Research Article

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Effects of quinestrol and levonorgestrel on populations of plateau pikas, *Ochotona curzoniae*, in the Qinghai-Tibetan Plateau

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Abstract

BACKGROUND: Plateau pikas (*Ochotona curzoniae*, Hodgson, 1858) are viewed as a pest in the Tibetan Plateau meadow ecosystem when their population densities are high. Traditional culling using rodenticides often poses a high risk to non-target species and even to humans. In this study, an investigation was made of the infertility effects of quinestrol (E), levonorgestrel (P) and a combination of the two (EP, ratio E : P = 1 : 2) on plateau pikas during 2007 and 2008.

RESULTS: Treatment with E or EP significantly decreased the pregnancy rate of female pikas in 2007. In 2008, there was a cross-year effect that still suppressed male reproduction in treated groups. Treatment with E obviously reduced the reproduction of pikas but not their population abundance in 2007; the reduction in population size was significant in 2008.

CONCLUSIONS: Single baiting of quinestrol in early breeding season reduced the reproduction and population size of pikas throughout 2007. The effect of infertility lasted into the next breeding season through a cross-year effect, which resulted in a significant reduction of population size in 2008. Quinestrol is a very promising non-lethal approach to managing pika populations; however, several factors need to be investigated further to improve the practicality of this method.

Keywords: fertility control; quinestrol; levonorgestrel; plateau pika; ecologically based rodent management

1 INTRODUCTION

Plateau pikas are small dominant herbivores in the meadow ecosystem of the Qinghai-Tibetan Plateau, China. The average lifespan of a plateau pika is 2–3 years, but a few, usually less than 15%, can live 4–5 years. In the past few decades, the Qinghai-Tibetan Plateau has experienced serious overgrazing from an increasing population of domestic livestock, including sheep and yaks. Initial overgrazing was thought to favour the invasion of plateau pikas, which further accelerated degradation of plateau meadows.[1] Pikas may play an important role in maintaining ecosystem functions as a keystone species for providing food for predators and underground nests for small birds, and in promoting nutrient recycling within alpine ecosystems.[2,3]

Fertility control is considered a non-lethal and sustainable method for managing rodent populations.[4] An effective fertility control method would satisfy the demands of both damage control and ecosystem conservation. Rodenticides, such as botulin toxin C, are widely used to control pika populations in this region. These rodenticides often pose a high risk to non-target animals such as birds, mammal predators, livestock and even to humans. Poisoning is also not a sustainable method for pika control because pika populations recover rapidly.[5,6] Killing is also not welcome by local Tibetan people with their Buddhist philosophy. Fertility control as a non-lethal method is a potential method for managing pika populations. As simulation modelling predicts, fertility control is effective in reducing populations of rodents,[7–9] either directly through unsuccessful mating of infertile males with fertile females or through territorial effects by preventing successful mating by other fertile males.[8] However, supporting field evidence remains unavailable.

Some synthetic hormone compounds have been tested for rodent control.[10] Most of these tests failed because a successful control often needs successive baiting, which is not only expensive but is also difficult to achieve in the field.[9] Recent studies reported

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Table 1. Locations of the experimental plots near Dawu Town, Guoluo County, Qinghai Province

Sampling plots	Longitude	Latitude	Altitude (m)	Treatment
1	100° 21.064′ E	34° 25.342′ N	3893	Quinestrol
2	100° 21.953′ E	34° 24.828′ N	3908	Control
3	100° 23.078′ E	34° 24.372′ N	3909	Mixture
4	100° 23.808′ E	34° 24.111′ N	3937	Levonorgestrel
5	100° 24.611′ E	34° 24.112′ N	3968	Mixture
6	100° 25.524′ E	34° 23.841′ N	3972	Quinestrol
7	100° 25.898′ E	34° 23.527′ N	3915	Control
8	100° 26.658′ E	34° 22.063′ N	3897	Levonorgestrel
9	100° 20.480′ E	34° 25.674′ N	3863	Levonorgestrel
10	100° 20.264′ E	34° 25.382′ N	3841	Control
11	100° 19.253′ E	34° 25.922′ N	3789	Levonorgestrel
12	100° 18.527′ E	34° 26.238′ N	3785	Quinestrol
13	100° 17.847′ E	34° 26.456′ N	3764	Mixture
14	100° 16.936′ E	34° 26.930′ N	3756	Mixture
15	100° 12.270′ E	34° 28.389′ N	3757	Control
16	100° 13.040′ E	34° 28.737′ N	3737	Quinestrol

that the mixture of two compounds, quinestrol and levonorgestrel (coded as EP, in a ratio of 1 : 2) could be a promising alternative for rodent fertility control.[11–17] The compounds have been commercially used in contraceptive pills for women.[18] Both laboratory and field experiments have confirmed the antifertility effects of EP and its individual components, levonorgestrel (synthetic progesterone, P) or quinestrol (synthetic oestradiol, E) on several wild rodent species, for example Brandt's voles (*Lasiopodomys brandti*),[14,17] grey hamsters (*Cricetulus migratorius*), midday gerbils (*Meriones meridianus*),[14] greater long-tailed hamsters (*Tscherskia triton*),[15,16] Djungarian hamsters (*Phodopus campbelli*)[13] and Mongolia gerbils (*Meriones unguiculatus*).[11,12] Antifertility effects of EP and its separate compounds on reproduction and populations of small mammals have not yet been investigated under field conditions.

