Effects of perceived predation risk on individual great tit life history and behavior

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Summary

Non-consumptive effects of predators (perceived predation risk) has been demonstrated to have pronounced effects on prey populations, inducing changes in morphology, behavior, ecology, and social interactions. The perception of predation risk alone affects the optimal expression of various phenotypic traits. Optimality theory predicts that individuals should be plastic in order to respond best to the current environment, therefore studies mainly focus on within individual responses to change in predation pressure. However, recently among-individual variation has gained interest because consistent individual differences across time and contexts has been documented for a wide range of behaviors and taxa. Theses consistent differences indicate behavioral types, and such types should differ in both the perception and susceptibility to risk. With this dissertation I aim to investigate individual level behavioral, morphological, and life history responses to manipulations of predation pressure and to determine if behavioral type influences such responses. Specifically, I use multiple long-term study populations of individually marked free living great tits (*Parus major*) to explore both within and among individual responses.

The **first chapter** examines how great tits respond vocally to increased perceived predation risk and validates our experimental manipulation methods. Great tits mainly communicate using vocal signals and vocal behaviors are relatively labile. We confirmed that our predator playback manipulations were perceived by the great tits as risky by examining individual birds' immediate response. Additionally we monitored the overall vocalizations in our study populations across the season, and found that birds also responded to our treatments at the population level and on longer time scales. Finally, we measured individual birds' vocal response to differences in predation pressure when also faced with the need to defend their territory from a potential intruder and found that the risk of territory loss was greater than the risk of predation.

Chapters 2 and 3 explore the effects of perceived predation risk on great tits behavior, morphology, and physiology during the winter when they face both starvation and predation risks. Body mass, exploratory behavior, and metabolic rate are related in great tits- individuals with high metabolic rate have high energy needs and tend to be more active and also heavier. Increased predation should favor decreases in all three traits. In chapter 2, we found that mass and exploratory behavior responded as expected, birds in areas exposed to predator playback tended to decrease in body mass and exploratory tendency compared with controls. In chapter 3, we found some evidence that metabolic rate may change as a function of treatment, with predator exposed birds potentially adaptively suppressing the normal seasonal increase in metabolic rate. Additionally, we found individual difference in response to treatment, and that the change in mass was dependent upon an individual's behavioral type.

My **fourth chapter** investigates the behavioral and life history responses of great tits to increased perceived predation risk during the breeding season. Despite recent research that indicates that environmental stress can influence the expression of variance components that shape the heritability of traits, few studies test how exposure to predators may alter the among and within individual variance. Our study allowed us to determine if predation influenced selection by separating within and between individual differences in variance and determining how repeatability, the upper limit of heritability, differed with increasing predation pressure. We found that predation pressure increased the repeatability, and potentially heritability, of clutch initiation in great tits by increasing among individual differences and decreasing variation within individuals. Additionally, our design allowed for a comparison of the same individuals across the two treatments (predator and control) and found that exploration was consistent across contexts, validating the use of exploratory behavior as a behavioral type.

These chapters provide evidence that individuals of different behavioral types experience changes in perceived predation risk differently and respond adaptively based on their level of perceived risk. This implies that predators may non-consumptively alter selection pressures on behavioral types because certain types of individuals must change more to reduce the threat of predation. In addition to influencing selection via differential costs for behavioral types, we also found that perceived predation potentially altered the heritability of certain life history traits by increasing differences among individuals and decreasing variability within individuals. The results of this dissertation highlight the importance of considering individuality in predator-prey dynamics and microevolutionary processes.

General Introduction

Predation is one of the most important selection pressures acting on phenotypic traits. Predators are known to alter prey morphology and demography (Volterra 1926, Huffaker 1958, Edmunds 1974). For example, prey can evolve camouflaged coloration to avoid predator detection or develop appendages to deter predators. Demography can be altered when predators preferentially cull certain individuals, such as gape limited predators that selectively consume small/younger individuals. These selective pressures shape the evolutionary trajectory of prey species. However, predation pressure varies in time and space and an individual's risk of consumption should vary greatly over its lifetime. Additionally, anti-predator responses at the wrong time are costly for prey as they require energy and preclude other important behaviors such as mating or foraging. Therefore, it behooves prey to be able to assess the current level of risk and respond adaptively. Predators' influence on prey behavior is particularly interesting because it is these behavioral responses of prey that allow individuals to respond and adapt to predators on ecological time scales (e.g. Lima and Dill 1990, Nelson et al. 2004, Creel and Christianson 2008).

These behavioral responses are often deemed 'non-consumptive' effects, as they occur in response to the threat of predation, and not as a result of selective consumption by predators. Research now demonstrates that these non-consumptive effects can have pronounced effects on prey (Lima 1998, Brown and Kotler 2004, Lind and Cresswell 2005, Preisser et al. 2005, Zanette et al. 2011). For example, the presence of predators may induce prey to live in groups, which in turn may favor selection for phenotypic traits allowing prey to cope with intra-specific competition (Werner and Peacor 2003). Predators thereby can affect a large range of phenotypic traits either directly or indirectly related to predation, including morphology (Relyea and Werner 2000), life-history (Eggers et al. 2006, Zanette et al. 2011), foraging (Macleod et al. 2005), vigilance (Brown et al. 1999) and parental care (Tilgar et al. 2011, Zanette et al. 2011, Bonnington et al. 2013, Ghalambor et al. 2013). These phenotypic responses to predation risk suggest that the perception of predation risk alone affects the optimal expression of various phenotypic traits.

Individual responses to perceived predation risk

Anti-predator response often precludes other behaviors. Therefore, individuals' decisions regarding which behavioral option to pursue should depend on the costs of predation and the benefits associated with each behavioral option (Lima 1998). Predation pressure varies spatially and temporally, and thus the optimal decision should also vary depending on such conditions. Additionally, individuals can differ in their current state or motivation, influencing the relative costs and benefits. For example, an individual at greater risk of starvation is more likely to forage despite predation risk (Lima 1986, Mathot et al. 2015). Thus, predation risk should only affect individuals when the benefits of anti-predator behavior outweigh the missed opportunity costs.

Traditionally, studies have focused on population level responses to predation risk; how members on average adjust their behavior to match environmental conditions (Westneat and Fox 2010). Optimality theory predicts that individuals will be plastic in order to respond best to the current environment

(Krebs and Davies 1997). However, phenotypic variation exists both among and within individuals (Westneat et al. 2015) and recently repeatable (so-called' 'consistent') among-individual variation has gained interest in behavioral ecology research (Dall et al. 2004, Réale et al. 2007). Individuals have been shown to demonstrate consistent behavioral differences in a wide range of taxa, from invertebrates to mammals (Wilson et al. 1994, Gosling 2001, Dingemanse et al. 2002, Bell et al. 2009). This variation among individuals is increasingly assumed to be adaptive and maintained by natural selection (Clark and Ehlinger 1987, Wilson 1998) and more recent studies now show it is heritable (e.g. Dochtermann et al. 2015). Specifically, recent research has demonstrated repeatable differences among individuals in boldness, risk perception, and risk susceptibility (Bouskila and Blumstein 1992, Luttbeg and Schmitz 2000, Stankowich and Blumstein 2005, Luttbeg and Sih 2010, Wolf and Weissing 2012). If individuals differ in susceptibility or perception of risk they can be expected to respond to risk differently. Specifically, those facing more risk should respond more strongly as their costs outweigh their benefits. This results in adaptive among-individual variation in phenotypic plasticity, or greater plasticity in individuals farther from the behavioral optima (Nussey et al. 2007, Dingemanse and Wolf 2013).

