EXPERIMENTAL STUDY ON THE EFFECT OF COVER AND VACCINATION ON THE SURVIVAL OF JUVENILE EUROPEAN RABBITS

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ABSTRACT

In Mediterranean ecosystems, the European rabbit is a keystone species that has declined dramatically, with profound implications for conservation and management. Predation and disease acting on juveniles are considered the likely causes. In the field, managers usually manage these processes by removing predators, increasing cover to reduce predation risk and by vaccinating against myxomatosis. These manipulations can be costly and, when protected predators are killed, damaging to conservation interests. Our goal was to test the effectiveness of cover and vaccination on juvenile survival in two large enclosures, free of mammalian predators, by adding cover and vaccinating juveniles. Rabbit warrens were our experimental unit, with nine replicates of four treatments: control, cover, vaccination, and cover and vaccination combined. Our results showed that improved cover systematically increased juvenile rabbit survival, whereas vaccination had no clear effect and the interactive effect was negligible. Our experimental data suggest that improved cover around warrens is an effective way of increasing rabbit abundance in Mediterranean ecosystems, at least when generalist mammalian predators are scarce. In contrast the effectiveness of vaccination programmes is questionable.

KEYWORDS

Oryctolagus cuniculus, juvenile survival, predation risk, myxomatosis, management, Mediterranean ecosystems.

INTRODUCTION

Disease and predation can profoundly affect animal populations (e.g. Sinclair and Arcese 1995; Connors et al. 2010). Studies of their impact on fitness have tended to focus on one process, yet in reality both processes can operate simultaneously, and may well interact in the field. On one hand, disease is known to increase the likelihood of animals being killed by predators (Temple 1987; Møller and Erritzoe 2000); whilst on the other hand, the risk of predation can have severe sub-lethal effects, affecting the incidence of disease and long-term survival and fitness (e.g. Navarro et al. 2004; Sheriff et al. 2011). An understanding of the relative importance of such population processes is crucial to develop effective management strategies aimed at species conservation and recovery.

In Mediterranean ecosystems of southwest Europe, European rabbits (*Oryctolagus cuniculus* Linnaeus 1758) are considered a keystone species, mainly because they represent an important prey for nearly 40 predator species (Villafuerte 1994; Delibes-Mateos et al. 2007). Rabbits are also an important small-game species in Spain, being hunted in over 30.000 private hunting estates covering more than 70 % of the country (Villafuerte et al. 1998). Yet rabbit populations have declined dramatically in recent decades, with consequences for conservation and hunting (Angulo and Villafuerte 2003). Declines have generated expensive game management efforts to stabilize and increase populations, often with little supporting evidence (Delibes-Mateos et al. 2008).

Viral diseases, such as myxomatosis, and predation are thought to have played a major role in rabbit population declines (Villafuerte et al. 1994; Angulo 2003; Moreno et al. 2007; Cotilla et al. 2010). Both of these operate primarily on juvenile rabbit

72 survival (Villafuerte 1994; Angulo and Villafuerte 2003; Cotilla et al. 2010; Smith and
73 Trout 1994; Calvete et al. 2002). In the wild, the epidemiological pattern of
74 myxomatosis is characterized by a rapid increase of antibodies in juvenile rabbits just
75 after the outbreak, resulting in a high prevalence of antibodies in adult rabbits (Calvete
76 et al. 2004). Juvenile rabbits are virtually all infected in their first year of life and hence
77 the pattern of myxomatosis outbreaks is closely related to the recruitment of susceptible
78 juvenile rabbits during the breeding season (Calvete et al. 2002). Similarly, predation is
79 thought to represent a major threat to rabbit populations (Villafuerte 1994; Moreno et al.
80 1996) and acts predominantly on the younger age classes (Villafuerte 1994; Cotilla and
81 Villafuerte 2007; Tablado et al. 2012). This predation pressure on juvenile rabbits is
82 imposed mainly by raptors during winter and spring, potentially causing the loss of over
83 60% of the reproductive potential of the population (Villafuerte 1994).
84 Predation and disease are also known to interact in lagomorphs (Tablado et al.
85 2012). For example, diseases may make rabbits more vulnerable to predation and high
86 predation risk may influence physical condition, compromising immunity and making
87 rabbits more vulnerable to disease (Dunsmore et al. 1971; Villafuerte et al. 1997;
88 Moreno et al. 2007; Sheriff et al. 2011; Tablado et al. 2012).
89 Attempts to reduce levels of predation focus primarily on the direct legal control
90 of predators and, indirectly, on the increase of the extent of available cover, or on the
91 illegal killing of protected species (e.g. Moreno et al. 1996; Villafuerte and Moreno
92 1997; Villafuerte et al. 1998; Lombardi et al. 2003). Management to reduce the impact
93 of diseases focuses on vaccination campaigns using commercial vaccines (Calvete et al.
94 2004; Guitton et al. 2008). These commercial vaccines succeed in immunizing domestic
95 rabbits, but they appear to be less effective in the field (Ferreira et al. 2009). Rabbit

