BRILL

Behaviour 149 (2012) 369–390

Behaviour
brill.nl/beh

Territorial defence behaviour in red squirrels is influenced by local density

Julia Shonfield [a], **Ryan W. Taylor** [b], **Stan Boutin** [c], **Murray M. Humphries** [d] **and Andrew G. McAdam** [a,*]

[a] Department of Integrative Biology, University of Guelph, Guelph, ON, Canada
[b] Department of Zoology, Michigan State University, East Lansing, MI, USA
[c] Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada
[d] Department of Natural Resource Sciences, Macdonald Campus, McGill University, Ste-Anne-de-Bellevue, QC, Canada
*Corresponding author's e-mail address: amcadam@uoguelph.ca

Accepted 18 March 2012

Abstract

While many animals defend territories to secure resources such as food and mates, little is known about why territory owners of the same species vary in their territorial defence behaviour. We tested whether potential intruder pressure, defence of offspring, resource-holding potential or aggressiveness of the individual territory owner best explained intraspecific differences in territorial defence in a wild population of North American red squirrels (*Tamiasciurus hudsonicus*). We assessed territorial defence behaviour of individual red squirrels by recording whether or not they produced territorial vocalizations, known as rattles, both in response to a territorial playback and during natural observation sessions without an experimental stimulus. We compared the relative fit of four a priori models to explain territorial defence intensity in red squirrels and found that rattling behaviour in red squirrels under natural conditions was best explained by the intruder pressure hypothesis. Red squirrels were more likely to vocalize if they were surrounded by a higher density of conspecifics on neighbouring territories, indicating that they adjust territorial defence in response to potential intruder pressure. However, vocalization responses of red squirrels to the playback were not affected by local density, which was reflected in similar support for the four a priori models. The differing effects of local density on red squirrel vocalization rate during natural observations and following playbacks indicates that the effects of local density on the territorial behaviour of red squirrels depends on the particular context in which this behaviour is expressed.

Keywords

aggressiveness, intruder pressure, offspring defence, playback, resource-holding potential, vocal communication.

DOI:10.1163/156853912X637842

1. Introduction

Territoriality involves the behavioural exclusion of other conspecifics from an area and is exhibited across a broad range of animal taxa (reviewed in Maher & Lott, 1995). There has been a strong interest in understanding why animals defend territories (Brown, 1964; Gill & Wolf, 1975), and many studies have sought to explain the occurrence of territoriality by examining the costs and benefits of territorial defence compared to the alternative of not defending a territory (reviewed in Maher & Lott, 2000). Despite recent evidence documenting intraspecific variation in the intensity of territorial defence in both songbirds and fish species (Johnsson et al., 2000; Nowicki et al., 2002; Hyman et al., 2004; Kleiber et al., 2007), little is known about why individual territory owners of the same species vary in their investment in territorial defence.

Several hypotheses may explain intraspecific differences in territorial defence, including intruder pressure (Hyman et al., 2004), offspring defence (Wolff, 1993; Wolff & Peterson, 1998), resource-holding potential (Parker, 1974) and consistent individual differences in behaviour. These hypotheses are not mutually exclusive and range from more proximate to ultimate explanations for variation in territorial defence behaviour. The intruder pressure hypothesis proposes that local density affects an individual's territorial defence because of the increased effort required to retain resources when intruder pressure is high. Intruder pressure, frequently quantified as local density, has predominantly been examined for its effect on territory size (Myers et al., 1979; Norton et al., 1982; Norman & Jones, 1984; Keeley & McPhail, 1998). Few studies have examined the effect of intruder pressure on the intensity of territorial defence behaviour (but see Hyman et al., 2004).

The offspring-defence hypothesis proposes that territorial defence is due to the presence of offspring to protect them from infanticide (Wolff, 1993; Wolff & Peterson, 1998). This hypothesis was originally proposed to explain why only females of some small mammals are territorial, and has not yet been tested to explain variation in territorial defence within a species. There is evidence, however, to suggest that aggressiveness of females with offspring is associated with the threat of infanticide (e.g., Balfour, 1983; Hoogland, 1985; Wolff, 1985), suggesting that territorial defence behaviour may be affected by the presence of offspring.

The resource-holding potential (RHP) hypothesis proposes that an animal's ability to retain possession of a resource (Parker, 1974) is reflected in the intensity with which they defend their territory, such that individuals with greater RHP defend more vigorously. RHP has predominantly been studied from the perspective of attempting to predict the winner of territorial contests (e.g., Gherardi, 2006; Humphries et al., 2006). Few studies have looked directly at the effects of RHP on defence behaviour, although a study on male song sparrows, *Melospiza melodia*, used age as a measure of RHP and found it to be an important factor influencing territorial defence behaviour (Hyman et al., 2004). The RHP hypothesis could provide an ultimate explanation for the evolution of territorial signaling, since retaining a territory can be crucial for survival (e.g., Larsen & Boutin, 1994) and reproductive success (e.g., Smith & Arcese, 1989).

Behavioural differences in individuals that are consistent across time and contexts are often referred to as animal personalities (Réale et al., 2010). Recent studies of animal behaviour have shown that consistent individual differences in aggression and boldness often covary (Tulley & Huntingford, 1988; Koolhaas et al., 1999; Boon et al., 2007; Careau et al., 2010). We hypothesized that territorial defence may be a part of this aggressive-boldness behavioural syndrome. This hypothesis differs from the others in that it proposes a proximate cause underlying territorial defence behaviour. The only study we are aware of that has tested whether territorial defence is part of a behavioural syndrome is the recent study by Amy et al. (2010) that found exploration was correlated with territorial responses of individual great tits, *Parus major*, to simulated intruders.

A comprehensive assessment of the relative importance of these four hypotheses to explain territorial defence behaviour has not been done in previous studies. The potential causes of differences in territory defence are not necessarily mutually exclusive and assessment of the importance of each hypothesis individually could be complicated by correlations among variables important to each hypothesis (e.g., individuals with more aggressive personalities might have higher resource-holding potential and settle in areas with high social density). In the absence of being able to individually manipulate these variables, considering the relative contributions of multiple hypotheses within the same analysis is the best way to avoid misassigning causation and to fully understand what factors affect territorial defence behaviour.