Behavioral types

Among individual differences in behavior have been categorized along a variety of axes, such as low to high aggressiveness (Benus et al. 1992), shy to bold anti-predator behavior (Clark and Ehlinger 1987, Wilson et al. 1994) and slow to fast exploration (Winkler and Leisler 1999). Certain behavioral axes are of particular interest to ecological and evolutionary studies of perceived predation risk. Specifically, exploration has been found to correlate with boldness and aggression (e.g. Gosling 2001, Herczeg and Garamszegi 2012), and individuals differ consistently in exploration tendency, ranging from "slow" to "fast" explorers in great tits (*Parus major*) (Dingemanse et al. 2012) and other species (Réale et al. 2007, Bell et al. 2009, Réale et al. 2010). Exploration behavior (defined as how much animals move through novel environments) is widely used as a proxy for risk-taking behaviors (e.g. Nicolaus et al. 2012) and is often linked to an individual's perception of risk, level of risk, and response to risk (e.g. van Oers et al. 2004, Jones and Godin 2010, Quinn et al. 2012). For example, fast exploring great tits are more willing to resume foraging following threatening disturbances as compared to slower exploring individuals (van Oers et al. 2004). Furthermore, animals that explore their environment more quickly are expected to be more conspicuous and have higher encounter rates with predators (Lima and Dill 1990, Brown et al. 1999), making exploration particularly costly in risky environments (e.g. Moses and Sih 1998, Hedrick and Kortet 2006). Consequently, individuals of different exploratory tendencies are generally expected to respond differently to changes in perceived predation risk.

Implications for evolutionary processes

Due to the rise in perceived importance of among-individual differences in behavior, behavioral ecologists increasingly study among- and within-individual variation within a single evolutionary framework (Nussey et al. 2007, Dingemanse et al. 2010, Westneat et al. 2015). This is achieved by using reaction norms: functions to describe the dependency of a phenotype on the environment (Schlichting and Pigliucci 1998). Therefore, the framework describes the variation across individuals (e.g. Dall et al. 2004, Sih et al. 2004, Dingemanse and Réale 2005, Nussey et al. 2005, Wilson et al. 2005, Dingemanse et al. 2010, Kluen and Brommer 2013) and allows researchers to understand how variation may be adaptive both within (Westneat and Fox 2010) and between individuals (Dingemanse and Wolf 2010). Measuring multiple individuals repeatedly across an environmental gradient, allows researchers to capture variation across individuals in the manner they adjust phenotypic traits in response to environmental conditions and to determine if individuals respond the same or differently (the latter called 'individual by environment interaction', I × E). This allows researchers to create behavioral reaction norms with an intercept that represents an individual's average behavior and a slope that represents its degree of phenotypic plasticity (Figure 1). Analysis of the intercepts allows for understanding of among-individual differences across environmental gradients while comparison of the slopes allows for understanding of among-individual differences in within-individual responses to the environmental gradient. Specifically, if all individuals respond in the same manner, their reaction norms will be parallel and behavior will be repeatable both within and between contexts (Figure 1a). Conversely, if individuals respond differently, their reaction norms will cross and repeatability may differ across contexts (Figure 1b). I × E interactions are of interest to behavioral ecologists in part because in situations where genetic relatedness is unknown, consistent differences among individuals can be used as a proxy for gene by environment interactions, as among individual differences represent the upper limit of possible genotypic differences. Additionally, I × E interactions are of interest specifically because they do incorporate non-genetic sources of variation that occur in nature, e.g. individuals differ in quality or quantity of food consumed at a given time. Therefore, the study of I × E is appealing because researchers can quantify an individual's response to the predation gradients it encounters while also incorporating other aspects of the individual and its environment, providing a highly realistic understanding of how phenotypes differ in response to changing predation risk.

A growing body of evidence indicates that environmental stress can greatly affect the expression of variance components (Hoffmann and Merila 1999, Charmantier and Garant 2005), yet few studies test how exposure to predators affects the variance components that shape the heritability of ecologically relevant traits, i.e. the among- and within-individual variance (Relyea 2005, Kraft et al. 2006, Dingemanse et al. 2009, Izhar and Eilam 2010, Dammhahn and Almeling 2012, Niemelä et al. 2012, Stein and Bell 2012, Briffa 2013, Furtbauer et al. 2015, Brown and Robinson 2016). These variance differences alone can influence selection because evolution depends on heritability (h^2), the fraction of phenotypic variance (V_P) owing to additive genetic variance (V_A) (Falconer and Mackay 1996). Phenotypic variance (V_P) is the total variance measured in a population, which is often separated into a number of components such as additive genetic (V_A), permanent environment (V_{PE}), and residual (V_R). However, determining additive genetic variance requires pedigree data that is not available for all study populations, so often among-individual variance (V_I) is used as a proxy for V_A (Dochtermann et al. 2015). The few studies exploring this topic have shown that different phenotypes within the same population respond differently to predators, thus, predators can induce short-term effects on prey populations in the lab by influencing the expression of V_{I} (Quinn 2005, Dingemanse et al. 2009, Izhar and Eilam 2010, Stein and Bell 2012, Brown and Robinson 2016). Additionally, it has been suggested that high predation risk may favor reduced predictability if unpredictable behavior helps ameliorate predation risk (intraindividual variability; Stamps et al. 2012, Briffa 2013). Specifically, predators may increase within-individual variation (V_{R}) in the behavior of their prey, consequently decreasing heritability of prey traits (Briffa 2013). This means that regardless of the direction or strength of selection induced by predation, predation exposure can strongly influence population dynamics because changes in heritability alone can alter the response to selection. Thus, even in the absence of a predator-induced selection, the more cryptic response of changes in the magnitude of variance components as a function of perceived predation risk is still expected (Brommer et al. 2008). Consequently, my dissertation evaluates myriad aspects of perceived predation risk influences prey, from populations to individuals to evolutionary processes.

