping), when they are recruited into the adult class of the population. The proportion of young rabbits surviving over all treatments, 0·264 ± 0·1789 (mean ± SE), differed among sterility treatments ($P = 0.030$). Further analysis by GLM regression showed that each sterilized group differed significantly ($P \leq 0.009$) from the unsterilized group, the proportions surviving and therefore recruited being, respectively, 0·150, 0·319, 0·269 and 0·317 for the 0%, 40%, 60% and 80% sterility levels. Therefore, young rabbits also benefited collaterally from the sterility of females in the populations.

The actual numbers of rabbits recruited to the adult class, adjusted for the numbers of young rabbits trapped during the breeding seasons (ANCOVAR, covariate $P < 0.001$), differed significantly among the sterility treatments ($P = 0.033$) but not among years ($P = 0.724$) and without interaction ($P = 0.992$). Over all treatments, 7·3 ± 5·41 (adjusted mean ± SE) young rabbits site^{-1} survived to be recruited each year. An adjusted mean of only 1·31 young rabbits site^{-1} year^{-1} was recruited to the unsterilized populations, whereas, respectively, 8·17, 10·28 and 9·46 were recruited where 40%, 60% and 80% of females were sterilized.

LIFETIME SURVIVAL AMONG POPULATIONS

The survival rates of adults and juveniles were analysed by grouping rabbits regardless of sex and sterility. Mean survival per month between trapping sessions was analysed by separate ANOVAs for adults and young because chi-square tests within JOLLYAGE showed that the estimates of lifetime survival of adult (≥ 1200 g) and young (< 1200 g) rabbits differed significantly ($P < 0.001$). The overall mean monthly survival was 0·910 for adult rabbits (see Table S4 in the supplementary material) and 0·680 for young rabbits (see Table S5 in the supplementary material). The variation in survival per month for adult rabbits was very small, with a coefficient of variation 9·8%, whereas it was greater for young rabbits, 44·3%; many young rabbits died very young while others survived, whereas all adult rabbits had already survived the high risk of early death.

Survival rates of adult rabbits per month (see Table S4 in the supplementary material) differed significantly among sterility treatments, increasing from controls (0·884) to 40% sterility (0·909) to higher combined 60% (0·921) and 80% (0·924) sterility (isotonic regression, $P = 0.02$). This analysis also supported a compensatory survival benefit for sterile females and increased survival of cohabiting untreated adults.

Similarly for young rabbits, the analysis of lifetime survival agreed with the above analysis of their survival over summer. Young rabbits also survived better in sterility-treated populations, with monthly survival (see Table S5 in the supplementary material) increasing from controls (0·608) to higher equal survival in 40% (0·696), 60% (0·704) and 80% (0·711) sterility levels (isotonic regression, $P = 0.05$).

Adult survival varied seasonally ($P < 0.001$), conforming with expectations based on pasture responses to summer–autumn aridity and the added presence of young. Adult survival was higher in the transitions from autumn to winter, 0·937, and from winter to spring, 0·944, than in the transitions from spring to summer, when young rabbits abounded, 0·902, and from summer to autumn, when pastures were depleted, 0·856 (see Table S4 in the supplementary material).

FLEA ABUNDANCE

Sterility reduced the intensity of infestation of fleas (flea count per rabbit) for adult female rabbits (ANCOVAR $P < 0.001$). The unsterilized populations had an adjusted mean flea count of 48·9 female^{-1}, the 40% treatment was similar, with 49·0 fleas female^{-1}, while the 60% sites had 33·7 and the 80% sites had only 23·7. The flea counts for adult female rabbits fluctuated with the seasons ($P < 0.001$) in all 3 years ($P = 0.165$), with a tendency to be lower in the drought year. The mean adjusted flea count per adult female for the seasons over the 3 years was 24 in late summer (February), declining to a minimum of 16 in early autumn (March), increasing to 32 in winter (July) and increasing further to 84 in spring (November), during the rabbit's breeding season. The contrast in flea counts between the breeding and non-breeding samples was most marked in the first year, least in the second (drought) year, and only marginally higher in the third year (season × year, $P = 0.001$).

The flea counts for adult sterilized females, averaging 41·0, exceeded those of the unsterilized females, 28·4 ($P = 0.003$), on the 40%, 60% and 80% sterility sites. This was apparent mainly in the 40% sterility treatment and more pronounced in the breeding season (surgical treatment × sterility level × season, $P = 0.024$). Apparently, fleas were more attracted to, or remained longer with, females that were probably in better health, had hormone profiles of pseudopregnancy or did not share fleas with nestling progeny.