We tested the four hypotheses outlined above to explain territorial defence behaviour in a wild population of North American red squirrels (*Tamiasciurus hudsonicus*). Red squirrels of both sexes defend exclusive territories throughout the year to secure food resources using highly conspicuous territorial vocalizations (Smith, 1968). These vocalizations, known as rattles, are used to establish territories and alert other conspecifics to a territory owner's presence on a territory, as well as to expel intruders (Smith, 1978; Lair, 1990). Although territory intrusions leading to physical interactions with other red squirrels occur infrequently (Gorrell et al., 2010; Dantzer et al., in press), red squirrels use territorial vocalizations regularly to maintain territory boundaries (Lair, 1990). Neighbouring squirrels will quickly intrude when an owner is temporarily removed (Donald & Boutin, 2011), their absence presumably detected from the lack of rattles coming from the territory. We tested the four proposed hypotheses by observing red squirrel territorial defence during natural observations (without any experimental stimulus) and in response to a territorial vocalization playback. This was done to test the hypotheses in two contexts: under natural conditions in the absence of any threat, and in the event of a simulated territory dispute. The playbacks were designed to simulate a squirrel intruding onto and attempting to claim a territory to observe the defence behaviour of the owner (the focal squirrel) in response to this intrusion.

We made the following predictions for each hypothesis. If territorial defence behaviour was affected by intruder pressure in red squirrels, then we predicted that individuals in an area of higher local density would be more likely to rattle than individuals in an area of lower local density. If territorial defence was affected by offspring defence, then we predicted that female red squirrels with offspring on their territory would be more likely to rattle than males or females without offspring. We used both body mass and age of individual red squirrels as measures of their resource-holding potential (RHP). Body mass is the most commonly used measure of RHP (reviewed in Kelly, 2008) and has been found to be an important predictor of territorial defence in other mammals (e.g., striped mice, *Rhabdomys pumilio*, Schradin, 2004). We included body mass as a measure of RHP in red squirrels and predicted that larger squirrels would be more likely to rattle. Age has previously been found to affect territorial defence behaviour (in song sparrows, Hyman et al., 2004), and in some cases older individuals have showed signs of senescence in their territory defence (Arcese, 1987). Red squirrels exhibit senescence in survival and reproductive traits (McAdam et al., 2007; Descamps et al., 2008), so they might also show senescence in their territorial defence. We, therefore, predicted that the probability of rattling would increase with age for younger squirrels but decrease with age in older squirrels. Finally, if territorial defence was related to inherent individual differences in aggressiveness in red squirrels, then we predicted that squirrels with higher aggression scores from standardized behavioural trials would be more likely to rattle than less aggressive individuals.

2. Methods

2.1. Study site and subjects

Red squirrels were studied on three sites in the southwestern Yukon (61°N, 138°W). The sites (approximately 40 ha each) have similar habitat, open boreal forest with white spruce, *Picea glauca*, as the dominant tree species (LaMontagne & Boutin, 2007). Red squirrels cache white spruce cones on their territory each fall in underground larder hoards known as middens (Fletcher et al., 2010), these defended food resources are essential for their survival (Larsen & Boutin, 1994; Larivée et al., 2010). All individual red squirrels in the population were enumerated with numbered metal ear tags (Monel #1, National Tag and Band) and coloured wires were threaded through the ear tags to allow for visual identification at a distance. Female reproductive status was monitored by individually targeted live-trapping using Tomahawk live traps baited with peanut butter (Tomahawk Live Trap, Tomahawk, WI, USA), and parturition dates were determined based on the timing of mass loss and the initiation of female lactation as well as the size of pups during an original nest entry (McAdam et al., 2007). Offspring were ear-tagged at 25 days of age while still in the nest. The study areas were staked at 30-m intervals, which provided spatial coordinates for behavioural observations in the field.

2.2. Field protocol

We conducted focal observations of individual squirrels and in-field playbacks between 700 and 1200 h from 24 May to 26 July 2009 to quantify territorial defence behaviour under natural conditions (no playback) and in response to a simulated territory intrusion (rattle playback). Squirrels were located on their territories by sight, sound or radio telemetry. Telemetry was used to locate squirrels fitted with radio collars (model PD-2C, 4 g, Holohil Systems, Carp, ON, Canada) for data collection not related to this particular study. Ownership of territories was determined by observations of territorial behaviour (i.e., rattling) and extensive live-trapping (Larsen & Boutin, 1994). The unique coloured markings on each squirrel's ear tags allowed for positive identification of the territory owner before the start of data collection. All natural observations and playback trials were completed by a single observer (J.S.). During natural observations individual squirrels were observed at a distance of approximately 10 m for three minutes. All occurrences of the squirrel rattling were counted during the three-minute period as a measure of territorial defence (Altmann, 1974). The behaviour of the squirrel was also recorded every 30 s (instantaneous sampling, Altmann, 1974), categorized as feeding, foraging, traveling, grooming, resting, being vigilant or in a nest.

Playbacks are frequently used to quantify territorial defence by observing an animal's response to a playback of a territorial vocalization (e.g., den Hartog et al., 2008; Amy et al., 2010). We measured red squirrel territorial defence behaviour in response to a rattle playback during a three-minute observation period following the playback. The playback consisted of a single rattle ranging between 1.5–12.3 s in duration (mean = 4.3 s). The recorded rattles were not edited to standardize the duration of calls, but there was no effect of duration of the playback on the responses of focal squirrels (generalized linear model with whether or not a squirrel rattled as the response variable, binomial error distribution, logit link function: $N = 84$, $Z = -0.39$, $p = 0.7$). Rattles were recorded using a Sennheiser K6 condenser microphone connected to a Marantz Professional Solid State Recorder PMD660 (frequency response 0.01–22.5 kHz). Calls were recorded at a 44.1 kHz sampling frequency with a 16-bit resolution. The speaker used for the playbacks was a special order Saul Mineroff SME-AFS field speaker with a frequency response that was extended by the manufacturer to include frequencies from 0.01 to 22.5 kHz. The rattle playback simulated a territorial dispute because it was played from within the focal squirrel's territory. Rattles used in the playback trials were recorded in the field from adult squirrels on their own territory within the study areas, either by opportunistic observation or by playing a rattle from the speaker to provoke a response. Recorded rattles used in the playback trials were from a mix of males and females, related and unrelated individuals from neighbouring and distant territories, but the identity of the calling squirrel did not affect the response of the focal squirrel (Shonfield, 2010).