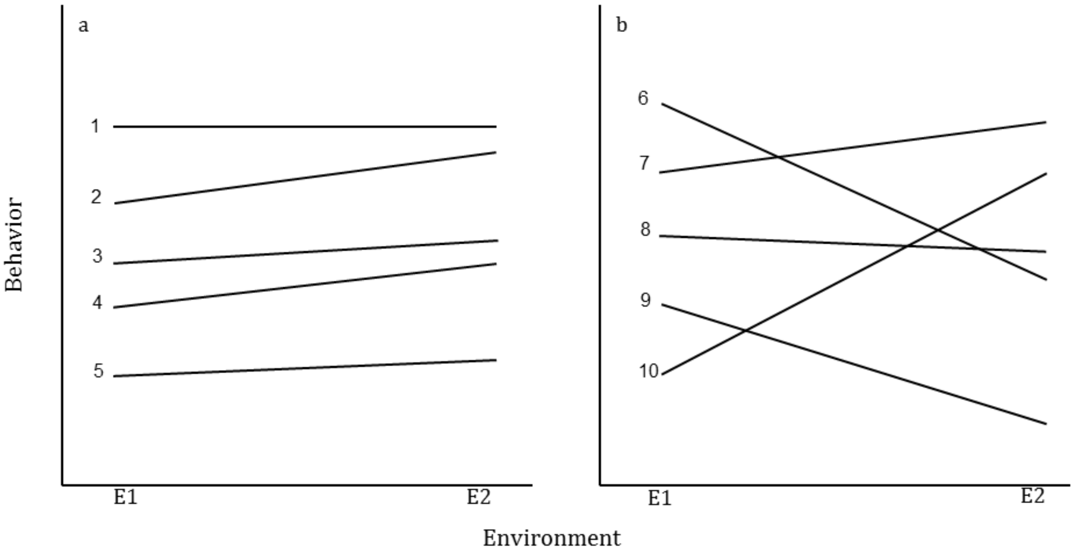

Figure 1. Illustration of variation viewed from the reaction norm approach, showing expressed values of the same behavior in two different environments. Each individual (number) is represented by a line. Each environment (E1, E2) represents a repeated measure within an individual. (a) Depicts a situation where all individuals respond the same to the environment and consistent individual differences exist. (b) Depicts a situation where individuals respond differently to the environment. The elevation of each line represents an individual's average behavior and the slope of each line represents the degree of phenotypic plasticity.

Study population and field sites

I used the great tit (*Parus major*) as a model organism to study the varying effects of perceived predation risk. Great tits are a common non-migratory passerine in Eurasia. Great tits are natural cavity-nesters and readily breed in nest boxes. Field work was conducted in conjunction with a larger study of great tits in Bavaria, Germany, across 12 study sites approximately 9-12ha each. Plots were established in 2009 and each contains 50 nest boxes placed in a grid approximately 50m apart from each other (Figure 2) for a total of 600 monitored nest boxes.

Figure 2. Study area between lakes Starnberg and Ammersee showing the 12 study populations in white (a) . Each site consists of 50 nest boxes (b) spaced 50m apart in a grid. Experimental manipulations involved 4 speakers evenly placed in each plot (c, gray points in b). Field sites are mixed forests, comprised predominantly of beech trees (*Fagus sylvatica*). (Source: Google Earth)

General field methods

Manipulation of perceived predation risk

We used playback to manipulate perceived predation risk at the plot level. Plot level manipulations were used in order to look at long term responses to manipulations of predation risk of a whole population, and to simulate a resident predator which represents long term changes in predation level. Four speakers (Shockwave, Foxpro, Pennsylvania, USA, Figure 2c) were placed evenly within each of our 12 plots, approximately 150-250 m apart, such that there was good coverage of the entire plot. Each year, speakers were placed in February (prior to the onset of breeding) and removed in July (after all first broods had fledged). In the first year, six plots received the control treatment and six plots received the predator treatment, half of the plots switched treatment for the second year. This design allowed us to examine repeatability, a proxy for heritability, within individuals both within and across treatments. We used the Eurasian blackbird (a sympatric, avian non-predator species, *Turdus merula*) as our control treatment and the sparrowhawk (a sympatric, avian predator species, *Accipter nisus*) as our predator treatment. All speakers were programed following the same scheme to match the normal timing and frequency of vocalization of our species. For the 3 hours after dawn and the 3 hours before dusk, speakers broadcast approximately 60% of the time; during the daylight hours between these two intensive periods, speakers broadcast approximately15% of the time. Playback was given for 4 consecutive days, followed by 4 consecutive days of non-playback, the cycle was repeated throughout the season to prevent habituation (Zanette et al. 2011).

An additional manipulation was conducted in the winter of 2013-2014. During this period blackbirds do not sing, hence the sounds of 2 nonthreatening heterospecifics (Eurasian wren, *Troglodytes troglodytes*, and common chaffinch, *Fringilla coelebs*) were broadcast in control plots. In predator plots we also added great tit mobbing calls because the sparrowhawk does not call frequently enough in these months to be used alone. Speakers were also programmed with a lower call frequency, 12 min of sound per plot per hour from dawn and dusk, to match the lower frequency during this season.

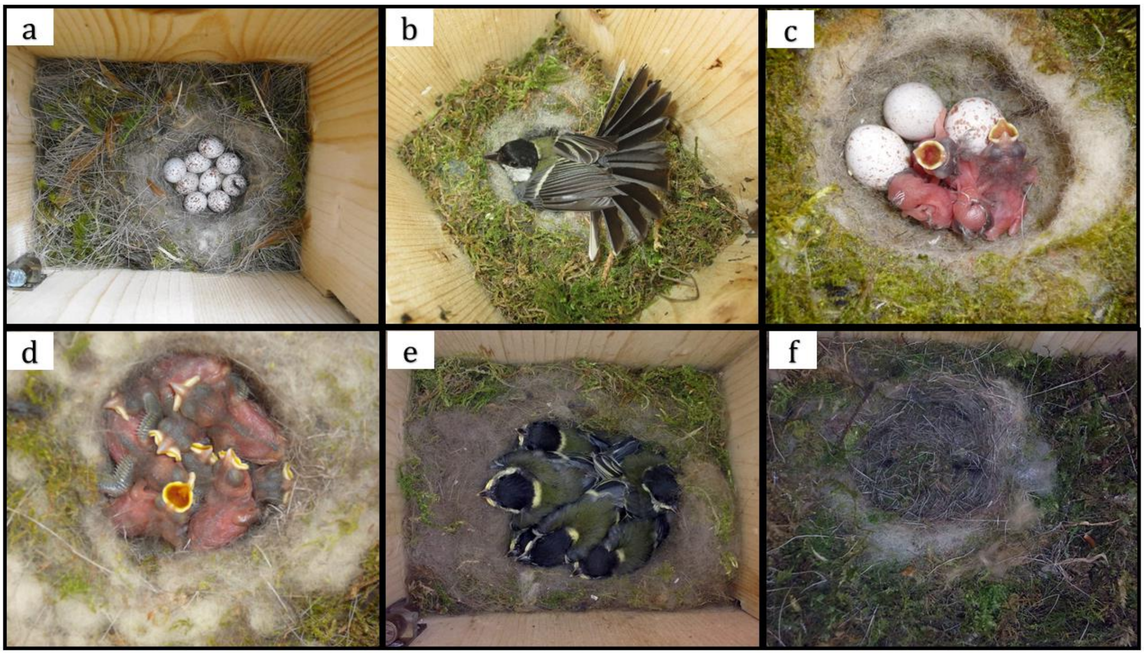

Figure 3. Great tit reproductive behavior. Great tits are common, short-lived, non-migratory passerines which breed throughout Europe. They are cavity-nesters that prefer deciduous forests but also readily accept nestboxes provided by humans for breeding. During the breeding season, which lasts from April through July, they generally form monogamous pairs. They usually produce clutch of approximately eight eggs (a), which is then incubated (b) for about two weeks. Once the first offspring hatch (c), the nestlings stay in the nest just under three weeks until fledging (f). We measure them twice during this nestling phase, at 6 (d) and 14 (e) days of age.