management can be very costly (e.g. Delibes-Mateos et al. 2008) and in the case of illegal predator control have important conservation implications (Villafuerte et al. 1998; Ferreira et al. 2009). Yet little attempt has been made to understand the relative influence of both processes (predation and disease) and on the effectiveness of legal forms of management.

The goal of this study was to experimentally manipulate cover and susceptability to disease through vaccination and test their effectiveness at improving juvenile rabbit survival. Here we focus on juvenile rabbit survival, since this age class is the most vulnerable to the effects of both disease and predation (Smith and Trout 1994; Villafuerte 1994; Calvete et al. 2002; Angulo and Villafuerte 2003; Cotilla et al. 2010), and, for this reason, its survival is usually considered an indicator of population quality and a crucial parameter for population persistence (Smith and Trout 1994; Angulo and Villafuerte 2003; Cotilla and Villafuerte 2007). We worked in large enclosures, where mammalian predators were excluded, as is the case in many managed hunting estates, and where there was grass, but little other cover. We increased cover around rabbit warrens and manipulated susceptability to disease by vaccinating juvenile rabbits against myxomatosis using a standard, commercial vaccine.

We expected that cover would improve juvenile survival directly by reducing predation by raptors, and indirectly by reducing the impact of myxomatosis. We anticipated that myxomatosis would outbreak half way through the experiment. We expected that vaccination would improve juvenile survival directly by reducing the impact of myxomatosis, and indirectly by reducing the levels of predation. Specifically, our predictions were that: 1) rabbits in control plots would always have lower survival; 2) rabbits in control plots would have higher survival before than after the disease

outbreak; 3) cover would always improve juvenile survival relative to control plots, equally before and after the outbreak; 4) vaccination would increase survival only after the outbreak, being similar to control before the outbreak, and 5) combined cover and vaccination treatments would have similar survival to that of cover alone before the outbreak, but the highest survival after the outbreak.

METHODS

Study area

The study area (Los Melonares) is situated in the south of the Sierra Norte Natural Park of Seville, Sierra Morena, SW Spain. It is characterised by a typically Mediterranean climate, with hot, dry summers and temperate, wet winters. The area consists mainly of grassland and scrubland including *Cistus ladanifer*, *Pistacia lentiscus, Myrtus communis*, *Lavandula stoechas* and *Retama sphaerocarpa*. The subspecies of wild rabbit occurring in the study area is the *O. cuniculus algirus*. Eleven species of raptor nested in the area, many of which preyed extensively on rabbits (Delibes-Mateos et al. 2007).

Experimental design

In 2002, four 200 x 200 m experimental plots were built, approximately 1-km from each other in the grassland area, in the context of a rabbit recovery program (see Rouco et al. 2008, 2011 for more details). No natural or artificial warrens were previously present in any plot. Two of these plots were provided with an exclusion fence to prevent the entry of terrestrial mammalian predators. Fenced enclosures are an increasingly used management technique in southwest Europe that allow for high

densities of rabbits (Ferreira and Delibes-Mateos 2010). Additionally, these are convenient systems to simulate legal predator control, one of the most important management measures implemented in this region to boost rabbit populations (Angulo 2003). For this reason, in this paper we focus on the two fenced plots only. Fences were 3-m tall and 1-m underground (4 x 4 cm mesh), with an electric wire at the top, to prevent mammalian predators entering. Small terrestrial predators were excluded by attaching another fence of smaller mesh size at the base (120 cm tall, 1.5 x 1.5 cm mesh).

In each plot 18 artificial warrens were built and were regularly distributed in four alternate lines of four or five warrens approximately 40 m apart (Rouco et al. 2011). Two different warren sizes were built: large (6 per plot) and small (12 per plot). Large warrens were exactly four times bigger than the small ones. Each warren was constructed using wooden pallets, wood, stones and soil (Rouco et al. 2008) and surrounded with a wire net (approx. 1 m high, 0.5 m underground, 1.5 cm mesh). Three rabbit traps were placed around the small warrens and five around the large ones. Rabbits could only leave or enter warrens by passing through these traps. Food and water were provided *ad libitum* next to each warren in both plots throughout the experiment ensuring that these resources were never limiting. Rabbits were live-trapped in all warrens in the two plots over 2-3 consecutive nights every month (usually the last week of each month) from March to October 2007. At their first capture animals were marked with individually numbered ear tags and measured (sex, weight, tarsus and ear length).