Recorded rattles were amplified in Raven Pro 1.3 Sound Analysis Software (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA) to ensure all recordings had a similar power level (dB). The rattles were saved as uncompressed 16 bit .wav files to preserve call characteristics, and transferred to a SanDisk mp3 player with .wav format support for use in the field. All rattles were played between 68–75 dB measured 2 m from the speaker (a similar range was used in a playback study of collared pikas, *Ochotona collaris* (Trefry & Hik, 2009)). Each recording was checked in the field using a Radio Shack digital sound level meter to ensure it fell within that range, although the volume was not noted for each individual playback. To test the distance that rattles would travel when played from the speaker at that volume, an observer listened for the rattles at increasing distances from the speaker. Rattles played from the speaker were audible to the human ear up to 120 m away, which was comparable to the only published account stating that red squirrel rattles can be heard up to 130 m away (Smith, 1968), indicating that the playbacks were at an appropriate volume to simulate a rattle from a squirrel.

To start a playback trial, the speaker was set up approximately 10 m from the focal squirrel and concealed behind a tree, fallen log or dense vegetation. The observer was positioned perpendicular to the focal squirrel and the speaker, at a distance of approximately 10 m from the squirrel. The rattle was played approximately 3 min after the speaker was set up, and no squirrels approached or investigated the speaker prior to the playback. The behaviour of the focal squirrel was recorded every 30 s throughout a three-minute period immediately following the playback (as above), and the audio recorder was turned on during the trial to record any rattles from the focal squirrel and to identify when neighbouring squirrels rattled. Some rattle recordings from focal squirrels during trials were later used as playback rattles in other playback trials.

In both the natural observations and playback trials, trials were not started if the focal squirrel alarm-called (known as a bark, Lair, 1990) as the observer approached or was observed interacting with (e.g., chasing) another squirrel. Trials were discarded if an interaction with another squirrel occurred or if the observer lost sight of the squirrel for longer than 1 min. Discarded trials were attempted again after three days. In total, the dataset comprised 93 natural observations and 84 playback trials. This final dataset is a reduction from the initial amount of data collected, comprising 105 trials of each type, because we randomly selected one trial for each individual in instances in which multiple trials were performed on the same individual. Although individual squirrels were used in both the natural observations and playback trials, each squirrel was included only once within each of these trial types in the final dataset. We also did the same analysis (described below) on a dataset with the first trial performed on each squirrel as opposed to a randomly selected trial from each squirrel, but the results did not differ. Animal use protocols were approved by the University of Guelph Animal Care and Use Committee (permit number 09R006).

2.3. Predictor variables

To test each of the four proposed hypotheses we used the following predictor variables: local density, reproductive condition, age and body mass, and two measures of aggressiveness from two separate behavioural tests (described below). Local density (squirrels/ha) was used as a measure of potential intruder pressure and was calculated for each focal squirrel as the number of squirrels that owned a midden within a 130 m radius (5.31 ha) of the midden of the focal squirrel. The distance of 130 m was chosen because rattles from neighbouring squirrels have been reported to be audible up to this distance (Smith, 1968) and this distance is consistent with the scale at which the effect of local density is strong (Dantzer et al., in press).

Reproductive condition was a categorical variable consisting of three groups: males, females with pups, and females without pups at the time of the trial. Females were placed in the 'with pups' group if they had pups younger than 70 days old, the approximate age when pups are weaned (Humphries & Boutin, 1996). The group of females 'without pups' consisted of yearling females that did not breed in 2009, pregnant females, females that had weaned pups, and one female that lost her litter earlier in the season and did not breed again. Natural observations were done on 52 males, 13 females without pups, and 28 females with pups. Playback trials were done on 48 males, 10 females without pups, and 26 females with pups.

Ages of most squirrels used in this study (64/93 in the natural observations and 61/84 in the playback trials) were known from enumerating squirrels with uniquely numbered ear tags while still in the natal nest (McAdam et al., 2007) as part of an ongoing long-term study. In cases where ages were not known with certainty, males that were first caught and tagged as adults were assumed to be one year old, and females that were first caught and tagged as adults were assumed to be either one or two years old based on nipple condition. Nipples become pigmented during a female's first estrus and remain that way for life (Steele, 1998), and most females in our study area delay breeding until they are two years old (McAdam et al., 2007). Body mass was measured in the field during live-trapping events throughout the 2009 season by weighing squirrels to the nearest gram using a Pesola spring balance (Pesola, Baar, Switzerland). For breeding females we used their average weight during lactation, and for non-breeding females and males we used their average weight between 4 April and 24 August, 2009, which corresponded to the same cumulative time-frame as when the breeding females were lactating. Squirrels were caught and weighed an average of 7.03 times (±2.83 SD) during the time-frame.

Following Boon et al. (2007) and Taylor et al. (2012), we measured aggression using a mirror-image stimulation (MIS) test (Svendsen & Armitage, 1973). In 2005, 2008, 2009 and 2010 we performed 105 MIS trials on 65 of the 93 squirrels in the observation trials and 57 of the 84 squirrels in the playback trials. The portable testing arena was a 60 × 80 × 50 cm white corrugated-plastic box with a clear acrylic lid through which the behaviour of the squirrel was recorded with a digital video camera. Individual squirrels were tested within one hour of being trapped on their territory. After trapping, the squirrel was handled as normal and ear-tag number, mass, and reproductive status were recorded. The test squirrel was then transferred into the portable arena on their territory through a sliding door to begin the habituation period. After a 7.5-min habituation period, a 45 × 30 cm mirror at one end of the arena was exposed to start the 5-min MIS trial. At the conclusion of the session the squirrel was released where it was trapped and the arena was cleaned with 70% ethanol.