Breeding season

We checked nestboxes twice weekly, beginning April 1st each year. Standard life history parameters and two behaviors were monitored using standard methods (Dingemanse et al. 2002, Nicolaus et al. 2009) (Figures 3 and 4). First, simulated territory intrusions were applied to each breeding male during egg laying and incubation to determine male aggressive behavior (for exact procedure, see Araya-Ajoy and Dingemanse 2014). Then, based on incubation onset and clutch size, we checked nestboxes daily to determine hatch date (day 0). At day 6, nestling were weighed, bled and given an aluminum ring with a unique identifying number. At day 7, parents were caught using a spring trap in the nestbox, tested for exploratory behavior (for exact procedure, see Stuber et al. 2013), weighed, bled, measured and given an aluminum ring with a unique identifying number (if necessary). At day 14, standard body (body mass ± 0.1 g, tarsus ± 0.1 mm, wing length ± 0.5 mm) and behavioral (breathing rate, number of breaths per minute; docility, number of struggles per minute) measurements of nestlings were recorded. Starting on day 19, boxes were checked every other day to determine fledge date and number of fledged nestlings.

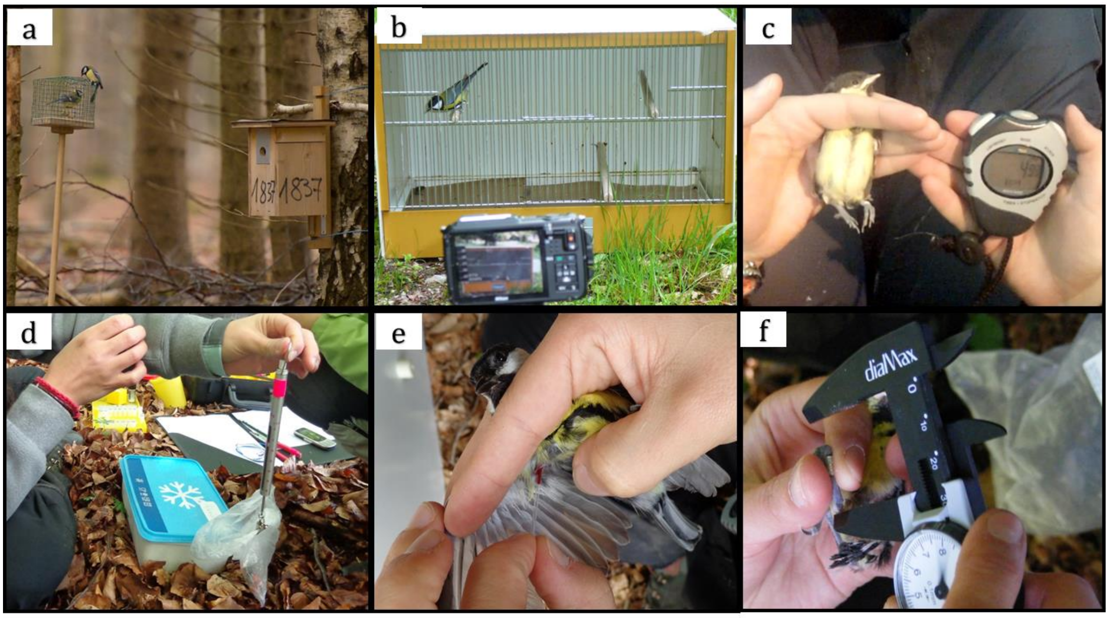

Figure 4. Field measurement process. In addition to life history (Figure 3) We also measure behavior and morphology of the great tits. Behavioral measures include simulated territory intrusions to determine adult aggressive behavior (a), adult exploration behavior (b), and chick docility and breathing rate (c). Both chicks and adults are also weighed (d), bled (e), measured (f), and given an aluminum ring with a unique identifying number (visible in f).

Winter

In early January, we captured all birds roosting in nestboxes following standard methods (Dingemanse et al. 2002). Birds were collected from the field and transported to the laboratory where they were individually housed in holding cages overnight. A subset of birds were held in respirometry chambers overnight (procedure detailed in Mathot et al. 2015) while their basal metabolic rate (BMR) was scored. The following morning, we measured exploratory behavior of each bird in a novel environment room (Dingemanse et al. 2002, Dingemanse et al. 2012). Following the behavioral test, we also recorded body mass and other standard morphometric measures. In late February, plots were visited again to collect birds roosting in boxes to acquire post-manipulation data.

Figure 5. Observation room in which we tested the exploratory behavior of great tits. Along the right hand wall are 24 sliding doors connecting the holding cages to the room. The front wall had a door at the left side and a one-way window through which the birds could be observed. Five artificial 'trees' made of wood with a trunk and four branches were placed in the room. The exploration score is a count of movements between perches during a 2-min period.

Dissertation overview

In this dissertation I use manipulative experiments to investigate how perceived predation risk influences the behavior and life history of great tits. Specifically, I explore changes in mean phenotypic response, individual variation in plasticity, and changes in variance components, repeatability, and trait correlations.

In Chapter 1 (Abbey-Lee et al. 2016a), I examined how great tits responded vocally to increased perceived predation risk. Communication is an important phenotypic trait that is involved in a variety of intraspecific and interspecific contexts. Communicating the wrong information, or to the wrong receiver, or at the wrong time, can all be costly, and therefore vocal behaviors are relatively more labile than other behavioral traits. In order to confirm that our playback manipulations were perceived by the great tits as risky we examined individual birds' immediate response to playback. Changes in vocal behavior should be relatively less costly than other behavioral or life history responses. Therefore, we conducted this study to determine if great tits perceive our treatment, so that if further studies would not find a response it could be determined that this was due to no response, not a failure of our design. Additionally, anti-predator response should vary depending on the predictability or longevity of risk. Therefore, if prey can predict the hunting scheme of the predator, then anti-predator response to a resident predator should persist even after immediate predator cues cease. Traditionally, studies of vocal responses to predators focus on brief play backs and immediate responses. Our study is unique in that we performed playback for multiple months and measured prey vocal response to playback up to 4 days after playback cessation. This allowed us to explicitly examine the temporal dynamics of anti-predator responses; exploring potential habituation across the season and if anti-predator response was immediate or persisted after cessation of the predator cue.