The pattern of flea infestation of pre-puberal male and female young rabbits (< 1200 g) differed from that of the adult females. Sterility did not affect the average number of fleas counted per young rabbit ($P = 0.126$)

and average numbers were low, being, respectively, 8·0, 11·9, 7·3 and 8·0 for the 0%, 40%, 60% and 80% sterility treatments. Flea counts on young rabbits did not peak in the breeding season ($P = 0.105$) and declined on young rabbits in the drought year ($P < 0.001$), the average being 10·2 in the first year, 5·1 in the drought year and 11·3 in the third year.

The pattern of flea infestations on male rabbits was intermediate between those of adult females and young rabbits. Female sterility reduced flea counts on males ($P < 0.001$), respectively 14·5, 11·3, 8·4 and 5·6 for 0%, 40%, 60% and 80% sterility. Flea counts on males increased during the breeding season ($P = 0.008$), from 8·9 in February and 7·6 in March to 12·7 in July and 10·6 in November. The drought year reduced flea counts on males as well ($P < 0.001$), being 12·5 in the first year, 5·2 in the drought year and 12·1 in the third year. Male rabbits always carried far fewer fleas than females.

TRANSMISSION OF MYXOMATOSIS

Antibody prevalence was similar between the two years ($P = 0.919$), which both included long dry periods, but sterility level and season interacted significantly ($P = 0.029$). Figure 4 shows that rabbits became infected mainly after spring births (November), with maximum prevalence of disease-recovered rabbits in late summer–autumn (March). During the spring breeding season, prevalence was lower in the unsterilized treatment, where relatively more young rabbits naive to myxoma virus were emerging from underground. By late summer, the prevalence had risen markedly in the unsterilized treatments to levels similar to those of the 40% and 60% sterility groups, while it had risen only a little in the 80% sterility groups, which had lower prevalence than the other groups. Nevertheless, by early autumn all sterility groups had a similar high prevalence of antibody; very few rabbits remained seronegative in all treatments, including those on sites with low numbers of rabbits. This result indicated very effective transmission.

MYXOMA VIRUS RFLP TYPES

Ten RFLP types of myxoma virus (designated A to J) infected rabbits on the study sites. Each site had a unique combination and temporal pattern of infecting types. From nil to three types occurred successively on a site in any one year. No type was confined to only one sterility treatment, only one type was restricted to two sterility treatments, four types occurred in only three treatments and two types affected all treatments. These patterns suggested that sterility level (proportions of susceptible young) was not a major determinant of the infecting virus types on sites. The infecting types on the treatment sites over all years were: 0% sterility, A, B, F, G; 40% sterility, A, B, C, E, F, H, I; 60% sterility, A, B, C, D, F, H, I, J; and 80% sterility, B, C, F, G, H, I, J.
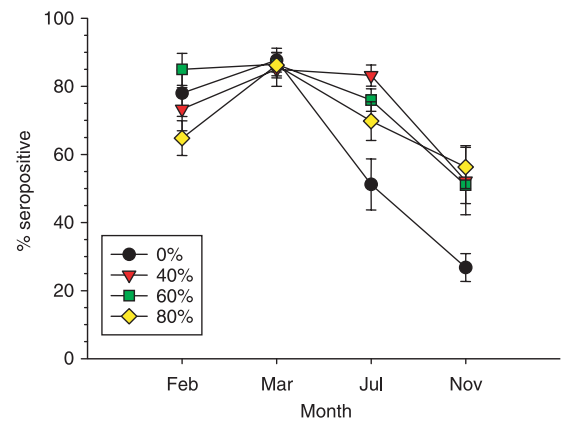

Fig. 4. Prevalence of antibodies to myxoma virus (% seropositive) in rabbits (> 500 g) in late summer (February), early autumn (March), winter (July) and spring (November) from February 1994 to November 1995. Error bars ± 1 SE.

Discussion

HOW DO RABBIT POPULATIONS RESPOND WHEN SOME FEMALES ARE STERILIZED?

Sterility of proportions of females did not reduce the abundance of adult rabbits but, during breeding seasons, high levels of sterility (80%) constrained the ephemeral abundance of young rabbits. During summer–autumn, sterilized populations, particularly the 40% sterility group, did not decline as unsterilized populations did. Thus sterility dampened seasonal population fluctuations.

DOES IMPOSED STERILITY INDUCE COMPENSATORY CHANGES IN RABBIT POPULATION FECUNDITY OR MORTALITY?

Survival of adult and young rabbits compensates for sterility. Sterility substantially improved survival of sterilized females and enhanced survival collaterally in the rest of the population of adults and young rabbits. We have no information on survival of nestlings or reproductive compensation before birth.

WHAT MINIMUM PROPORTION OF FEMALE RABBITS MUST BE STERILIZED TO REDUCE POPULATIONS?