Our experiment was conducted from March to October 2007. Predation and disease were manipulated as follows. Warrens were randomly allocated to one of the

following four treatments: control (no treatment), cover, vaccination, or both cover and vaccination. In total, there were nine warrens (3 large and 6 small) in each treatment split between the two plots. The impact of raptor predation was manipulated by adding cover to the surroundings of the appropriate warrens (e.g. Richardson and Wood 1982). Cover was added in February 2007 and consisted of six wooden pallets (2x1 m) placed in the immediate vicinity of the warren exits. These provided cover for rabbits to move to and from their feeding areas. To manipulate the impact of myxomatosis, all juvenile rabbits (weight < 900 g; Soriguer 1981; Villafuerte 1994) were either injected with 0.5 ml of a commercial vaccine against myxomatosis (POX-LAP from OVEJERO Laboratories, León, Spain), or a 0.5 ml saline control solution, at their first capture. Myxomatosis was known to be consistently present in the population with typical annual outbreaks in the summer (Villafuerte et al. 1994; Calvete et al. 2002; Rouco et al. 2008), in contrast to RHD (Rabbit Hemorrhagic Disease, for which outbreaks are extremely irregular). In 2007, the myxomatosis peak was detected in July when nearly 50% of juvenile rabbits showed symptoms of the disease, regardless of treatment (Ferreira et al. 2009), and so, for analyses purposes, we considered this month to represent the disease peak. Finally, blood samples were collected in two occasions (April and October 2007, pre- and post-outbreak periods, respectively) to detect antibodies against myxomatosis in juvenile rabbits as a way to check if vaccination provided additional protection. The details on the seroprevalence analysis and results are throroughly presented in Ferreira et al. (2009).

Capture-mark-recapture survival analysis

We used capture-mark-recapture techniques (Lebreton et al. 1992) to test our predictions about the effects of cover and vaccination on juvenile survival. First we built an initial capture history database spanning all sampling occasions (March to October 2007) with all juvenile rabbits grouped by treatment in order to test that the dataset met the assumptions underlying capture-mark-recapture analyses (Lebreton et al. 1992). We tested these assumptions by applying the goodness-of-fit tests available in the program U-CARE 2.3 (Choquet et al. 2005). Then we modified the structure of the capture-recapture dataset according to the biases detected, and performed further goodness-of-fit tests of dispersion in MARK 6.0 (White and Burnham 1999). Once we had a suitable general starting model that fitted the data adequately, we incorporated plot, warren size and time varying age (since some juveniles became adults during the experiment) as covariates of both survival and detection probability.

Subsequently we used MARK 6.0 to model survival and detection probability, using the Akaike's Information Criterium modified for small sample sizes (AICc) in order to assess model fit (Burnham and Anderson 2002). We started by investigating the influence of covariates, infection period and experimental treatment on detection probability. Models accounting for infection period were designed to fit a hypothetical difference in estimates before and after the outbreak of myxomatosis in July. This was achieved by merging pre-outbreak (March-June) and post-outbreak (July-October) time dependent parameters separately. We then investigated the influence of covariates, infection period and experimental treatment in survival rates.

To test for an effect of myxomatosis on juvenile survival, we assessed whether infection period explained a significant part of the temporal variation in survival using an ANODEV test (Grosbois et al. 2008). The test included a model with constant

survival for all treatments, a model with time dependent survival for all treatments, and a model where pre-/post-outbreak survival parameters differed additively for all treatments.

To assess differences in juvenile survival between experimental treatments, we used treatment contrasts, where model fit was assessed using AICc. Differences between pairs of treatments were assessed by comparing models with 1) equal survival parameters for the pair of treatments, 2) with different parameters before and after the outbreak of myxomatosis, 3) with different parameters only before the outbreak, or 4) with different parameters only after the outbreak. In addition, similar contrasts were used to assess whether cover and vaccination had an additive or interactive effect in the combined treatment before and/or after the outbreak. Because transience in juvenile survival was detected (see Electronic Supplementary Material), estimates reported in the results and discussion sections refer to the non-transient class. Estimates for the transient class are provided in the Electronic Supplementary Material. In order to account for model uncertainty, parameter estimates reported in this manuscript are model averaged across the best set of models with refined detection probability and survival rates (Burnham and Anderson 2002). Results and contrasts reported are based on differences on the logit scale, since we used the logit link throughout the analysis in MARK (White and Burnham 1999).