The purpose of this study was to assess the antifertility effect of P, E and EP on the fertility of plateau pikas in the field with a single baiting during the early breeding season. The study was conducted over 2 years in 2007 and 2008, and aimed to examine the cross-year effects of P, E and EP on pika fertility and population abundance. This research aimed to establish a non-lethal method for reducing animal damage and conserving biodiversity in the Qinghai-Tibetan Plateau.

2 EXPERIMENTAL METHODS

2.1 Experimental design

Sixteen plots with an area of 1 km^2 were selected along a roadside to the east and south of Dawu Town, Maqin County, Qinghai Province (Table 1). The distance between plots was at least 500 m to minimise potential effects of pika dispersal among plots. The sixteen plots were randomly divided into four groups: quinestrol group (E) treated with baits containing quinestrol (0.005%); levonorgestrel group (P) treated with baits containing levonorgestrel (0.005%); mixture group (EP) treated with baits containing a mixture of quinestrol and levonorgestrel (ratio 1 : 2, 0.005%); control group treated with baits containing no quinestrol or levonorgestrel. The concentrations of E, P and EP were determined on the basis of previous studies of many other rodent species.[11–17]

One of the four plots in each treatment group was used to study physiological and morphological changes of the reproductive organs by sampling male and female pikas (removal sampling plot); the other three plots were used to monitor the changes in population abundance by visually counting pikas along 20 × 100 m line transects (visual counting plots). Sampling and surveys were conducted in late April (5 days after bait delivery finished), early and late May, early and late June, late July and late August in 2007, and in late April, early and late May, late June, late July and late August in 2008.

2.2 Baiting protocol

Each compound was dissolved in alcohol, diluted in water and mixed with plain oats to produce baits containing either quinestrol (0.005%) or levonorgestrel (0.005%), or a mixture of quinestrol and levonorgestrel (ratio 1 : 2, 0.005%).[19] Control baits contained no quinestrol or levonorgestrel. Sugar (2%) was added to the baits to improve palatability. Baits were dried and stored for later experiments.

Baits were delivered by hand to each study plot once only in late April 2007 when pikas normally begin to breed.[20] Operators followed parallel line transects, approximately 20 m apart, across each study plot, and baits of approximately 5 g were placed next to all the burrow entrances. In each plot of 1 km^2, approximately 300 kg of baits was delivered. To measure the rate of bait consumption or removal by pikas, six small quadrants (10 m × 10 m) were selected and monitored in the centre of plots 1, 2, 3 and 4. Bait consumption was surveyed on days 1, 3 and 6 after bait delivery.

2.3 Physiological changes

2.3.1 Reproductive organs

Male and female adult pikas were captured by string nooses[21] from the removal sampling plots (plots 1, 2, 3 and 9). The captured pikas were euthanised and dissected in order to measure physiological and morphological changes in reproductive organs. Reproductive organs, including each of the testes, left cauda epididymis and seminiferous vesicles of adult male pikas were collected and weighed immediately. The number of embryos in the uteri of female pikas was counted to calculate the pregnancy rate and litter size, and the weight of each ovary was measured separately. At least 15 adult males and 15 adult females were collected from each removal sampling plot at each sampling time from late April to early June in both 2007 and 2008. In July and August, when pikas stopped breeding, the sampling size was reduced to five adult males and five females. The pregnancy rate was defined as: number of pregnant females/total number of adult females. The litter size was defined as: number of embryos/number of pregnant females.

2.3.2 Sperm concentration

The cauda epididymides were cut into small pieces and placed in 10 mL Ringer–Lock fluid (9 g NaCl, 4.2 g KCl, 0.5 g Na_2CO_3 and 0.24 g $CaCl_2$ dissolved in 1000 mL water) to determine sperm density. The fluid was shaken gently before use, and the sperm number was counted using a hemocytometer.

Table 2. Differences in bait consumption between control (Con) and treatment groups (E, EP, P) in the field within the first 6 days after bait delivery

	Summary of baiting ratio						
	Means (mean ± SEM)				*P*-values		
Time	Control group	E group	P group	EP group	Con versus E	Con versus P	Con versus EP
Day 1	56 ± 12.1%	50 ± 6.1%	38 ± 4.6%	26 ± 4.3%	0.969	0.553	0.208
Day 3	75 ± 7.7%	72 ± 7.5%	46 ± 5.6%	52 ± 6.4%	0.995	0.07	0.19
Day 6	91 ± 2.9%	91 ± 0.5%	88 ± 4.6%	93 ± 0.9%	>0.999	0.921	0.95

2.3.3 *Hematoxylin and eosin (H& E) stain*

Pika testes were fixed in 10% neutral buffered formalin immediately for 24 h. A small incision was made in the testes from the breeding seasons (>1 g) in order for the fixative to infuse into the tissue. No incision was made for testes of non-breeding seasons because they were very small (<0.2 g). The fixed tissues were then gradually dehydrated in ethanol and embedded in paraffin. Sections of 6 μm thickness were collected on polylysine embedded slides. The sections were deparaffinised, rehydrated and then rinsed in Mayer's hematoxylin staining buffer for 5 min. After a water wash, the sections were briefly treated with 1% hydrochloric acid (in 75% alcohol), followed by 0.1% ammonia, and dehydrated to 95% alcohol. The slides were counterstained with 0.05% eosin and subjected to dehydration and mounting in neutral balsam. The slides were observed under light microscope with a charge-coupled device (CCD; Spot, USA).