Increased survival sufficiently compensated for the imposed sterility to sustain adult abundance for 3 years when 80% of females were sterilized. However, during winter–spring breeding, 80% sterility significantly constrained population peaks each year, while 40% and 60% sterility reduced population peaks only in some years. During summer aridity, unsterilized populations declined from high levels to low, while 80% sterility alone stabilized populations. Thus, over 3 years, 80% sterility constrained rabbit numbers to late summer (nadir) densities.

DOES ABUNDANCE OF EUROPEAN RABBIT FLEAS DECLINE WHEN PROPORTIONS OF FEMALE RABBITS FAIL TO BREED?

Sterility reduced the abundance of fleas on adult females and the small numbers on adult males, but did not reduce the few fleas on young pre-puberal rabbits.

DOES STERILITY OF RABBITS INTERFERE WITH TRANSMISSION OF MYXOMATOSIS?

A high level of sterility slowed transmission of myxoma virus among rabbits in summer, but by autumn similar high levels of antibodies prevailed in all sterility treatments. In Western Australia as well, the similar sterility treatments had no significant effect on transmission of myxoma virus (Kerr *et al.* 1998). Sterility did not seem to determine the RFLP type of myxoma virus infecting the treatment sites.

In summary, compensatory survival made the rabbit populations resilient to up to 80% female sterility over 3 years. The production of young rabbits exceeded losses of adults; adult numbers remained relatively constant throughout the trial, irrespective of drought and annual myxomatosis epizootics. The annual attrition of young suggests the populations became constrained by depletion of essential resources, for example food of adequate moisture (Cooke 1982) or nutrient quality (White 1978, 1993; Richardson & Osborne 1982; Richardson & Wood 1982). Sterility probably extended the availability of these resources, benefiting survival. Sterility reduced flea populations on adult rabbits without reducing the penetration of myxomatosis in rabbit populations. Twigg *et al.* (2000) reached similar conclusions for rabbits in the Mediterranean climate of south-western Australia. Consequently, we consider the conclusions robust for variation and variability in seasonal and environmental conditions.

Our results contrast with those of a similar study on New Zealand populations of the introduced brushtail possum (Ramsay 2005). This arboreal marsupial differs from the rabbit in having low fecundity and high survival of established adults. The imposed sterility reduced local recruitment but greater immigration of yearling possums compensated, and sustained population abundance. Therefore sustained utilization of resources precluded improved survival of possums. The different outcomes for possums and rabbits arise from differing demographies, immigration propensities and spatial characteristics of the study sites.

HOW DOES STERILITY INDUCE COMPENSATION IN SURVIVAL?

High levels of sterility reduced the population density in spring, increased survival of the sterilized females and collaterally increased survival of cohabiting rabbits, probably because of better supplies of food (*sensu* White 1978) in summer and autumn, when food quality

declines (Parer & Libke 1991) and most young rabbits die. The longitudinal studies of Gibb (1977) and Rödel *et al.* (2004), although equivocal, concur with the notion of a depleted food supply reducing survival of rabbits at increased population density. An experimental contemporary comparison (Garson 1986) links the food supply of rabbits to improved survival, although other causes are possible. Stodart & Myers (1966) showed experimentally that better food supply and quality increased the production of rabbit litters and improved the health of the progeny; we imply improved survival follows.

Sterilized females benefit individually and survive longer by not giving birth or lactating, thereby conserving body reserves. The collateral benefit, increased survival in all classes of non-sterilized rabbits, could have three main causes: (i) rabbit density relative to the limited essential resources of food, moisture and shelter would be lower; (ii) rabbit biomass, growth rates, metabolism and consumption may be lower; (iii) rabbits may exploit resources more efficiently and effectively where fewer young rabbits reduce competitive strife and attraction of predators (Williams *et al.* 1995), enabling improved foraging, nutrition and survival. This interpretation, implicating limited food resources, agrees with principles espoused by White (1978, 1993, 2004) and falls within Krebs' mechanistic paradigm rather than the density dependence paradigm (Krebs 1995). We cannot test our interpretation, as data are lacking on the availability of the food components that limit reproduction (Poole 1960; Myers & Poole 1962; Stodart & Myers 1966; Cooke 1981) and survival (Myers & Bults 1977; Cooke 1981).

COST OF REPRODUCTION

Theoretical deductions that reproduction is costly have been inferred largely from observed survival rates of breeding and non-breeding individuals in field populations, rather than through experimental manipulations of sterility (Roff 1992). Some data are available for castrated male mammals (Jewell 1997 for Soay sheep) but only limited data for experimentally sterilized females. Our experiment has shown a strong effect of sterility on female survival rates, an increase of 67%, or 95% when discounting the collateral benefit, demonstrating a cost of reproduction greater than that observed in female Soay sheep (Tavecchia *et al.* 2005).