RESULTS

In total, between March and October 2007, 1312 juveniles were live-trapped, 595 of which corresponded to new captures (details in Table 1.1 in Electronic Supplementary Material). The mean number of juvenile rabbits captured per warren per

239 month was 16.6 ± 1.30 (standard error). Initial models suggested that neither treatment
240 nor infection period affected probability of detecting rabbits (Table 1.4 in Electronic
241 Supplementary Material). Refinement of survival parameterisation indicated a large
242 impact of plot and age, and a small impact of warren size on juvenile survival (models
243 10-14, Table 1). Accounting for treatment improved model fit (compare models 17 and
244 20, 19 and 22, 15 and 18, Table 1).

245 Model fit was not improved by accounting for infection period (models 20-22,
246 Table 1). However, examination of monthly survival estimates from an additive time
247 dependent model (model 17 in Table 1) showed a marked decrease in juvenile survival
248 in August, suggesting that the impact of a July outbreak of myxomatosis might be
249 reflected with one month delay in juvenile survival (Table 1.3 in Electronic
250 Supplementary Material). Thus, we fitted a further set of models with a delayed impact
251 of the outbreak in survival, e.g. where the pre-outbreak period lapsed from March to
252 July (instead of June as previously considered), while the post-outbreak period lapsed
253 from August to October. This set of models showed a better fit than previous models
254 (compare models 15, 16 and 18 with their time varying/infection period equivalents,
255 Table 1).

256 The ANODEV test indicated that myxomatosis explained a significant part of
257 the time variation in survival ($F_{(1,22)}$ =18.90, P<0.001), causing a substantial reduction in
258 mean survival rates across all treatments (Figure 1). Between treatment contrasts (Table
259 2) suggested that cover improved juvenile survival compared to control, especially
260 before the outbreak (estimates for non-transients rabbits pre-outbreak cover = 0.939,
261 95% CI [0.848, 0.977], control = 0.907 [0.807, 0.958]; post-outbreak cover = 0.325
262 [0.211, 0.464], control = 0.25 [0.163, 0.380]). However, vaccination did not improve

juvenile survival in relation to controls, (pre-outbreak vaccinated = 0.906 [0.809, 0.957]; post-outbreak vaccinated = 0.271 [0.180, 0.387]). In the combined treatment, juvenile survival was similar to control pre-outbreak (0.903 [0.790, 0.958]) and higher than control post-outbreak (0.326 [0.189, 0.502]). Estimates above show that survival in the combined treatment was similar to control and vaccination treatments before the outbreak and similar to that of cover after the outbreak.

DISCUSSION

This experiment demonstrated that, in the absence of mammalian predators, juvenile rabbit survival was highest in warrens with additional cover. However, the level of improved survival was relatively modest in the pre-outbreak period (with a 3.5% increase relative to controls) but rather important during the post-outbreak phase (26.3% increase relative to controls). In contrast, vaccination had no measurable effect on juvenile survival, despite the fact that the myxomatosis outbreak had a large impact on juvenile survival across all treatments.

The unexpected observation that vaccination did not improve juvenile survival could be related to different causes. For example, it has been shown that vaccination can have adverse effects on rabbit physiology (Peeters et al. 1995; Twigg et al. 1997). Some secondary effects include mild fevers (Marlier et al. 2000) and lethargy, making juveniles less responsive and more vulnerable to predation or even death. On the other hand, there is a possibility that vaccination failed to immunize juvenile rabbits or that the latter may have not been sufficient to impact the survival of this age class at the population level. The fact that in a previous work (Ferreira et al. 2009) the proportion of juveniles seropositive to myxomatosis was similar between vaccinated vs. non-

vaccinated, both before and after the disease outbreak, may corroborate this hypothesis. In fact, in the post-outbreak period (October 2007), all of the juveniles sampled were seropositive to the disease regardless of whether they had been vaccinated or not against myxomatosis prior to the outbreak, which suggests that, in our experiment, vaccinating against myxomatosis was redundant. Vaccination campaigns in the field can additionally be influenced by the highly variable spatial-temporal pattern exhibited by the virus (Villafuerte et al. 2000), which is a function of a panoply of factors such as the virulence of circulating strains or population density (Arthur and Louzis 1988), providing paradoxical effects at the individual level. It is therefore possible that the vaccine we used (developed for domestic rabbits), which is the only one available against myxomatosis (regardless of the source laboratory of production), might be innefective to protect wild specimens against all the strains of the virus. The latter is supported by the report of cases where highly virulent strains have decimated even vaccinated rabbits in rabbitries, e.g. in Greece (Kritas et al. 2008). Whatever the mechanism it seems clear that vaccination programmes in wild populations are likely to be costly (e.g. average 4 790 euros/year per 2 000 ha; Angulo 2003) and potentially ineffective (Ferreira et al. 2009).