We quantified each squirrel's aggressive behaviour during the trials by scoring the videotaped trials using JWatcher Video 1.0 (Blumstein & Daniel, 2007) and the same ethogram as Boon et al. (2007) and Taylor et al. (2012). During the MIS trials we recorded proportions of time spent in the third of the arena closest to the mirror, and farthest from the mirror, and the proportion of time spent stretching towards the mirror. We also recorded the rate of aggressive contact with the mirror (attacks), the rate of grunting vocalizations, and the rate of crouches (tail positioned over head with hairs erect). MIS trials were performed by R.W.T. and four assistants and analyzed by R.W.T. and five assistants. We tested the inter-observer reliability of our measurements by calculating the correlation between two observers' scores of the same trial (56 trials were scored by multiple observers), and removed measurements with a reliability of <0.7 (Martin & Bateson, 1993; Taylor et al., 2012). We used principal component analysis using a correlation matrix to identify the dominant axes of behavioural variation in the MIS trials (Martin & Réale, 2008). All further analyses used the scores calculated from the first principal component loadings for each trial and were interpreted as aggression scores. We obtained single aggression scores for each individual by extracting the best linear unbiased predictors (BLUPs) from a linear mixed model (Kruuk, 2004; Boon et al., 2007). To control for effects of habituation we ran the model with a fixed effect term for trial number and a quadratic term for trial number to account for a non-linear response to repeated trials. To control for effects of seasonality and study area we included day of year and study area as fixed effects. We assigned mean aggression scores of the natural observation or playback trial dataset to individuals for which we were not able to measure aggressiveness to maintain the size of the dataset. Aggression scores are repeatable between years (Taylor et al., 2012).

The second behavioural test quantified a squirrel's response to being handled as the number of seconds spent struggling in a mesh handling bag out of a total of 30 s. This test has previously been correlated with aggressiveness in red squirrels and represents boldness in captivity (Carere & van Oers, 2004; Boon et al., 2007). A total of 1063 struggle tests were done in 2005, 2008, 2009 and 2010 on 92 out of 93 squirrels in the natural observations and 83 out of 84 squirrels in the playback trials. We obtained single scores for each individual by extracting the best linear unbiased predictors (BLUPs) from a linear mixed model. Boon et al. (2007) found an effect of observer on red squirrel struggle rates, so we included a fixed effect term for the 36 observers in the model. To control for habituation to being handled we ran the model with a fixed effect term for the number of days since the squirrel was last handled, and for the number of times the squirrel had been handled that year. To control for effects of study area we included study site as a fixed effect term. We assigned mean values to the two squirrels that were not tested for struggle rate instead of deleting the data to maintain the size of the dataset. As with the aggression scores, struggle rate was highly repeatable for individuals between years (Taylor et al., 2012).

Several other variables were analyzed in a separate exploratory analysis as potential covariates of rattling behaviour by focal squirrels. These variables included study site, time of day, Julian date, distance of the focal squirrel to the centre of its midden, and activity level of the squirrel during both the natural observations and the playback trials. Each squirrel's overall activity level was calculated as the proportion of behaviours that were recorded every 30 s during the three-minute observation period that were classified as feeding, foraging, traveling, and grooming. Non-active behaviours, thus, included resting, being vigilant, and in a nest. Additionally for only the playback trials, we assessed the effects of the duration of the playback, and whether or not a neighbour was heard rattling at any point during the three-minute observation period on the rattling behaviour of the focal squirrel.

2.4. *Statistical analyses*

All statistical analyses were performed using R version 2.10.1 (R Development Core Team, 2009). The response variable in all cases was whether or not the focal squirrel rattled during the 3-min observation session. We used a binary response of whether or not the focal squirrel rattled as opposed to the frequency of rattling, since it was rare for squirrels to rattle more than once in a three-minute period (only 3% of natural observations and 7% of playback trials). Red squirrels do not emit different rattles in response to an intruder than they do in unelicited defence of territory boundaries (Goble, 2008), and the rattles are the primary means by which squirrels defend their territory (Smith, 1968), so this binary response was the best measure of territory defence.

We assessed the relative importance of the four hypotheses to red squirrel territorial defence behaviour by comparing the fit of four a priori generalized linear models (binomial error distribution, logit link function) using Akaike's Information Criterion (AIC; Burnham & Anderson, 2002). The intruder pressure model included local density as the only predictor variable. The offspring-defence model included only reproductive condition (a categorical variable with three groups: males, females with pups, and females without pups) as a predictor variable. The resource-holding potential model included as predictor variables mass, age and a quadratic age term (age^2) to account for potential non-linear effects of age due to senescence. Models with only mass or age (both with and without the quadratic age term) as predictor variables gave very similar results and did not affect the relative importance of this model (results not shown). The aggressiveness model included two measures of aggressiveness: the struggle test score and aggression score from the MIS test. Models with only one of the two aggressiveness variables were also fitted, but these did not affect the relative importance of the aggressiveness model and are not shown. The fit of these four a priori models were compared and evaluated based on differences (Δ_i) in Akaike's Information Criterion corrected for small sample sizes (AIC_c) for the natural observations and playback trials separately (Burnham & Anderson, 2002). We also conducted a global model analysis using a generalized linear model with all of the predictor variables related to the four a priori hypotheses tested above for the natural observations and playback trials separately to compare the relative importance of each predictor variable.

In addition to the a priori model comparison approach, we also used an exploratory forward stepwise model selection approach to determine whether other unanticipated combinations of predictor variables could explain red squirrel territory defence behaviour. We ran separate generalized linear models (binomial error distribution; logit link function) for the natural observations and playback trials. For the natural observations we included study site, time of day, Julian date, distance of the focal squirrel to its midden, and activity level as potential predictor variables. For the playback trials, we included these five potential predictor variables (listed above), as well as the duration of the playback rattle, and a categorical variable of whether or not a neighbouring squirrel was heard rattling during the post-playback period.

All continuous predictor variables were checked for non-linearities using a cubic spline with the R package mgcv (Wood, 2006), and no non-linearities were detected. We assessed the dispersion parameters of all models using the family quasibinomial (Faraway, 2006) and there were no signs of over-dispersion (dispersion parameters were between 1.003 and 1.047).

3. Results

3.1. Rattling behaviour in the absence of a stimulus

Red squirrels rattled in 34% of the 3-min natural observations without an experimental stimulus. Model selection using AIC_c revealed that the intruder pressure model was the best fit to the data, while the other three models had considerably less support (Δ_i values were between 3.84 and 7.28, Table 1). Squirrels were more likely to rattle during a natural observation when they had more neighbours and, therefore, a presumed higher probability of intruder pressure (generalized linear model $N = 93$, $Z = 2.16$, $p = 0.03$). To

Table 1.
Generalized linear models and $\mathrm{AIC_c}$ results predicting the rattle probability of focal squirrels during natural observations (binary response, logit link).