In Chapters 2 (Abbey-Lee et al. 2016b) and 3 (Mathot et al. In Press), I examined the effects of perceived predation risk on great tits during the winter when they face both starvation and predation risks. Specifically, we monitored the change in body mass, exploratory behavior, and metabolic rate, and if these changes depended on an individual's behavioral type. These studies fill a large gap in the literature by examining whether individual differences in vulnerability to predation (inferred from exploration tendency) predict how individuals respond phenotypically to changes in perceived predation risk. Importantly, the design of the experiment allows for distinctions between responses to risk via plastic responses within individuals versus specific types of individuals selectively leaving the area. If individuals respond differently to risk depending on their vulnerability, then those types of individuals that respond more likely pay higher costs (as responding is costly). This would lead to differential selection pressures on behavioral types, implying that predators may non-consumptively alter selection pressures.

In Chapter 4 (unpublished manuscript), I examined the behavioral and life history responses of great tits to increased perceived predation risk during the breeding season. How perceived predation risk influences life history in free living species is greatly understudied. Also, despite recent research indicating that environmental stress influences the expression of variance components, few studies test how exposure to predators may alter the variance components that shape the heritability of ecologically relevant traits, and therefore their environment-specific evolutionary potential. Specifically, we monitored individual great tits' life history patterns and conducted behavioral assays across two years. The design of our experiment allowed us to examine plasticity within and among individuals exposed to the same treatment across the years (6 of 12 plots), to test if repeatability differed in the different predation regimes. Using these same individuals, we tested if predation treatment altered correlations between behavior and life history. This relationship should change as a result of predation pressure because individuals of certain behavioral types are more susceptible to predation risk (e.g. via increased encounter rates). Thus, certain individuals should shower greater anti-predator response and modify their life history more than others, altering the relationship between behavior and life history. Additionally, our experimental design also allowed us to examine individuals that were exposed to a different treatment in each year (6 of 12 plots), to determine if traits were repeatable across contexts and if different types of individuals responded differently to predation risk. Notably, with this study we were able to determine if perceived predation risk has the potential to alter evolutionary processes and if different behavioral types were under differential selection pressures, validating the importance of incorporating individual differences into the study of perceived predation risk for a complete understanding of micro-evolution in natural populations.

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Chapter 1

Immediate and carry-over effects of perceived predation risk on communication behavior in wild birds

Abstract

Predation induces strong selection on phenotypic traits, affecting prey via consumptive and nonconsumptive effects. Communication is important for territorial behavior, but can increase susceptibility to predators. Therefore, predation risk should influence prey communication patterns, territoriality, and fitness. We evaluated how this trade-off is resolved via 2 manipulations of perceived predation risk using audio playback in wild great tits (Parus major). In the first experiment, we examined the immediate response of individuals to playback; the second experiment, replicated across 2 years, examined both immediate and carry-over effects (over days) of playback broadcast for a 5-month period (March–July) in 12 nest box populations. Birds exposed to predator sounds showed decreased singing and increased alarming compared to controls, both immediately and on days without play back exposure (carry-over effects). Perceived predation risk did not affect how birds responded to simulated territory intrusions. In combination, these studies' findings imply that individuals perceive predator vocalizations as "risky" up to days after exposure to predator cues, and adjust their behavior to minimize this risk. However, the lack of effects on territorial aggression implies that individuals are able to weigh the costs of predation against the benefits of each type of vocal behavior. Acknowledging that context changes the relative costs and benefits of antipredator behavior has important consequences for understanding predator–prey dynamics.

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Introduction

Predation is one of the most important selection pressures acting on phenotypic traits. Traditionally, studies have focused on selection induced by the direct killing of prey by predators; however, predators can also affect prey through non-consumptive effects (Brown and Kotler, 2004; Cresswell, 2008; Lima, 1998a; Lind and Cresswell, 2005; Preisser et al., 2005). Specifically, predation risk affects the costs associated with the expression of various behavioral and morphological traits; prey often adjust such traits in response to changes in risk as a form of adaptive phenotypic plasticity (Lima, 1998b). Such effects can either represent adaptations to predation risk *per se* or to predator-induced changes in prey ecology. For example, the presence of predators may induce prey to live in groups, which in turn may favor phenotypic traits allowing prey to cope with intra-specific competition (Werner and Peacor, 2003). Predators thereby can affect a large range of phenotypic traits either directly or indirectly related to predation, including morphology (Relyea and Werner, 2000), life-history (Eggers et al., 2006; Zanette et al., 2011), foraging (Macleod et al., 2005), vigilance (Brown, 1999) and parental care (Bonnington et al., 2013; Ghalambor et al., 2013; Tilgar et al., 2011; Zanette et al., 2011).

Communication is an important phenotypic trait that is likely affected by perceived predation risk. Communication is involved in a variety of intra- and interspecific contexts with obvious benefits but also energetic costs (Stoddard and Salazar, 2011). Importantly, the production of signals at the wrong time can put the emitter at risk. This potential risk arises from eavesdropping, the use of information by individuals other than the primary target (Peake, 2006). Eavesdropped information can be used by predators to locate prey, assess prey quality, and predict attack success. Indeed, acoustic signals are often exploited by predators (Burk, 1988; Zuk and Kolluru, 1998). For example, fringe-lipped bats (*Trachops cirrhosis*) are better able to track their prey when they are chorusing (Tuttle and Ryan, 1981), and models of crested tits (*Lophophanes cristatus*) are attacked more frequently by Eurasian sparrowhawks (*Accipiter nisus*) when accompanied by calls (Krams, 2001).

One important context for communication is territory and mate defense (Bradbury and Vehrencamp, 2011; Catchpole and Slater, 2008; Krebs et al., 1978; McGregor, 1993). In many bird species, males defend breeding territories. Territory defense often involves vocal communication in the form of singing (to advertise quality, deter other males, and attract females), calling (alarm or contact, sharing information between partners or neighbors), as well as activities such as patrolling and aggressive behavior (physically fighting with other males in the area) (Krebs, 1971; Krebs et al., 1978; Naguib, 2005; Todt and Naguib, 2000). Successfully defending a territory and mate is often essential for male fitness, however, these very same behaviors simultaneously increase an individual's visibility, and therefore susceptibility, to predators (Kim et al., 2011; Lima and Dill, 1990). Consequently, the demography of predators should affect the use of behaviors involved in territorial communication.

Given the predation cost induced by communication, one may expect birds to alter their behavior in the presence of predators. Individuals can use direct or indirect cues, or a combination of both, to acquire information about predation risk (Nersesian et al., 2012). For example, experimental increases in perceived predation risk led to a reduction of singing in blue petrels (*Halobaena caerulea*) and veeries (*Catharus fuscescens*) (Mougeot and Bretagnolle, 2000; Schmidt and Belinsky, 2013), while crested tits switch to short-range calls when feeding in risky places (Krama et al., 2008). Empirical studies thus imply that mobbing and alarm calls are generally used in response to the presence of predators, despite the potential costs to the caller, likely because alarm calls are important for communicating risk with mates and the properties of the calls make them less localizable (Klump and Shalter, 1984; Marler, 1955). At the same time, there is some evidence that mobbing calls may attract nest predators and result in nest predation (Krama and Krams, 2005). Nevertheless, overall, predators usually influence prey demography via consequences to communication and territoriality.