Our results clearly show that cover improves juvenile rabbit survival in areas where raptors are their main predators. Avian predation is particularly heavy on juveniles up to 3 months of age (Villafuerte and Viñuela 1999) and this could explain the success of the cover treatment in our study. Cover is fundamental for juvenile rabbits as a resource that increases refuge opportunities from predators (Moreno et al. 1996), decreases the need for group vigilance (Villafuerte 1994), and reduces individual distances to forage (Villafuerte and Moreno 1997).

311 Across the Iberian Peninsula rabbits seem to be recovering better in areas where
312 several management activities have been carried out simultaneously and regularly
313 (Delibes-Mateos et al. 2008). In particular, improved rabbit recovery has been observed
314 in hunting estates where both mammalian predator control and habitat management
315 activities are frequently applied (Angulo 2003; Delibes-Mateos et al. 2008). Conversely,
316 rabbit populations did not change in places where restocking or vaccination were the
317 main management activities (Delibes-Mateos et al. 2008). Rabbits are such an important
318 component of Mediterranean ecosystems (Delibes-Mateos et al. 2007) that there is an
319 urgent need to restore healthy, wild populations. This will benefit both conservation and
320 human wellbeing and livelihoods. Whilst rabbit populations are at low density,
321 protected species of predators are likely to continue to be vulnerable to direct or indirect
322 killing by hunters (Márquez et al. 2013). Identifying the most effective management
323 techniques to improve rabbit abundance is therefore urgently needed. The results from
324 this study suggest that habitat management to improve cover is likely to be most
325 effective at improving survival of juvenile rabbits. There is now a need to understand
326 the optimum strategies for managing cover and other habitat features targeted at the
327 European rabbit (Ferreira et al. 2013).
328 Despite unequivocal, our results need to be carefully extrapolated to natural
329 populations, since they are based on only two enclosures studied over 8 months and
330 during one single epidemic outbreak. Our rabbits were free from mammalian predators
331 and were provided with *ad libitum* food and water at all times. They were therefore in
332 good condition, and may have higher survival than wild populations. For example, the
333 average juvenile survival in Doñana National Park was 0.45 (Villafuerte 1994), which is
334 considerably lower than in our study. Also the concomitant influence of viral

hemorraghic disease RHD was not explored in our study, although this disease was not detected in our study area during 2007. Therefore, further research should explore 1) the effect of improving cover in open areas with mammalian predators, 2) alternative techniques to minimize the effects of diseases, including RHD, in the field, and 3) optimum strategies for improving cover.

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477 Table 1. Summary of the model selection process. For every successive modelling step,
478 the complete set of models considered are given, with decreasing level of support based
479 on Akaike's Information Criterium (AICc) scores.

Modelling step	**Model no.**	**Model specification**	**AICc**
General starting model	1	Φ(a2+t)+Age+Plot+WSize, p(t)+Age+Plot+WSize	2861.77
Detection probability covariates	2	Φ(a2+t)+Age+Plot+WSize, p(t)+Age+Plot	2859.99
	3	Φ(a2+t)+Age+Plot+WSize, p(t)+Age+Plot+WSize	2861.77
	4	Φ(a2+t)+Age+Plot+WSize, p(t)+Age+WSize	2867.41
	5	Φ(a2+t)+Age+Plot+WSize, p(t)+Plot+WSize	2947.49
	6	Φ(a2+t)+Age+Plot+WSize, p(t)	2950.61
Detection probability infection period and treatment	7	Φ(a2+t)+Age+Plot+WSize, p(t)+Age+Plot	2859.99
	8	Φ(a2+t)+Age+Plot+WSize, p(t+treat)+Age+Plot	2861.57
	9	Φ(a2+t)+Age+Plot+WSize, p(t+inf)+Age+Plot	2870.68
Survival rates covariates	10	Φ(a2+t)+Age+Plot+WSize, p(t)+Age+Plot	2859.99
	11	Φ(a2+t)+Age+Plot, p(t)+Age+Plot	2861.44
	12	Φ(a2+t)+Age+WSize, p(t)+Age+Plot	2863.71
	13	Φ(a2+t)+Plot+WSize, p(t)+Age+Plot	2871.40
	14	Φ(a2+t), p(t)+Age+Plot	2877.53
Survival rates infection period and treatment	15	Φ(a2+delayinf+treat)+Age+Plot+WSize, p(t)+Age+Plot	2855.96
	16	Φ(a2+delayinf*treat)+Age+Plot+WSize, p(t)+Age+Plot	2856.06
	17	Φ(a2+t+treat)+Age+Plot+WSize, p(t)+Age+Plot	2856.83