2.4 Serum testosterone levels

Serum testosterone levels were measured by specific RIA in Beijing Northern Biotechnology Company using their kit (S10940093). Testosterone levels of serum were measured without extraction. Briefly, 100 μL of rabbit antitestosterone and 100 μL of ^{125}I-testosterone were mixed together with 50 μL of pika serum. The mixture was incubated at 37 °C for 1 h and further mixed with 500 μL of donkey antirabbit IgG. After incubation at room temperature for 15 min, the total mixture was centrifuged at 3500 r min^{-1} for 15 min. The supernatant was carefully discarded, and the result was read from a γ-counter. The standard curve ranged from 0.05 to 20 ng mL^{-1}.

2.5 Population abundance

Line transects were used to estimate the population abundance of plateau pikas following the method of Pech *et al.*[6] Each transect was 100 m long and 20 m wide. In each visual counting plot, 20 line transects were selected to cover the area of the plot. Two teams scanned and counted ten transects independently at the same time in the same plot to correct for errors due to different observers. It took 3–4 days to finish the survey of the 12 plots for each time. All surveys were conducted between 8:30 am and 12:30 am, when pikas were active and easily observed. Surveys were delayed on rainy days. The data from the line transects were used to calculate the population density in each plot, and the population densities of the three plots of each group were used to calculate the average population density of each group.

2.6 Statistics

For data showing non-normal distribution [i.e. relative weight of reproductive organs (g g^{-1}), sperm density, pregnancy rate and litter size], the Kruskal–Wallis test was used to analyse whether significant differences existed for each time point. If a significant difference existed, the Mann–Whitney *U*-test was employed to analyse the significant differences in variables between controls and each treated group for this time point. For ovary weight analysis, data of absolute weight (g) were used. For data showing a normal distribution, the least significant differences (LSD) method was used as the *post hoc* analysis of one-way ANOVA to test the significant differences of variables among control and treatment groups. All statistical analysis was conducted using the SPSS (v.17.0) software package.

3 RESULTS

3.1 Bait consumption

Bait consumption in both control and treated groups was measured for six successive days after bait delivery in the field. By day 6, about 90% of baits were consumed by pikas. There was no significant difference in bait consumption between control and treatment groups (Table 2).

3.2 Testis weight

The testis weight of group E was significantly lower than that of the control group in late April ($P = 0.02$), early May ($P < 0.001$), late May ($P < 0.001$) and early June ($P < 0.001$) of 2007 (Fig. 1A). The testis weight of group EP was significantly lower than that of the control group in late April ($P = 0.016$), early May ($P < 0.001$), late May ($P < 0.001$) and early June ($P = 0.001$). The testis weight of group P was significantly lower than that of the control group in early May ($P = 0.006$).

The testis weight of group E was significantly higher than that of the control group in late April ($P = 0.008$), early May ($P = 0.001$) and late June ($P = 0.01$) of 2008. The testis weight of group EP was significantly higher than that of the control group in late April ($P < 0.001$) and late June ($P < 0.001$). The testis weight of group P was significantly higher than that of the control group only in late April ($P = 0.02$).

3.3 Cauda epididymis weight

The cauda epididymis weight of group E was significantly lower than that of the control group in late April ($P < 0.001$), early May ($P < 0.001$), late May ($P < 0.001$), early June ($P < 0.001$) and late June ($P < 0.001$) of 2007 (Fig. 1B). The cauda epididymis weight of group EP was significantly lower than that of the control group in early May ($P < 0.001$), late May ($P < 0.001$), early June ($P < 0.001$) and late June ($P = 0.017$). There was no significant difference between the control and group P.

The cauda epididymis weight of group E was significantly higher than that of the control group in late June ($P = 0.018$) of 2008.