Model	K	Log-likelihood	$\mathrm{AIC_c}$	Δ_i	w_i
Intruder pressure: local density	2	−57.39	118.91	0.00	0.76
Offspring defense: reproductive condition	3	−58.24	122.75	3.84	0.11
Aggressiveness: struggle rate + aggression score	3	−58.25	122.77	3.86	0.11
Resource-holding potential: mass + age + age^2	4	−58.87	126.19	7.28	0.02

Number of parameters is represented by K. One parameter is used in all models for the intercept. Models were evaluated based on differences between $\mathrm{AIC_c}$ scores (Δ_i) and $\mathrm{AIC_c}$ weights (w_i). Δ_i is the difference between the observed model ($_\mathrm{i}$) and the best model as determined by the lowest $\mathrm{AIC_c}$. Models are named after the four hypotheses tested.

illustrate this effect, we divided local density into three categories: low (0.4–1.2 squirrels/ha), medium (1.3–2.1 squirrels/ha) and high (2.2–3.2 squirrels/ha), and calculated the rattle probability for each group (Figure 1). In the global model analysis, the effect local density was marginally non-significant ($p = 0.055$), and all other predictor variables were not significant (all p values > 0.1).

In the exploratory analysis, the characteristics of when (time of day, date) and where (study site, distance of the squirrel to their midden) the trial was conducted, and the proportion of time a squirrel spent active during the three-minute observation period had no effect on the probability of a squirrel rattling ($N = 93$, all $|Z|$ values < 1.5, all p values > 0.1). In addition, none of the original variables other than local density (e.g., aggressiveness, mass, age, reproductive status) significantly affected the probability of the focal squirrel rattling regardless of which other variables were included in the model ($N = 93$, all $|Z|$ values < 1.5, all p values > 0.1).

3.2. Rattling behaviour in response to a stimulus

The probability of a squirrel rattling in response to the playback simulating a territory dispute was 39%. Differences in $\mathrm{AIC_c}$ scores between each model and the best model (Δ_i) were all less than 4 (Table 2). There was similar support for the intruder pressure, offspring-defense and aggressiveness models

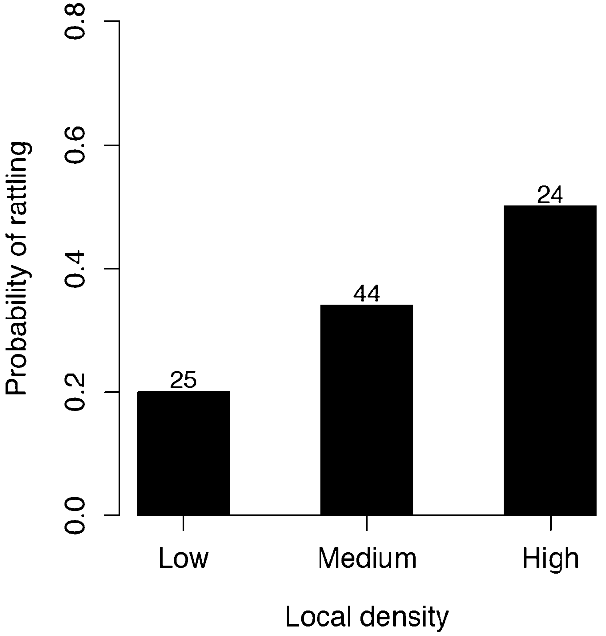

Figure 1. Rattle probabilities of focal squirrels during natural observations across three local density levels. Local density was calculated as the number of squirrels with a midden within a 130-m radius (5.31 ha) of the midden of the focal squirrel, and ranged between 0.4 and 3.2 squirrels per ha. Squirrels were more likely to rattle when they had more neighbours (generalized linear model $N = 93$, $Z = 2.16$, $p = 0.03$). To illustrate this effect, we divided local density into three categories: low (0.4–1.2 squirrels/ha), medium (1.3–2.1 squirrels/ha) and high (2.2–3.2 squirrels/ha), and calculated the rattle probability for each group. Sample sizes are indicated above each bar.

($\Delta_i < 2$), but somewhat less support for the RHP model ($\Delta_i = 3$; Table 2). However, none of the predictor variables included in the generalized linear models testing the four hypotheses significantly affected whether or not a squirrel rattled in response to the playback ($N = 84$, all $|Z|$ values < 1.5, all p values > 0.1), and the proportion of the deviance explained in each of the four models was less than 2%. In contrast to the natural observations with no experimental stimulus, squirrels with more neighbours were not more likely to rattle in response to the playback ($N = 84$, $Z = 0.12$, $p = 0.91$). In the global model analysis all predictor variables were not significant (all p values > 0.1).

In the playback trials a neighbouring squirrel was heard rattling in 32 out of 84 trials. In our exploratory analysis, the probability of a squirrel rattling

Table 2.
Generalized linear models and $\mathrm{AIC_c}$ results predicting the rattle probability of focal squirrels in response to a territorial playback simulating a territory dispute (binary response, logit link).

Model	K	Log-likelihood	$\mathrm{AIC_c}$	Δ_i	w_i
Intruder pressure: local density	2	−56.26	116.67	0.00	0.39
Offspring defense: reproductive condition	3	−55.32	116.94	0.27	0.34
Aggressiveness: struggle rate + aggression score	3	−55.90	118.10	1.44	0.19
Resource-holding potential: mass + age + age^2	4	−55.58	119.68	3.01	0.09

Number of parameters is represented by K. One parameter is used in all models for the intercept. Models were evaluated based on differences between $\mathrm{AIC_c}$ scores (Δ_i) and $\mathrm{AIC_c}$ weights ($\mathrm{w_i}$). Δ_i is the difference between the observed model ($_\mathrm{i}$) and the best model as determined by the lowest $\mathrm{AIC_c}$. Models are named after the four hypotheses tested, and are followed by the predictors included in each model.

in response to the playback was affected by the date of the trial, but the direction of this effect differed depending on whether or not a neighbour rattled (Julian date × neighbour rattle: $N = 84$, $Z = -3.6$, $p < 0.001$). In the early part of the breeding season (late May to early June), squirrels had a high probability of rattling in response to the playback if a neighbour was also heard rattling (88%), and a low probability of rattling if no neighbour rattled (9%). This pattern was reversed later in the breeding season (mid to late July) when squirrels were less likely to rattle if a neighbour rattled after the playback (27%) and more likely to rattle if no neighbour rattled (50%). Other characteristics of the trial including study site, time of day, distance of the squirrel to their midden, the proportion of time a squirrel spent active during the 3-min observation period and duration of the playback rattle had no effect on the probability of a squirrel rattling in response to the playback ($N = 84$, all $|Z|$ values < 1.5, and all p values > 0.1).