	18	Φ(a2+delayinf)+Age+Plot+WSize, p(t)+Age+Plot	2857.52
	19	Φ(a2+inf+treat)+Age+Plot+WSize, p(t)+Age+Plot	2859.63
	20	Φ(a2+t)+Age+Plot+WSize, p(t)+Age+Plot	2859.99
	21	Φ(a2+inf*treat)+Age+Plot+WSize, p(t)+Age+Plot	2863.02
	22	Φ(a2+inf)+Age+Plot+WSize, p(t)+Age+Plot	2863.40
	23	Φ(a2+treat)+Age+Plot+WSize, p(t)+Age+Plot	2889.66

480 *Note*: Symbols: Φ=survival rate; p=detection probability; a2=two *time since marking*
481 subclasses (1 month since marking and > 1 month); inf=two myxomatosis infection
482 periods (pre-outbreak vs. post-outbreak); delayinf=two infection periods with one-
483 month delay in the impact of myxomatosis; t=time dependent parameter; Age=time-
484 varying covariate age; Plot=covariate experimental plots (fenced enclosures);
485 WSize=covariate warren size.
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489 Table 2. Results of contrasts fitted to assess differences in juvenile survival rate (Φ)
490 between treatments. To account for model uncertainty in the model selection process,
491 two sets of models were fitted built upon general models with i) an interactive effect of
492 treatment and delayed myxomatosis (model 16 in Table 1), and ii) an additive effect of
493 treatment and delayed myxomatosis (model 15 in Table 1). For every contrast, models
494 were fitted with same survival parameters for treatments under consideration, with
495 different parameters in both pre-outbreak and post-outbreak periods, or with different
496 parameters in only one period. Lower AICc score for every contrast and set indicate
497 better fit. CV* = treatment with cover and vaccination combined parameterised as an
498 interaction between those treatments.

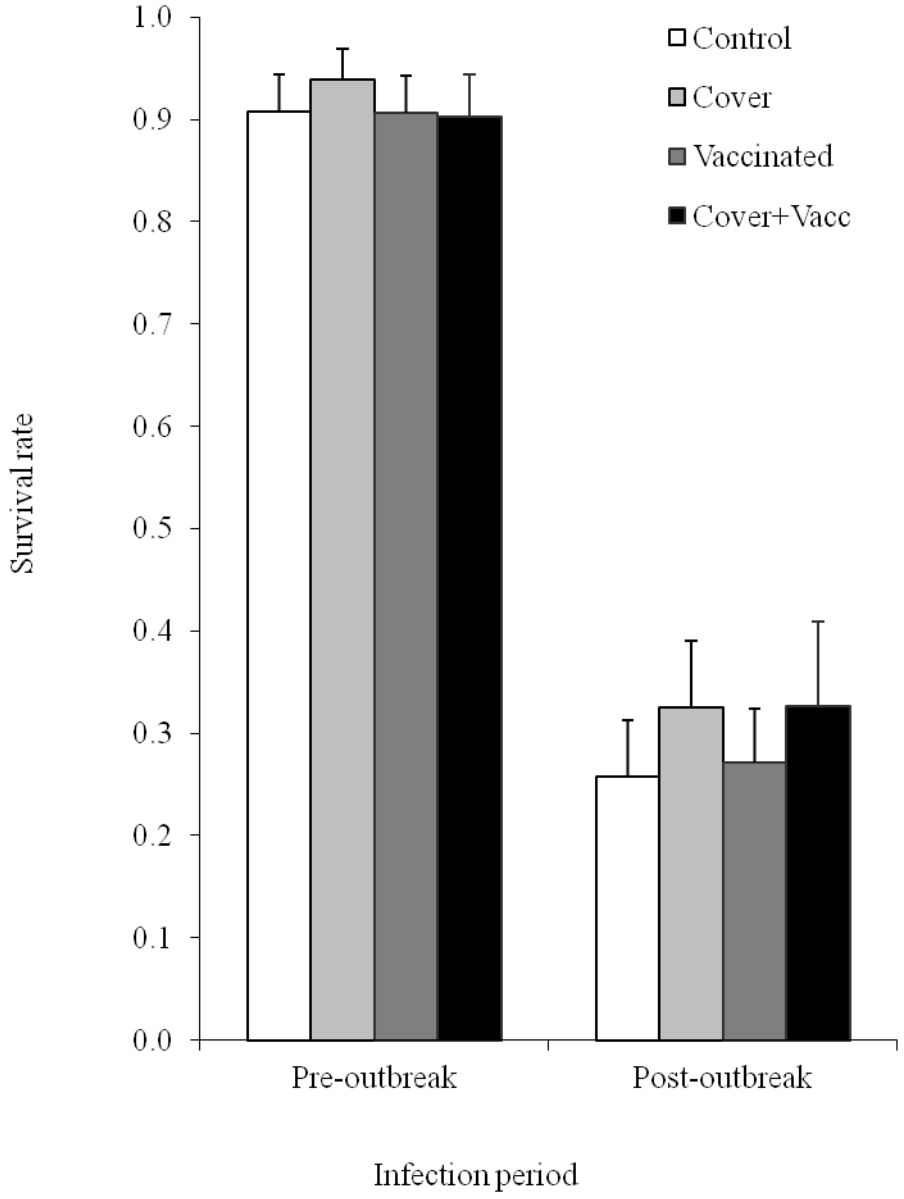
Contrast set	**Model fitted**	**AICc Interaction set**	**AICc Additive set**
Baseline model		2856.06	2855.96
Cover vs. Control	Φcover=Φcontrol	2858.91	2859.61
	Φcover≠Φcontrol both periods	2856.06	2855.95
	Φcover≠Φcontrol pre-outbreak only	2854.83	2852.96
	Φcover≠Φcontrol post-outbreak only	2858.61	2861.48
Vaccinated vs. Control	Φvacc=Φcontrol	2852.12	2853.91
	Φvacc≠Φcontrol both periods	2856.06	2855.95
	Φvacc≠Φcontrol pre-outbreak	2854.18	2855.97