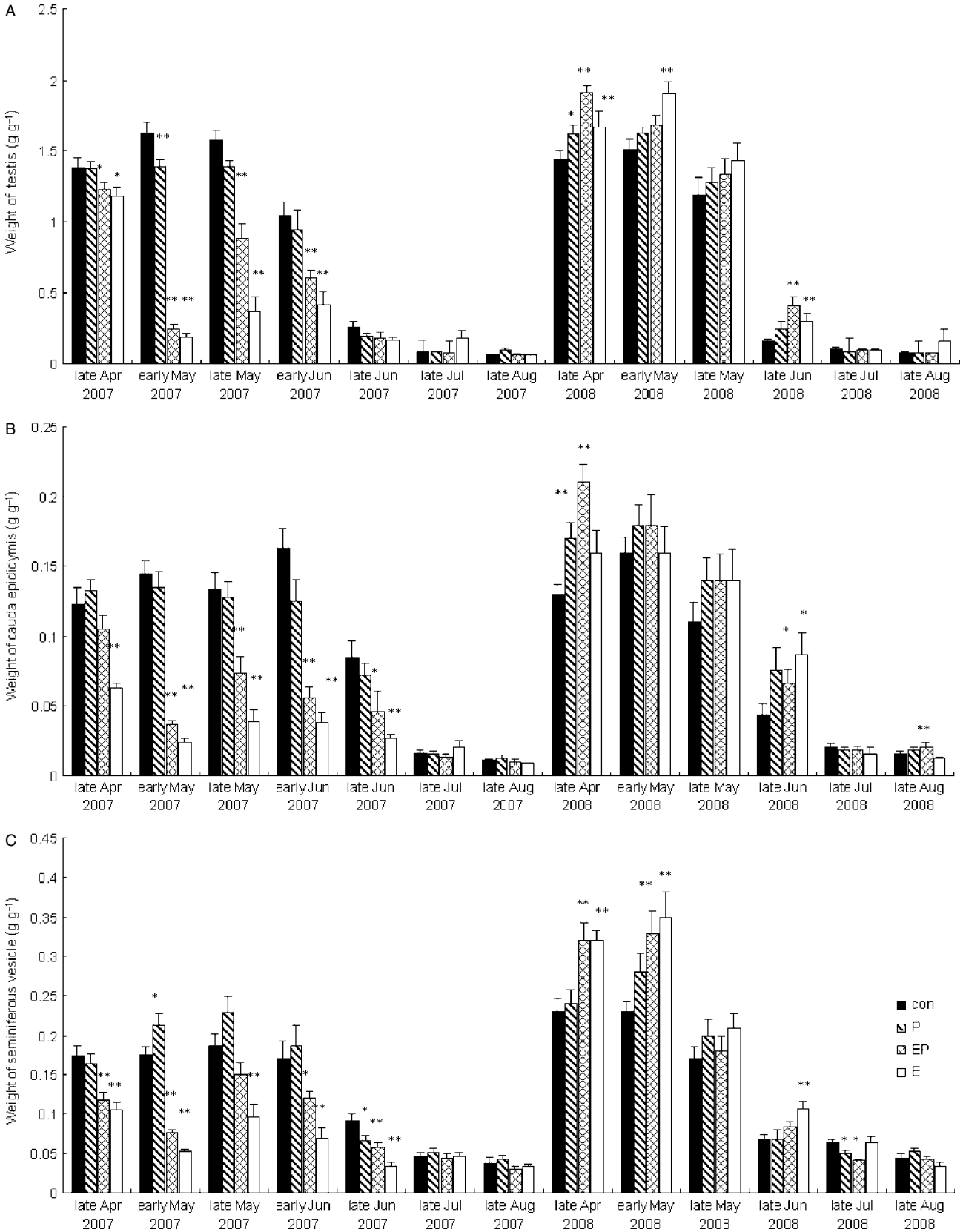

Figure 1. Differences in weight (g g^{-1}) of testes, cauda epididymis and seminiferous vesicle (mean + SEM) between control (Con) and treatment groups (P, EP, E) in 2007 and 2008. A: testes; B: cauda epididymis; C: seminiferous vesicle. $n = 15$ for each sampling point except late July and late August ($n = 5$) in both 2007 and 2008. *: $P < 0.05$; **: $P < 0.01$.

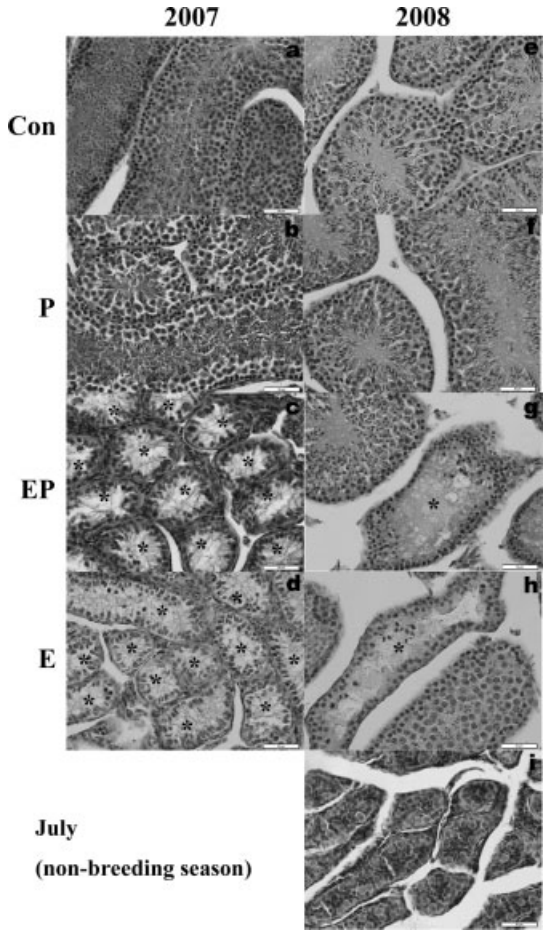

Figure 2. Morphological changes in the testes of male adult pikas in late April of 2007 and 2008: (a) control group in 2007; (b) group P in 2007; (c) group EP in 2007; (d) group E in 2007; (e) control group in 2008; (f) group P in 2008; (g) group EP in 2008; (h) group E in 2008; (i) in non-breeding season (late July). * denotes damaged seminiferous tubules. Bar = 50 μM. Sections (6 μM) stained with H& E.