4. Discussion

Territorial defence behaviour has been found to differ between individual territory owners (Johnsson et al., 2000; Nowicki et al., 2002; Hyman et al., 2004; Kleiber et al., 2007), and several hypotheses could explain the observed differences. We used AIC model comparison to assess the relative importance of four a priori models to explain territorial behaviour in red squirrels, and found that the propensity to rattle under natural conditions (no experimental stimulus) was best explained by local density, lending support to the intruder pressure hypothesis. The offspring-defence, resource-holding potential, and aggressiveness hypotheses had considerably less support. Red squirrels rattled in 50% of the 3-min natural observation sessions when they were surrounded by higher numbers of neighbouring conspecifics (high local density: 2.2–3.2 squirrels/ha), but rattled in only 20% of trials when they had fewer neighbours (low local density: 0.4–1.2 squirrels/ha). Other studies have found that increased local population density results in more frequent aggressive interactions with conspecifics (Turpie, 1995; Meadows, 2001), and reduced territory size (Myers et al., 1979; Norman & Jones, 1984; Keeley & McPhail, 1998). Bannertail kangaroo rats (*Dipodomys spectabilis*) have been found to increase drumming rates (a territorial signal) with increased population density (Randall, 1984). Hyman et al. (2004) found a spatial autocorrelation in territorial aggression of neighbouring song sparrows in response to playbacks, but this effect was not consistent across the entire length of the trial, and they did not specifically test the effect of the number of neighbours on territorial defence. So despite the fact that previous studies have suggested that intruder pressure affects territorial defence, ours is the first comprehensive study to have found that only local density (and not reproductive condition, RHP or aggressiveness) affects intraspecific differences in territorial defence. The number of surrounding conspecifics affects the propensity of individual red squirrels to vocalize, indicating they adjust territorial defence in response to intruder pressure.

We found little support for the intruder pressure, offspring-defence, resource-holding potential, and aggressiveness hypotheses in explaining territorial defence in response to a simulated territory dispute. The predictor variables were nonsignificant based on p values and each model explained a relatively low proportion of the null deviance. Previous studies have found effects of RHP (age and territory tenure, Hyman et al., 2004) and individual behavioural differences (exploratory behaviour in this case, Amy et al., 2010) on territorial responses to playbacks. We found that factors influencing the territorial responses of red squirrels were inconsistent between situations with and without the playback stimulus, and it was more difficult to predict whether a squirrel would rattle in the playback trials. Local density affected territorial defence behaviour in the absence of a playback, but was not an important factor influencing a squirrel's territorial response in the event of a territorial dispute. If the playback itself was ineffective, we would have expected similar results of the model selection analysis in both contexts (with and without playback), but this was not the case. In addition, previous analyses based on the entire dataset (including multiple observations per animal) and several behavioural measures (rattling, and whether the squirrel looked at or approached the speaker) indicated that focal squirrels responded behaviourally to the playback (Shonfield, 2010).

Some of the variability in territorial behaviour could have been due to unmeasured aspects of the trial or focal individual. For example, minor differences in the sound level of the playbacks may have affected responsiveness. However, it would have been difficult to ensure that each squirrel heard the call at the same sound level due to differences in habitat structure (e.g., tree density), which could affect how each squirrel heard the playback even if each recording was the exact same sound level. Responsiveness of focal squirrels may also have been affected by other untested factors such as the amount of cached food resources, but we did not have the data to test whether this affected territorial defence in this study. A study on territorial defence behaviour of brown trout found an effect of habitat preference (Johnsson et al., 2000), presumably due to the value of resources within different habitats. Resource availability is likely to affect territorial defence behaviour and should be further tested in future studies of intraspecific differences in territorial defence.

Our post hoc exploratory analyses uncovered an interaction between whether or not a neighbour rattled and the date on which the playback trial was performed. Earlier in the summer focal squirrels were more likely to rattle in response to a playback when a neighbour also rattled than when no neighbour rattled. That is, focal squirrels exhibited congruent rattle responses with their neighbours early in the breeding season. Later in the breeding season focal squirrels were less likely to rattle if a neighbour also rattled after the playback and, thus, showed a lack of congruence with their neighbours in rattle responses. We have shown that the probability of rattling is affected by local density, so it seems clear that neighbouring squirrels are affecting each other's rattling behaviour. Juvenile red squirrels emerge from their natal nest and make forays off their natal territory in search of a vacant territory later in the summer (Larsen & Boutin, 1994). It is possible that this influx of juveniles searching for territories in the population affected rattle responses of adult red squirrels to rattles from their established neighbours (i.e., a type of conspecific audience effect, Matos & Schlupp, 2005) and resulted in the significant interaction, but further investigations will be required to corroborate and further elucidate this finding.

In our analyses we used a combination of multi-model inference using AIC as well as forward stepwise model selection based on p values. Multi-model inference has been increasingly used in ecological studies as an alternative to traditional null hypothesis testing, especially when more than one a priori hypothesis is plausible (Johnson & Omland, 2004; Hobbs & Hilborn, 2006). Forward stepwise procedures have been criticized, as they have been shown to produce elevated Type-I error rates (Mundry & Nunn, 2009). Here we used both approaches to serve two distinct purposes. Our AIC models allowed us to compare the relative fit of four a priori models to assess the relative importance of four hypotheses to explain territorial behaviour. While the stepwise procedure did not test a hypothesis, it did serve two valuable purposes. First, it was able to exclude several factors that were not included in a priori models (e.g., time of day, squirrel activity and location). Second, it revealed a complex interaction between Julian date and the behaviour of a focal squirrel's neighbours on its probability of rattling in response to a playback that we would not have predicted a priori. While this interaction could represent a spurious relationship reflecting the inflated Type-I error rates that can plague stepwise procedures (Mundry & Nunn, 2009), it is also possible that further study will confirm and provide a more mechanistic understanding of this relationship. If so, the uncovering of this unanticipated complex pattern using a stepwise procedure will have proved quite useful.