It is expected that individuals minimize predation risk; however, such anti-predator response often precludes other behaviors. Theory predicts that the evolved decision of which behavioral option to pursue should depend on the costs of predation and the benefits associated with each behavioral option (Lima, 1998a). Therefore, predators should only affect communicative signals whose costs of predation outweigh the benefits. We thus expect to see the strongest anti-predator response in situations where communication has the fewest relative benefits. Conversely, we do not expect to find strong effects of increased perceived predation risk in situations where the communicative signal has major benefits. For example, males of territorial species such as great tits (*Parus major*) cannot afford to lose their territory when challenged by intruders, since they cannot expect to easily acquire another. Owners usually win territory contests, therefore acquiring a replacement territory is difficult once territories are established (Krebs, 1982). Additionally, great tits tend to occupy their previous year's territory, making it difficult for a replaced bird to establish a territory in subsequent years as well (Harvey et al., 1979). The context in which behavior is expressed should thus influence the strength of the observed response to perceived risk of predation.

Anti-predator response is also predicted to vary depending on the predictability or longevity of risk (Bosiger et al., 2012; Kronfeld-Schor and Dayan, 2003; Lima and Dill, 1990). Species of predators vary in many aspects such as when and where they forage, and whether they tend to re-visit the same location. Therefore, prey should perceive predation cues as predicting risk on different temporal scales (from seasonal to daily to hourly) depending on predator types in the area (Lima and Bednekoff, 1999; Lima and Dill, 1990). Many forms of temporal risk are predictable enough for prey to respond adaptively to changes (Kronfeld-Schor and Dayan, 2003). Therefore, if prey can predict the predator scheme (i.e. whether or not a detected predator typically remains in the area) then anti-predator responses to a predator that remains should persist after immediate predator cues cease. This notion has received considerable support in the literature regarding other predator cues and developmental plasticity of morphological traits (e.g. Bytheway et al., 2013; Coslovsky and Richner, 2011; Lima and Bednekoff, 1999). However, to our knowledge, only one other study has examined the vocal response of birds to predator playbacks on a time scale longer than immediately after playback: Schmidt and Belinsky (2013) examined vocal response for one evening post predator playback. Most studies broadcast brief playbacks (under 5 minutes) and record the prey's immediate response. We found two other studies that broadcast playback for extend periods (Gursky, 2002, 2003), however response data was only collected immediately after playback bouts. Our study is unique in that it measures prey vocal response to playback up to 4 days after playback cessation.

Using a playback experiment, we investigate how calls of an avian predator (Eurasian sparrowhawk) affect communication behavior in great tits, whether those effects are immediate vs. long-lived, and how they are affected by the context in which the communicative signal is expressed. The sparrowhawk was chosen for two reasons. First, it is the main avian predator of great tits (Geer, 1978; Perrins and Geer, 1980) and has been shown to alter great tit breeding success (Götmark, 2002). Second, its territorial hunting style predicts that great tits should continue to act as if predation is possible even after cessation of the predator cue, allowing for examination of long term effects throughout an entire breeding season (Newton, 1993; Newton et al., 1977). Great tits mainly communicate via songs and alarm calls. They use songs to advertise quality and attract a mate and they use a variety of alarm calls to communicate with partners and neighbors (Latimer, 1977; Marler, 1957). Vocalizing in the presence of avian predators is risky. Therefore, we expected birds exposed to an increased perceived predation risk to reduce the rate of song bouts and increase alarming. Additionally, we expect that fewer individuals will choose the risky option of vocalizing in areas with increased risk. We expect that this pattern will remain throughout the season. Finally, we predict that this general pattern will hold across contexts, although in situations where the benefits of communication are great (i.e. territory intrusions) the response to predation risk will be weaker.

Materials and Methods

Short-term manipulations focusing on immediate responses

To investigate the immediate effect of predator vocalizations on behavior of great tits, we conducted a playback experiment in a 15 km^2 area around the Max-Planck Institute for Ornithology, Seewiesen, Germany (47°58'17’’N, 11°14'10’’E) (Fig. S1A). The area used for this study was composed of mixed deciduous forests, agricultural sites and small towns outside the forest plots used for our plot-level manipulations detailed below. Playbacks and observations were conducted between March 1 and April 15, 2014. A total of 50 different birds were exposed to one of two experimental treatments: 25 individuals to a playback of Eurasian blackbird song (a sympatric, avian non-predator species, *Turdus merula*), and 25 individuals to a playback of Eurasian sparrowhawk call (a sympatric, avian predator species). An additional 46 birds were lost during the observation period and are not discussed in this study. All observations were made by the same observer (AK). Bird sounds were acquired from the Xeno-Canto database (www.xeno-canto.org) or provided by Hans Heiner Bergmann. Twelve unique files were made for each sound type; each file was normalized with the software program Audacity 2.0.5 and edited to last 10 seconds. Sounds were played through a powered portable speaker (Shockwave, Foxpro, Pennsylvania, USA) at a distance of 15 m. The order of sound type was random. The observer broadcast white noise into headphones during the playback. In this way, the observer was blind to the treatment and avoided potential bias (Milinski, 1997). Data were collected when birds were most active, between sunrise (approximately 0620h-0650h) and 1400h. Experiments were applied to 3-8 individuals daily.

Experimental birds were selected haphazardly when located (either visually or acoustically). To avoid problems related to pseudo-replication (Hurlbert, 1984), each site was visited only once and the minimum distance between two observations was 150 m (Krebs, 1971). Birds were observed during 130 seconds divided into three periods: a 60-s baseline period (before), followed by a 10-s playback period (during) and another 60-s period (after). During the 'before' and 'after' periods, behavioral traits were recorded. The observer scored the number of song bouts and alarm calls emitted by the subject. For each observation period, the observer additionally recorded the subject's initial behavior (silent, singing, alarming or contact calling) and whether or not the focal bird was lost.

Repeated plot-level manipulations focusing on immediate and carry-over effects

To simultaneously investigate immediate and carry-over behavioral effects of predator vocalizations, we conducted a playback experiment during spring (March-July) 2013 and 2014. The experiment was performed in 12 forest plots that were established in a 15 km^2 area around the Max-Planck Institute for Ornithology, Seewiesen, Germany (Fig. S1B Araya-Ajoy and Dingemanse, 2014; Mathot et al., 2015; Nicolaus et al., 2015; Stuber et al., 2013). Plots consisted of 50 nest boxes arranged in a regular grid spanning approximately 9-12 ha. In order to manipulate perceived predation risk, four speakers (Shockwave, Foxpro, Pennsylvania, USA) were evenly distributed in each plot in February 2013 and 2014 (Fig. S2). Six plots received a control treatment and six plots received a predator treatment. The same species were used as in the immediate response project (detailed above), in control plots, speakers were programed to play sounds of blackbirds. In predator plots, speakers were programmed with calls from sparrowhawks. All speakers were programed following the same scheme, matching the normal timing and frequency of vocalization of our playback species. That is, for the first 3 hours after dawn, and for the last 3 hours before dusk, speakers broadcast, on average, six 6-minute song/call bouts per hour (i.e., 60% of the time). During the daylight hours between these two intensive periods, speakers broadcast approximately 1.5 bouts per hour (i.e. 15% of the time). The amount of silence between playbacks was determined randomly to avoid habituation. Playback was given for 4 consecutive days ("on"), followed by 4 consecutive days of non-playback (speakers were off), after which the cycle was repeated, throughout the season; this design is known to prevent habituation (Zanette et al., 2011), and is used here to investigate whether treatment effects were specific to speaker status (i.e. "on" vs. "off" days). This design thereby enables investigation of immediate and carry-over effects of predator cues. As part of another study (in progress), we were interested in applying these treatments until all nestlings had fledged, hence playbacks were continued until late July.