EFFICACY OF A MYXOMA VIRUS STRAIN AS A VECTOR FOR IMMUNOCONTRACEPTION OF RABBITS

IC myxoma, theoretically, would kill some rabbits and sterilize all those recovering from infection. The seropositive rabbits had survived infection and recovered, but lethality was unknown. Myxoma virus isolates from this study were classified into 10 RFLP types. Some types were successional, indicating they occupied different Hutchinsonian niches, and therefore probably varied in lethality and transmissibility. Cumulatively,

by autumn they had infected almost all susceptible rabbits, irrespective of the level of sterility. IC myxoma would need to out-compete these field strains. It should be more highly transmissible (Williams 1996; Merchant *et al.* 2003a,b) and would need appropriate moderately high lethality (Mead-Briggs & Vaughan 1975).

HOW COULD FERTILITY CONTROL HELP CONSTRAIN THE IMPACTS OF RABBITS?

Rabbit control has two aims: (i) to prevent losses to agricultural production, where the benefit is assumed to be proportional to the level of reduction of rabbits; (ii) to conserve palatable native perennial vegetation, where rabbits must be reduced to very low densities for several years (Williams *et al.* 1995). How could fertility control advance these aims?

Fertility control reduced the peaks of rabbit abundance proportionally during breeding seasons. The 80% sterility treatment reduced rabbit abundance in spring by 48% relative to controls. This big reduction in grazing pressure during the southern Australian growing season would increase pasture reserves during summers and droughts. Perennial plants may benefit from dilution within the conserved annual vegetation, with influence from dietary selection. Importantly, native perennials may be conserved near the margins of suitable rabbit habitat within regions, and near distribution boundaries at a continental scale.

Would fertility control of rabbits add its effects to those of myxomatosis, RHD, poisoning, fumigation and warren ripping? Would it interact positively with these methods (Williams 1997), mutually enhancing positive effects and exceeding simple addition? Even if it did, would the benefits of introducing fertility control justify the costs?

UTILITY OF FERTILITY CONTROL FOR RABBITS

The benefit–cost ratio could indicate the utility of fertility control for rabbits, but it involves values of conserving native biota. Alternatively, use of cost-effectiveness (*sensu* cost per effectiveness) enables comparison of methods, their combinations and integration (for a methodology see Williams & Moore 1995); values and cultural aspects can be judged separately (Norton & Pech 1988).

Cost-effectiveness may be reduced beneficially if fertility control is (i) cheaper or more effective than existing methods or (ii) interacts positively with them. Existing methods include effective biological controls, RHD virus and myxomatosis, which spread naturally and are cost-free. Fertility control seems less effective and no cheaper than these diseases and may add a cost if not self-sustaining (Hood 2000). Thus to reduce cost-effectiveness, fertility control must interact positively with existing methods and compensate for any added cost, or be used where RHD and myxomatosis are infrequent or insufficiently effective, provided IC myxoma

can spread sufficiently and infect and permanently sterilize 80% or more of females over many years.

PRESENT NEEDS FOR RABBIT CONTROL AND PROSPECTS FOR IC MYXOMA

Myxomatosis and RHD do not control rabbits in Australia to a sufficient extent. Each disease effectively reduces populations but rarely eliminates them. Resistant or recovered survivors thrive, and populations may resurge between epizootics. Each disease varies geographically in effectiveness, myxomatosis because of insufficient insect vectors in arid regions (Sobey & Conolly 1971; Cooke 1984, 1990) and RHD in higher rainfall regions, probably because a related benign virus immunizes rabbits (Cooke *et al.* 2002). In mesic parts of Australia, the two diseases benefit spring regeneration of annual flora but rabbit densities later in summer deter regeneration of perennials (Mutze *et al.* 2002). In arid regions, even very low densities of rabbits prevent regeneration of suckering perennial trees and shrubs, and, at least in dry periods, some seed-setting perennial shrub species (Denham & Auld 2004). Additional controls or further positive interactions are needed to conserve Australia's dwindling suite of native perennial flora and dependent fauna.

We need information on the interaction of RHD and myxomatosis and the adaptive responses of rabbit populations in different climatic regions to help refine strategies for integrating poisoning and warren ripping with the actions of the diseases. We particularly need to investigate every opportunity for additional biological controls (viz. Hamilton *et al.* (2005). Some might have better, earlier prospects than IC myxoma; viral competition and the required sterilization of 80% of females ensure uncertain practicality, cost–benefit, cost-efficiency and schedule for deployment. Broad-scale suppression of rabbits in Australia requires a more immediate improvement in strategic combinations of varied forms of attrition.

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Supplementary material

The following supplementary material is available as part of the online article (full text) from http://www.blackwell-synergy.com.

Appendix S1. Surgical procedures.

Table S1. Attained sterility levels.

Table S2. Young per adult female.

Table S3. Survival of sex and sterility classes.

Table S4. Monthly survival of adults.

Table S5. Monthly survival of young.