Acknowledgements

We thank all field technicians for their assistance with field data collection and Ainsley Sykes for coordinating research efforts. We thank all members of the McAdam Lab for feedback on an earlier version of this manuscript. Research support was provided by the Natural Sciences and Engineering Council of Canada (NSERC), the National Science Foundation, the Northern Scientific Training Program, and the Arctic Institute of North America. This is publication number 63 of the Kluane Red Squirrel Project.

References

Altmann, J. (1974). Observational study of behaviour: sampling methods. — Behaviour 49: 227-267.

Amy, M., Sprau, P., de Goede, P. & Naguib, M. (2010). Effects of personality on territory defence in communication networks: a playback experiment with radio-tagged great tits. — Proc. Roy. Soc. Lond. B: Biol. 277: 3685-3692.

Arcese, P. (1987). Age, intrusion pressure and defense against floaters by territorial male song sparrows. — Anim. Behav. 35: 773-784.

Balfour, D. (1983). Infanticide in the Columbian ground squirrel, *Spermophilus columbianus*. — Anim. Behav. 31: 949-950.

Blumstein, D.T. & Daniel, J.C. (2007). Quantifying behaviour the JWatcher way. — Sinauer Associates, Sunderland, MA.

Boon, A.K., Réale, D. & Boutin, S. (2007). The interaction between personality, offspring fitness and food abundance in North American red squirrels. — Ecol. Lett. 10: 1094-1104.

Brown, J.L. (1964). The evolution of diversity in avian territorial systems. — Wilson Bull. 76: 160-169.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and multimodel inference: a practical information-theoretic approach. — Springer, New York, NY.

Careau, V., Réale, D., Humphries, M.M. & Thomas, D.W. (2010). The pace of life under artificial selection: personality, energy expenditure, and longevity are correlated in domestic dogs. — Am. Nat. 175: 753-758.

Carere, C. & van Oers, K. (2004). Shy and bold great tits (*Parus major*): body temperature and breath rate in response to handling stress. — Physiol. Behav. 82: 905-912.

Dantzer, B., Boutin, S., Humphries, M.M. & McAdam, A.G. (in press). Behavioral responses of territorial red squirrels to natural and experimental variation in population density. — Behav. Ecol. Sociobiol., DOI 10.1007/s00265-012-1335-2.

den Hartog, P.M., Slabbekoorn, H. & ten Cate, C. (2008). Male territorial vocalizations and responses are decoupled in an avian hybrid zone. — Philos. Trans. Roy. Soc. B 363: 2879-2889.

Descamps, S., Boutin, S., Berteaux, D. & Gaillard, J.M. (2008). Age-specific variation in survival, reproductive success and offspring quality in red squirrels: evidence of senescence. — Oikos 117: 1406-1416.

Donald, J.L. & Boutin, S. (2011). Intraspecific cache pilferage by larder-hoarding red squirrels (*Tamiasciurus hudsonicus*). — J. Mammal. 92: 1013-1020.

Faraway, J.J. (2006). Extending the linear model with R: generalized linear, mixed effects and nonparametric regression models. — Chapman and Hall/CRC Press, Boca Raton, FL.

Fletcher, Q.E., Boutin, S., Lane, J.E., LaMontagne, J.M., McAdam, A.G., Krebs, C.J. & Humphries, M.M. (2010). The functional response of a hoarding seed predator to mast seeding. — Ecology 91: 2673-2683.

Gherardi, F. (2006). Fighting behavior in hermit crabs: the combined effect of resource-holding potential and resource value in *Pagurus longicarpus*. — Behav. Ecol. Sociobiol. 59: 500-510.

Gill, F.B. & Wolf, L.L. (1975). Economics of feeding territoriality in the golden-winged sunbird. — Ecology 56: 333-345.

Goble, A. (2008). Signature signals in the territorial vocalizations of red squirrels (*Tamiasciurus hudsonicus*) and their use in kin recognition. — MSc thesis, Michigan State University, East Lansing, MI, USA.

Gorrell, J.C., McAdam, A.G., Coltman, D.W., Humphries, M.M. & Boutin S. (2010). Adopting kin enhances inclusive fitness in asocial red squirrels. — Nature Commun. 1: 22.

Hobbs, N.T. & Hilborn, R. (2006). Alternatives to statistical hypothesis testing in ecology: a guide to self teaching. — Ecol. Appl. 16: 5-19.

Hoogland, J.L. (1985). Infanticide in prairie dogs — lactating females kill offspring of close kin. — Science 230: 1037-1040.

Humphries, E.L., Hebblethwaite, A.J., Batchelor, T.P. & Hardy, I.C.W. (2006). The importance of valuing resources: host weight and contender age as determinants of parasitoid wasp contest outcomes. — Anim. Behav. 72: 891-898.

Humphries, M.M. & Boutin, S. (1996). Reproductive demands and mass gains: a paradox in female red squirrels (*Tamiasciurus hudsonicus*). — J. Anim. Ecol. 65: 332-338.

Hyman, J., Hughes, M., Searcy, W.A. & Nowicki, S. (2004). Individual variation in the strength of territory defense in male song sparrows: correlates of age, territory tenure, and neighbor aggressiveness. — Behaviour 141: 15-27.

Johnson, J.B. & Omland, K.S. (2004). Model selection in ecology and evolution. — Trends Ecol. Evol. 19: 101-108.

Johnsson, J.I., Carlsson, M. & Sundstrom, L.F. (2000). Habitat preference increases territorial defence in brown trout (*Salmo trutta*). — Behav. Ecol. Sociobiol. 48: 373-377.

Keeley, E.R. & McPhail, J.D. (1998). Food abundance, intruder pressure, and body size as determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*). — Behaviour 135: 65-82.

Kelly, C.D. (2008). The interrelationships between resource-holding potential, resource-value and reproductive success in territorial males: how much variation can we explain? — Behav. Ecol. Sociobiol. 62: 855-871.

Kleiber, D., Kyle, K., Rockwell, S.M. & Dickinson, J.L. (2007). Sexual competition explains patterns of individual investment in territorial aggression in western bluebird winter groups. — Anim. Behav. 73: 763-770.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W. & Blokhuis, H.J. (1999). Coping styles in animals: current status in behavior and stress-physiology. — Neurosci. Biobehav. Rev. 23: 925-935.