	only		
	Φvacc≠Φcontrol post-outbreak only	2854.03	2853.76
CV* vs. Control	Φcv=Φcontrol	2854.93	2854.32
	Φcv≠Φcontrol both periods	2856.06	2855.95
	Φcv≠Φcontrol pre-outbreak only	2856.87	2855.68
	Φcv≠Φcontrol post-outbreak only	2854.67	2852.36
CV* vs. Additive Cover+Vaccinated	Φcv*= Φcover+Φvacc	2888.50	2855.54
	Φcv*≠ Φcover+Φvacc both periods	2856.06	2855.95
	Φcv*≠ Φcover+Φvacc pre-outbreak only	2854.15	2855.68
	Φcv*≠ Φcover+Φvacc post-outbreak only	2880.75	2852.36

499

500

501 Figure 1.



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503 Figure 1. The combined effect of treatment and infection period (pre-outbreak: March-
504 July and post-outbreak: August-October) on juvenile survival across the whole
505 experiment and obtained from Time Since Marking (TSM) models. The graph shows
506 model averaged survival mean estimates (± SE) for non-transient juveniles.
507
508
509

510 **Electronic Supplementary Material**

511 Table 1.1 Number of juveniles live-trapped at each trapping session per treatment.

Month	Control	Cover	Vaccinated	Cover+Vacc	Total
March	29	39	45	27	140
April	70	51	74	47	242
May	54	62	64	37	217
June	74	89	62	62	287
July	27	55	51	30	163
August	21	42	24	27	114
September	11	29	11	15	66
October	12	28	20	23	83

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513

514 Table 1.2 Monthly juvenile survival estimates (mean [95 % CI]) obtained from an
515 additive time dependent model (model 17 in Table 1), showing a marked decrease in
516 survival rates in August across all treatments for the transient (= one month since
517 marking) and non-transient class (above one month since marking). Because captures
518 started in March, all individuals captured in April belonged to the transient class, thus
519 estimates for the non-transient class are not available for March.