The cauda epididymis weight of group EP was significantly higher than that of the control group in late April ($P < 0.001$), late June ($P = 0.04$) and late August ($P = 0.005$). The cauda epididymis weight of group P was significantly higher than that of the control group in late April ($P = 0.006$).

3.4 Seminiferous vesicle weight

The seminiferous vesicle weight of group E was very significant lower than that of the control group in late April ($P < 0.001$), early May ($P < 0.001$), late May ($P < 0.001$), early June ($P < 0.001$) and late June ($P < 0.001$) of 2007 (Fig. 1C). The seminiferous vesicle weight of group EP was significantly lower than that of the control group in late April ($P = 0.001$), early May ($P < 0.001$), early June ($P = 0.041$) and late June ($P = 0.003$). The seminiferous vesicle weight of group P was significantly lower than that of the control group in late June ($P = 0.022$), while in early May the seminiferous vesicle weight of group P was significantly higher than that of the control group ($P = 0.03$).

The seminiferous vesicle weight of group E was significantly higher than that of the control group in late April ($P < 0.001$), early May ($P < 0.001$) and late June ($P = 0.008$) of 2008. The seminiferous vesicle weight of group EP was significantly higher than that of the control group in late April ($P = 0.001$) and early May ($P = 0.009$), while in late July the seminiferous vesicle weight of group EP was significantly lower than that of the control group ($P = 0.022$). The seminiferous vesicle weight of group P was significantly lower than that of the control group in late July ($P = 0.016$).

3.5 The structure of seminiferous tubules

By late April of 2007, 2 weeks after bait delivery, spermatogenesis was obviously inhibited in male adults from groups E and EP (Fig. 2). The diameter of seminiferous tubules in groups E and EP were much smaller than in the control group. Germ cells, including elongating spermatids, elongated spermatids, round spermatids and even pachytene spermatocytes, disappeared in tubules, with only sertoli cells present in seminiferous tubules (Figs 2C and D). It is notable that, by late April of 2008, although the testis weight of male adults in groups E and EP increased, spermatogenesis in seminiferous tubules was still disrupted; some tubules displayed serious damage. There were no spermatids in the damaged tubules which had clearly lost the ability to perform spermatogenesis (Figs 2G and H).

3.6 Sperm concentration

The sperm concentration of group E was significantly lower than that of the control group in early May ($P < 0.001$), late May ($P < 0.001$) and early June ($P < 0.001$) of 2007 (Fig. 3A). The sperm concentration of group EP was significantly lower than that of the control group in early May ($P < 0.001$), late May ($P < 0.001$) and early June ($P = 0.001$). The sperm concentration of group P was significantly lower than that of the control group in early June ($P < 0.001$).

The sperm concentration of group E was significantly lower than that of the control group in late April ($P = 0.007$) and in early May ($P = 0.029$) of 2008. The sperm concentration of group P was significantly lower than that of the control group in late April ($P = 0.028$). There was no significant difference in sperm concentration between group EP and the control group.

3.7 Ovary weight

The ovary weight of group E was significantly higher than that of the control group in late April ($P < 0.001$) and early May ($P = 0.002$), but significantly lower in early June ($P = 0.001$) of 2007 (Fig. 3B). The ovary weight of group EP was significantly higher than that of the control group in late April ($P = 0.001$) and early May ($P = 0.004$).

The ovary weight of group E was significantly higher than that of the control group in late April ($P = 0.039$) and late May ($P = 0.001$) of 2008. Ovary weight was significantly higher in group EP than in the control group in late May ($P < 0.001$). There was no significant difference in ovary weight between group P and the control group.

3.8 Pregnancy rate and litter size

The average pregnancy rate of adult females in group P was significantly higher than that of the control group in late April ($P = 0.042$) and late May ($P = 0.009$) of 2007 (Fig. 3C). It is notable that there were no pregnant females found in late April or early May in group E, nor in early May in group EP ($P < 0.001$). In late May, the pregnancy rate was significantly lower in group E ($P = 0.001$) and group EP ($P = 0.006$). There was no significant difference in adult female pregnancy rate in 2008. There were no