Kruuk, L.E.B. (2004). Estimating genetic parameters in natural populations using the "animal model". — Philos. Trans. Roy. Soc. B 359: 873-890.

Lair, H. (1990). The calls of the red squirrel — a contextual analysis of function. — Behaviour 115: 254-282.

LaMontagne, J.M. & Boutin, S. (2007). Local-scale synchrony and variability in mast seed production patterns of *Picea glauca*. — J. Ecol. 95: 991-1000.

Larivée, M.L., Boutin, S., Speakman, J.R., McAdam, A.G. & Humphries, M.M. (2010). Associations between over-winter survival and resting metabolic rate in juvenile North American red squirrels. — Funct. Ecol. 24: 597-607.

Larsen, K.W. & Boutin, S. (1994). Movements, survival, and settlement of red squirrel (*Tamiasciurus hudsonicus*) offspring. — Ecology 75: 214-223.

Maher, C.R. & Lott, D.F. (1995). Definitions of territoriality used in the study of variation in vertebrate spacing systems. — Anim. Behav. 49: 1581-1597.

Maher, C.R. & Lott, D.F. (2000). A review of ecological determinants of territoriality within vertebrate species. — Am. Midl. Nat. 143: 1-29.

Martin, J.G.A. & Réale, D. (2008). Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*. — Anim. Behav. 75: 309-318.

Martin, P.R. & Bateson, P.P.G. (1993). Measuring behaviour: an introductory guide, 2nd edn. — Cambridge University Press, Cambridge.

Matos, R.J. & Schlupp, I. (2005). Performing in front of an audience: signallers and the social environment. — In: Animal communication networks (McGregor, P.K., ed.). Cambridge University Press, Cambridge, p. 63-83.

McAdam, A.G., Boutin, S., Sykes, A.K. & Humphries, M.M. (2007). Life histories of female red squirrels and their contributions to population growth and lifetime fitness. — Écoscience 14: 362-369.

Meadows, D.W. (2001). Centre-edge differences in behaviour, territory size and fitness in clusters of territorial damselfish: patterns, causes, and consequences. — Behaviour 138: 1085-1116.

Mundry, R. & Nunn, C.L. (2009). Stepwise model fitting and statistical inference: turning noise into signal pollution. — Am. Nat. 173: 119-123.

Myers, J.P., Connors, P.G. & Pitelka, F.A. (1979). Territory size in wintering sanderlings — effects of prey abundance and intruder density. — Auk 96: 551-561.

Norman, M.D. & Jones, G.P. (1984). Determinants of territory size in the pomacentrid reef fish, *Parma victoriae*. — Oecologia 61: 60-69.

Norton, M.E., Arcese, P. & Ewald, P.W. (1982). Effect of intrusion pressure on territory size in black-chinned hummingbirds (*Archilochus alexandri*). — Auk 99: 761-764.

Nowicki, S., Searcy, W.A., Krueger, T. & Hughes, M. (2002). Individual variation in response to simulated territorial challenge among territory-holding song sparrows. — J. Avian Biol. 33: 253-259.

Parker, G.A. (1974). Assessment strategy and evolution of fighting behavior. — J. Theor. Biol. 47: 223-243.

R Development Core Team (2009). R: a language and environment for statistical computing. — R Foundation for Statistical Computing, Vienna, available online at http://www.R-project.org

Randall, J.A. (1984). Territorial defense and advertisement by footdrumming in bannertail kangaroo rats (*Dipodomys spectabilis*) at high and low population densities. — Behav. Ecol. Sociobiol. 16: 11-20.

Réale, D., Dingemanse, N.J., Kazem, A.J.N. & Wright, J. (2010). Evolutionary and ecological approaches to the study of personality. — Philos. Trans. Roy. Soc. B 365: 3937-3946.

Schradin, C. (2004). Territorial defense in a group-living solitary forager: who, where, against whom? — Behav. Ecol. Sociobiol. 55: 439-446.

Shonfield, J. (2010). Territorial defence behaviour and a test of the mechanism of kin recognition in red squirrels. — MSc thesis, University of Guelph, Guelph, ON, Canada.

Smith, C.C. (1968). The adaptive nature of social organization in the genus of tree squirrels *Tamiasciurus*. — Ecol. Monogr. 38: 31-64.

Smith, C.C. (1978). Structure and function of vocalizations of tree squirrels (*Tamiasciurus*). — J. Mammal. 59: 793-808.

Smith, J.N.M. & Arcese, P. (1989). How fit are floaters? Consequences of alternative territorial behaviors in a nonmigratory sparrow. — Am. Nat. 133: 830-845.

Steele, M.A. (1998). *Tamiasciurus hudsonicus*. — Mamm. Species 586: 1-9.

Svendsen, G.E. & Armitage, K.B. (1973). Mirror-image stimulation applied to field behavioral studies. — Ecology 54: 623-627.

Taylor, R.W., Boon, A.K., Dantzer, B., Réale, D., Humphries, M.M., Boutin, S., Gorrell, J.C., Coltman, D.W., & McAdam, A.G. (2012). Low heritabilities, but genetic and maternal correlations between red squirrel behaviours. — J. Evol. Biol. 25: 614-624.

Trefry, S.A. & Hik, D.S. (2009). Eavesdropping on the neighbourhood: collared pika (*Ochotona collaris*) responses to playback calls of conspecifics and heterospecifics. — Ethology 115: 928-938.

Tulley, J.J. & Huntingford, F.A. (1988). Additional information on the relationship between intraspecific aggression and anti-predator behavior in the three spined stickleback, *Gasterosteus aculeatus*. — Ethology 78: 219-222.

Turpie, J.K. (1995). Non-breeding territoriality — causes and consequences of seasonal and individual variation in grey plover *Pluvialis squatarola* behavior. — J. Anim. Ecol. 64: 429-438.

Wolff, J.O. (1985). Maternal aggression as a deterrent to infanticide in *Peromyscus leucopus* and *P. maniculatus*. — Anim. Behav. 33: 117-123.

Wolff, J.O. (1993). Why are female small mammals territorial? — Oikos 68: 364-370.

Wolff, J.O. & Peterson, J.A. (1998). An offspring-defense hypothesis for territoriality in female mammals. — Ethol. Ecol. Evol. 10: 227-239.

Wood, S.N. (2006). Generalized additive models: an introduction with R. — Chapman & Hall/CRC Press, London.