Point counts were performed throughout the season in order to record great tit vocalization behavior; we collected 2.3 ± 0.9 (mean plus standard error; range 1 - 4) point counts per plot per each period (every four days). Point counts were conducted at a 10-m horizontal distance from the loudspeaker on both on and off days; on "on"-days only when the speakers were not actively broadcasting. Each point count consisted of one of 17 potential observers standing still and counting all songs and alarm calls made by great tits within a five minute period. Using information on the direction from which calls were emitted, the minimum number of great tits vocalizing during the observation was also determined. In order to prevent bias in our point count data (Milinski, 1997), the majority of observations (79%) were conducted by seasonal observers that were aware of the speakers in the plots, but not of purpose of the data collection, experimental design or theoretical predictions.

Simulated territory intrusions

Within the same 12 forest plots, simulated territorial intrusions (i.e. "aggression tests") have been applied to each breeding male from breeding season 2010 onwards (for exact procedure, see Araya-Ajoy and Dingemanse, 2014; briefly described below). Territorial intrusions were simulated by placing one of 17 taxidermic mounts of a male great tit as a visual stimulus with one of 20 playback songs as an acoustic stimulus one meter in front of a nest box. Each breeding male was subjected to four aggression tests: two during egg-laying (one and three days after the first was laid) and two during incubation (one and three days following the onset of incubation). Tests were performed for first breeding attempts only, and were conducted between 0700h and 1200h with the specific time for each nest semi-randomly assigned. One of 18 observers performed the observation at a distance of 15 m. Taxidermic mount, song, and observer, were all randomly assigned. Following the arrival of a focal male within a 15m radius of the nest box, we recorded the following behaviors within a 3-min period: number of calls, number of songs, and minimum distance from the mount ('approach distance'). For ease of interpretation, approach distance was multiplied by -1 throughout the paper (i.e. higher values thus represent a more aggressive response). Approach distance was included in this study because individuals that engage in physical contests are less alert to cues of predator approach and more susceptible to predator attack. Subjects that did not arrive within 15 minutes were scored as non-responsive. In the two years where we applied the predation treatment, we performed 811 (2013) and 870 (2014) tests. Only tests where male identity was known (n=1201 tests (71%), n=244 unique (ringed) males) were used for statistical analysis; in 806 (67%) of these tests, the male responded.

Statistical analyses

All statistical analyses were performed with R 3.1.1 (R Core Team, 2013). We applied generalized linear mixed-effects models to analyze our data (detailed below), for which we used the “glmer” function (package lme4). Additionally, we used the “sim” function (package arm) to simulate the posterior distribution of the model parameters and values were extracted based on 2000 simulations (Gelman and Hill, 2007). The statistical significance of fixed effects and interactions were assessed based on the 95% credible intervals (CI) around the mean (β). We consider an effect to be ‘significant’ in the frequentist’s sense when the 95% CI did not overlap zero (Nakagawa and Cuthill, 2007). We used visual assessment of the residuals to evaluate model fit.

Repeatability estimates of all variables were calculated following Nakagawa and Schielzeth (2010). For Gaussian response variables (approach distance) it was calculated as the between-individual variance divided by the sum of the between-individual and residual variances. For variables following Poisson distributions we used an observation-level random effect to estimate the dispersion parameter. Residual variance is taken to be π2/3 for binomial models (Nakagawa and Schielzeth, 2010).

Short-term manipulations focusing on immediate responses

To test the effect of the different sound stimuli on great tit behavior, we ran univariate linear mixed-effects models in which either the number of song bouts or the number of alarm calls was the response variable. Both variables were count data and were therefore modelled with Poisson errors. Type of playback (blackbird vs. sparrowhawk, categorical variable), period (before vs. after playback, categorical variable), and the interaction between period and playback type were included as fixed effects. Additionally, time since sunrise (in minutes; mean centered) and Julian date (mean centered) were included as fixed effects to account for temporal and seasonal patterns in birdsong. Individual identity was included as a random effect.

Repeated plot-level manipulations focusing on immediate and carry-over effects

We ran univariate generalized linear mixed-effects models with either the number of song bouts, the number of alarm calls, or the number of birds vocalizing as separate response variables to test the effect of the different sound stimuli on great tit behavior. Because our response variables were counts, we fitted Poisson errors. Treatment (control vs. predator, categorical) and speaker status (whether the playback was broadcasting that day: “on” vs. “off”, categorical), and their interaction, were included as fixed effects. Additionally time since sunrise and date were also included as covariates to account for temporal and seasonal patterns in birdsong. We accounted for various ways in which the data were non-independent by including three random effects. First, the unique combination of plot and year (‘PlotYear’; n = 24 levels) was included because the predator treatment varied at this level. Second, the unique combination of day, plot, and year (‘DayPlotYear’; n= 471 levels) was included because the interaction between predator and on/off treatment was specific to each unique combination of day, plot, and year. Finally, the unique combination of speaker identity and year (‘SpeakerYear; n = 96 levels) was included to account for year-specific micro-environmental spatial effects. Additionally, observer identity was added as a random effect.

We also ran the above model incorporating the three-way interaction between date, treatment, and speaker status in order to account for possible changes to the cost/benefit ratio over the season. Adding this interaction did not change any of our model results and produced an unstable estimate due to the ratio of parameters to degrees of freedom, and therefore we do not report these results in this manuscript.

Simulated territory intrusions

To test the effect of the different sound stimuli on responses to simulated territorial intrusions, we used the model structure described in the previous section, using the following response variables: probability to respond (with a binary error structure), number of song bouts (Poisson errors), alarm calls (Poisson errors), or approach distance (Gaussian errors). Two slight changes were made to the structure of the random effects. First, simulated territory intrusions were not associated with specific speakers, so that variable was not included as a random effect. Second, for this data individual identity was known, so it was included as a random effect. Observer identity was not added as a random effect for this model because previous data showed that it was non-significant (Araya-Ajoy and Dingemanse, 2014).