Class	Period	Control	Cover	Vaccinated	Cover+Vacc
Transient	March-April	0.591 [0.458, 0.712]	0.714 [0.591, 0.812]	0.607 [0.478, 0.724]	0.631 [0.491, 0.752]
	April-May	0.663 [0.523, 0.779]	0.772 [0.643, 0.865]	0.678 [0.534, 0.794]	0.699 [0.551, 0.815]
	May-June	0.620 [0.439, 0.773]	0.738 [0.572, 0.856]	0.636 [0.456, 0.784]	0.659 [0.476, 0.804]
	June-July	0.472 [0.305, 0.644]	0.606 [0.432, 0.757]	0.488 [0.322, 0.657]	0.513 [0.337, 0.686]
	July-August	0.472 [0.140, 0.831]	0.607 [0.220, 0.895]	0.489 [0.148, 0.841]	0.514 [0.160, 0.855]
	August-September	0.062 [0.021, 0.168]	0.102 [0.036, 0.256]	0.066 [0.022, 0.177]	0.072 [0.025, 0.190]
	September-October	0.166 [0.042,	0.255 [0.069,	0.175 [0.045, 0.490]	0.190 [0.050, 0.510]

		0.472]	0.612]		
Non-transient	March-April	n.a.	n.a.	n.a.	n.a.
	April-May	0.915 [0.832, 0.959]	0.949 [0.890, 0.977]	0.920 [0.839, 0.962]	0.927 [0.845, 0.967]
	May-June	0.899 [0.794, 0.954]	0.939 [0.867, 0.973]	0.905 [0.806, 0.956]	0.913 [0.814, 0.962]
	June-July	0.830 [0.653, 0.929]	0.894 [0.755, 0.958]	0.839 [0.661, 0.933]	0.852 [0.673, 0.941]
	July-August	0.830 [0.449, 0.967]	0.894 [0.583, 0.981]	0.839 [0.465, 0.969]	0.852 [0.485, 0.973]
	August-September	0.264 [0.159, 0.404]	0.382 [0.245, 0.541]	0.277 [0.170, 0418]	0.297 [0.182, 0.446]
	September-October	0.520 [0.237, 0.790]	0.651 [0.341, 0.871]	0.537 [0.250, 0.801]	0.561 [0.270, 0.816]

520

521

522 Table 1.3 Juvenile survival model averaged estimates (mean [95 % CI]) for the transient
523 class (= below one month since marking) for both the pre (March - July) and post-
524 outbreak (August-October) periods.

Period	Control	Cover	Vaccinated	Cover+Vacc
Pre-outbreak	0.616 [0.509, 0.713]	0.721 [0.567, 0.836]	0.613 [0.510, 0.708]	0.606 [0.465, 0.731]
Post-outbreak	0.054 [0.018, 0.150]	0.074 [0.025, 0.196]	0.058 [0.020, 0.154]	0.074 [0.023, 0.212]

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528 **Detection probability**

529 Initial goodness-of-fit tests in U-CARE indicated a lack of fit of a Cormack-
530 Jolly-Seber model to the capture histories dataset without covariates (quadratic
531 χ^2=124.815, df=78, P<0.001; overall model dispersion $\hat{c}$=1.600), with strong evidence
532 of transience in juvenile survival [Test3.SR, N(0,1) statistic for transience=6.0244,
533 P<0.0001)] but also some evidence of trap-dependence [Test2.CT, N(0,1) statistic for
534 trap-dependence=-3.435, P<0.001)]. Group-specific tests suggested that while
535 transience was common to all juvenile groups, trap dependence was only limited to one
536 group, and based on few degrees of freedom (df=5). Thus, we fitted *time since marking*
537 model structures [TSM models (Pradel *et al*., 1997)] in juvenile survival in MARK.
538 Specifically, a preliminary model included time varying monthly survival with two
539 TSM classes (i.e. one vs. above one month since marking), and time varying monthly
540 detection probability, but no treatment effects on either survival or detection probability.
541 Goodness-of-fit dispersion tests in MARK indicated that this model fitted the data
542 adequately, as was therefore used as our general starting model. To complement this
543 model, we incorporated plot, warren size and age as additive covariates of both survival
544 and detection probability, and merged the last two detection probability parameters in
545 order to allow estimation of all parameters (model 1 in Table 1).
546 Refinement of detection probability parameterisation indicated a substantial impact of
547 age and plot and no impact of warren size on detection probability (models 2-6, Table
548 1). Grouping detection probability parameters by treatment didn't improve model fit
549 either (models 7-8, Table 1), neither did merging pre-outbreak and post-outbreak time
550 dependent parameters separately to account for infection period (models 7 and 9, Table
551 1; for estimates of detection probability, see Table A1).

552

553 Table 1.4 Monthly juvenile model averaged detection probability estimates (mean [95

554 % CI]) for every capture session.

Capture session	Estimate
April	0.694 [0.584, 0.786]
May	0.484 [0.402, 0.567]
June	0.507 [0.426, 0.588]
July	0.380 [0.312, 0.453]
August	0.279 [0.218, 0.350]
September	0.278 [0.184,0.397]
October	0.278 [0.184, 0.397]

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