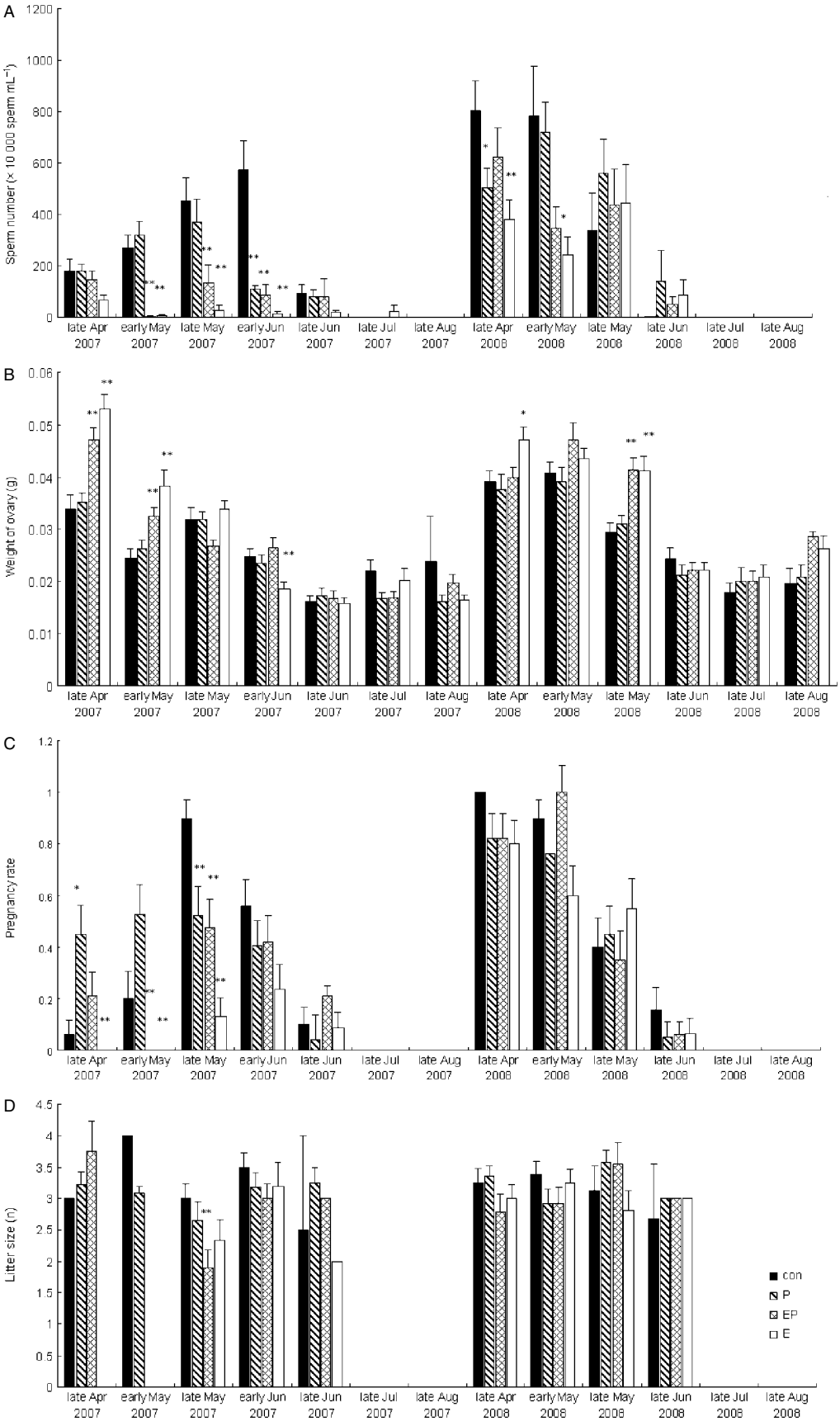

Figure 3. Differences in sperm concentration, ovary weight (g), pregnancy rate and litter size (mean + SEM) between control (Con) and treatment groups (P, EP, E) in 2007 and 2008. A: sperm concentration; B: ovary weight; C: pregnancy rate; D: litter size. $n = 15$ for each sampling point except late July and late August, $n = 5$ in both 2007 and 2008. *: $P < 0.05$; **: $P < 0.01$.

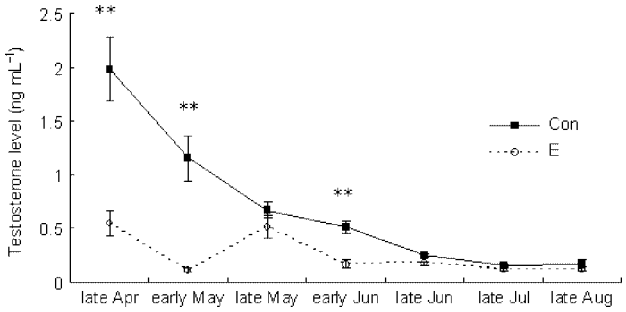

Figure 4. Serum testosterone levels in control and E groups in 2007 ($n = 15$ except for $n = 5$ for late July and late August). **: $P < 0.01$.

clear changes in litter size (non-pregnant pikas were excluded) observed, except for the litter size of group EP compared with that of the control group in late May ($P = 0.008$) (Fig. 3D).

3.9 Serum testosterone levels

During breeding seasons, the serum testosterone levels of male adult pikas in group E were significantly lower than those of the control group in late April, early May and early June (all $P < 0.001$) (Fig. 4). The serum testosterone levels of male adult pikas in both group E and the control group declined after late April. They were very low in non-breeding seasons, and the difference in serum testosterone levels between group E and the control group became non-significant.

3.10 Population abundance

There was no significant difference in population abundance between control and treatment groups in 2007. In 2008, the population abundance of groups P and EP was significantly lower than that of the control group in late June ($P = 0.041$, $P = 0.047$). The population abundance of group E was also significantly lower than that of the control group in late June ($P = 0.002$) and late July ($P = 0.033$). In general, the population density of group E was lower than that of the control group from early May 2007 onwards, and the population increase in early 2008 was noticeably reduced in group E (Fig. 5A). No significant differences were found in the rate of population increase between control and treatment groups in 2007 and 2008 (Fig. 5B).

4 DISCUSSION

The present results demonstrated that, after a single quinestrol (E)-containing bait delivery in the early breeding season, sperm concentrations of male pikas significantly decreased in both the current and the following year. The weight of male pika reproductive organs (testes, cauda epididymis and seminiferous vesicle) also significantly decreased in the current year, but increased in the following year. E significantly increased ovary weight of female pikas in both the current and following years, but it significantly reduced reproduction of female pikas (especially the pregnancy rate) in the current year. E significantly decreased population abundance in the current year, but not in the following year.

It has been suggested that oestrogen can cause negative feedback through the suppression of the hypothalamo-pituitary-gonad (H-P-G) axis,[22,23] which can result in low levels of testosterone and spermatogenesis failure.[24] It was found that E and EP (the mixture of quinestrol and levonorgestrel) significantly reduced the weight of reproductive organs in male pikas in the current year: spermatogenesis in male pikas was inhibited in groups E and EP, and serum testosterone levels of adult male pikas in group E were also significantly lower than in the control group. These results suggest that male pika infertility was achieved through suppression of the H-P-G axis mainly by quinestrol. Differences in population abundance for most months did not reach statistical significance because there were only three plots.

Progesterone has been used together with testosterone in the development of a new contraceptive for men.[25] In this study, a less predominant infertility effect of P (levonorgestrel) on male pikas by comparison with E was found in early June in both 2007 and 2008. P may have both positive and negative effects in females in the current year. The results suggest that P cannot reach a satisfying infertility effect in the current year, so single E or EP should be a better choice for the population control of pikas.

It is notable that a single baiting of E or EP in early 2007 caused lower sperm concentrations in adult male pikas the following year, but heavier (larger) reproductive organs. The enlargement of reproductive organs was probably a compensation effect induced by the extremely low sperm concentration in the first year. It was also found that nearly half of these male pikas were newborn in 2007, suggesting that a cross-year infertility effect was caused by female pikas from groups E or EP. Previous studies have shown that infant albino rats also have reproductive problems if they are exposed to oestrogen.[26–28] In groups E and EP, some females were found that bred in late May and early June of 2007, probably owing to the recovery of reproduction in male pikas after bait delivery in late April (Fig. 3). It is likely that spermatogenesis in offspring of female pikas treated with E or EP could be reduced. The maternal infertility effect of E or P through female rodents needs to be further investigated in future studies. The cross-year infertility effect might have resulted in lower population abundance in group E in 2008 after one single-baiting in April 2007. Thus, E may be a potential compound for sustainable control of pika populations.

In mice and rats, oestrogen can enhance follicle development,[29] while progesterone is essential for promoting embryo implantation.[30] Synthetic progesterone and oestrogen in female contraceptives are used at a dose that is usually high enough to suppress follicle-stimulating hormone (FSH) secretion and block ovulation. If the dosage is not high enough, the extra synthetic hormone intake may have positively affected female reproduction. In this study it was found that E (of both group E and group EP) showed significant positive effects on the weight of female reproductive organs (ovaries) in the current year, supporting the previous observations. However, E showed a significant negative effect on reproduction of female pikas (especially the pregnancy rate), which was caused by reproductive failure in male pikas treated with E or EP. The present behavioural studies showed that infertile male pikas significantly increase their territorial behaviour, preventing mating of fertile males with their females (Liu M *et al.*, submitted). Additionally, oestrogen can cause abortions in pregnant females.[31,32] In this study, E baits were delivered in late April when some female pikas could have been pregnant.[20] Quinestrol (E) might contribute to the very low (or even zero) pregnancy rate of female pikas through abortion of pregnant females in late April.

Although E significantly decreased pika reproduction in the current year after one single baiting in spring, the reduction in population abundance was not so obvious. Infertile males and females might have higher survival rates that compensated for reduced recruitment of juveniles. The climate was wetter in 2008

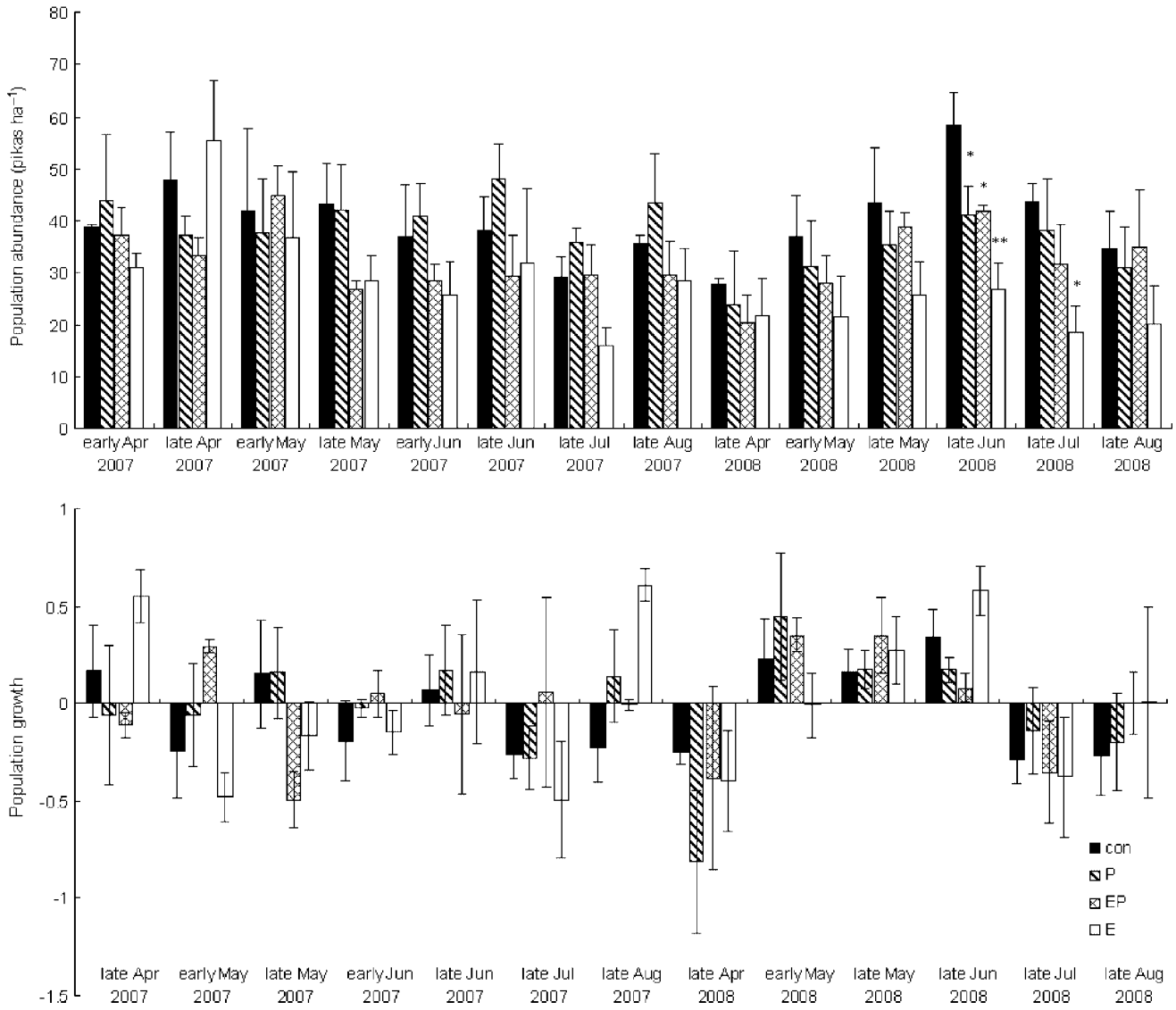

Figure 5. Differences in population abundance (pikas ha^{-1}, mean + SEM) and population growth rate (mean ± SEM) between control (Con) and treatment groups (P, EP, E) in 2007 and 2008. A: population abundance; B: population growth rate. Early April in 2007 indicates the population abundance before the baits were laid. Other time points indicate the population abundance after the baits were laid. *: $P < 0.05$; **: $P < 0.01$.

than in 2007, so the reproduction of both male and female pikas was higher in 2008 than in 2007 (Figs 1 and 3, and see below). The difference in population abundance between control and treatment groups became larger and more significant. It was difficult to detect significant differences in population abundance if the differences were not large because there were only three replicates for each treatment and control group and the variation among replicates was often large.

The overall reproduction rate of female pikas in 2007 was higher than in 2008. This was likely caused by climatic differences between these two years. Previous studies have shown that abundant precipitation increases the population growth of small rodents in many ecosystems.[33–38] The grass turned green much earlier in 2008 than in 2007 (Liu M, personal observation), which contributed to greater reproduction rates in 2008 than in 2007 (Figs 1 and 3).

As pikas are essential in maintaining the alpine meadow ecosystem of the Qinghai-Tibet Plateau, it is not necessary to eradicate all pikas. Fertility control using E or EP can satisfy damage management and biodiversity conservation needs in this region. The infertility effect was less obvious in group EP than in group E. This is likely due to the lower concentration of E in group EP than in group E. Elevating the concentration of E and/or the amount of bait delivery should be effective at increasing infertility, but, to avoid pollution, it is better to keep the E content and amount of bait at an effective low level. A satisfying result was that the main birth wave in May was completely inhibited, effectively decreasing the population size. The secondary birth wave in June was partially weakened to ensure a basic population density and biodiversity. In this study near Dawu Town, the single provision of a 3 day food supply of 0.005% quinestrol-containing baits achieved target infertility levels (over 90% of the male pikas exhibited severe testis degeneration), while in other lower-altitude places the content of E and/or the amount of bait should be increased 50–100% to ensure adequate infertility.

Environmental oestrogen has been of great concern to human health.[39–41] Unlike many persistent synthetic oestrogen

pollutants, quinestrol decomposes very quickly.[42,43] Preliminary tests showed that this compound quickly decomposed and was less of a threat to birds in their natural environment (Qu J *et al*., unpublished).

A number of factors need to be investigated further before the use of quinestrol can be seriously considered as a practical control technique. These factors should include: (1) whether a reduction in recruitment is offset by increased survival; (2) whether population abundance can be reduced significantly; (3) how initial density affects the results of fertility control; (4) optimising the amount of bait material and the method of distribution required for effective control; (5) providing a satisfactory explanation for the apparent cross-year effect into the second year.

The present study demonstrates that quinestrol is an effective compound for reducing male pika fertility. The infertility can last into the next breeding season through cross-year effects. Quinestrol has the potential to be a male sterilisation compound used to control the plateau pika population in the Qinghai-Tibetan Plateau. It is likely a potential alternative to lethal management of small mammals.

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