Results

Short-term manipulations focusing on immediate responses

Compared to controls, birds exposed to sparrowhawks tended to differ in the number of songs (β=1.14, 95% CI =-0.30, 2.66) and differed in the number of alarm calls (β= -1.85, 95% CI = -3.62, -0.09) prior to treatment application (Table 1), which was unexpected since treatment group was applied randomly. Both the number of songs and the number of alarm calls were influenced by the interaction between period (before vs. after) and playback type (blackbird vs. sparrowhawk) (Table 1). For number of songs, the interaction was due to individual birds exposed to sparrowhawk calls decreasing their song output after exposure to playback, whereas control birds did not (Fig. 1). For number of alarms, the interaction was due to individual birds exposed to control calls decreasing alarm calling after exposure to playback, whereas birds exposed to predator sounds did not (Fig. 1). Additionally, birds produced more songs and fewer alarm calls later in the day (Table 1). Both behaviors harbored (short-term) individual repeatability (0.89 (0.77, 0.95) and 0.74 (0.64, 0.83); respectively).

Repeated plot-level manipulations focusing on immediate and carry-over effects

We conducted 1,420 point counts (716 in control plots; 704 in predator plots), of which 326 (45%; control plots) and 331 (47%; predator plots) were on days that the speakers were actively broadcasting ("on"). In 525 out of 1,420 cases (37%), no bird vocalized; the probability of no vocalization did not differ between treatments: 266 out of 716 (37%; control treatment) vs. 259 out of 704 (37%; predator treatment).

For the 895 point counts where at least one bird was vocalizing, we found that the number of song bouts per bird and number of birds vocalizing were influenced by the playback treatment but the number of alarm calls per bird was not (Table 2). The treatment showed long-term effects on vocalization behavior: compared to the control plots, fewer birds vocalized in the predator treated plots on days the speakers were not actively broadcasting (β= -0.26, 95% CI =-0.39, -0.14). Additionally, birds exposed to predation playback sang less than birds exposed to control sounds during off days (β= -0.27, 95% CI = -0.50, -0.05). There was no difference in alarming behavior for birds exposed to predator playback compared to those in the control areas during off days (β=0.24, 95% CI = -0.32, 0.77). Compared to days where the speaker was not broadcasting, fewer birds vocalized in control plots on days the speakers were on (β= -0.12, 95% CI = -0.24, 0.00). In contrast, there was no difference in number of birds vocalizing in the predator plots based on speaker status (Fig. 2), creating a strong trend for number of birds vocalizing to be influenced by the interaction between treatment and speaker status (β= 0.17, 95% CI = -0.02, 0.35). The years differed: compared to 2013, there were fewer birds vocalizing, more alarms per bird, and fewer songs per bird in 2014.

Simulated territory intrusions

We performed 1,681 simulated territory intrusions. We analyzed data for the 1,201 cases where male identity was known. Of these, in 806 cases the male responded; 424 cases in control plots and 382 cases in predation treatment plots. We found no difference in probability of response between treatment groups on off-days (β= -0.18, 95% CI = -0.69, 0.32), no effect of speaker status (on/off) in control plots (β= -0.15, 95% CI = -0.60, 0.28), and no treatment-specific effect of on/off (β= 0.49, 95% CI = -0.12, 1.13) (Table 3). However, breeding context and time of day did have significant effects on probability of response. Males with nests in the incubating phase were more likely to respond than birds with nests in the laying phase (β= 1.43, 95% CI =1.14, 1.73), as well as birds tested earlier in the day (β= -0.20, 95% CI =-0.34, -0.07).

Of the 806 tests in which males responded, we found no effect of treatment, speaker status, or the interaction between treatment and speaker status for any of our response variables: number of songs, number of alarm calls, or approach distance (Table 3). Other effects were similar to those reported for these study populations in previous years (2010-2012; Araya-Ajoy & Dingemanse 2014): breeding context was an important predictor of responses to simulated territorial intrusions; the number of songs increased with breeding stage (β=0.45, 95% CI = 0.26, 0.63) while number of calls, and approach distance, decreased (β= -1.74, 95% CI = -2.27, -1.19 and β= -0.67, 95% CI = -0.86, -0.49; respectively). Additionally, the number of alarm calls was negatively influenced by time of day and sequence (nested within breeding context) (β= -0.27, 95% CI = -0.52, 0.00; β= -0.77, 95% CI = -1.31, -0.23).

Birds occupy nest boxes throughout the plots. Therefore, for the simulated territory intrusions there was no standard distance from observation point to speaker as in the other parts of this paper. It is possible that effects of the treatment are only detectable at short distances from the speaker. However, adding distance to nearest speaker (m) to these models had no effect: there was no effect of distance, treatment, speaker status, or any of the two- or three-way interactions among these variables (Results not shown).

Discussion

This study evaluated both immediate and carry-over effects of vocal responses of free-living great tits exposed to an experimental manipulation of perceived predation risk in different communicative contexts. We found that predator vocalizations influenced various aspects of acoustic behaviors. Effects were both immediate and persisted for a number of days after predator cues had last been broadcast. Specifically, birds exposed to avian predator sounds showed increased alarming and decreased singing tendencies compared to birds exposed to control sounds, both immediately and days later. However, we found that perceived predation risk treatment did not affect how birds responded vocally to simulated territory intrusions. These results suggest that individuals perceive predator vocalizations as “risky” and adjust their behavior accordingly. However, when facing the trade-off between territory defense and predation risk, the benefits of territory defense may greatly outweigh the costs of increased predation exposure, explaining why vocal communications used by territory owners was not affected by perceived predation risk. This supports previous work that shows that perceived predation risk alone can influence prey behavior (Mougeot and Bretagnolle, 2000; Schmidt and Belinsky, 2013) and implies that such effects occur only when the relative costs and benefits associated with a given behavior are greatly affected by predation risk.

Immediate versus carry-over effects

We found that in response to predator vocalizations birds decreased their singing and increased their alarming behavior immediately relative to those exposed to control sounds. This is in accordance with other playback experiments showing immediate effects of predator cues (Mougeot and Bretagnolle, 2000; Schmidt and Belinsky, 2013). Our findings imply that individual great tits are able to differentiate between playback types, and that predator vocalizations alone are enough to elicit a behavioral response.

Similarly, when we examined responses to predation risk treatments applied repeatedly over longer time frames (four consecutive days), we found that in areas with increased perceived predation risk, fewer birds vocalized and there were fewer songs produced per bird. The effect of fewer birds vocalizing was not due to a decrease in birds in the area; breeding densities were the same between predator and control plots (predator = 17.3, control = 17.7, β= -0.31, 95% CI= -3.70, 2.72; mixed-effect model with random intercepts for plot and treatment as a fixed effect). On days when the speakers were actively broadcasting, point count data collection took place between one minute and 1.5 hours after a sound bout. Indicating that, at least for sparrowhawk predators, great tits perceive risk as lasting for at least 1.5 hours. However, we found no effect of treatment on response in the simulated territory intrusion context. The set of experiments thereby demonstrates that great tits are capable of perceiving and responding to changes in predation risk but only choose to do so in certain contexts.

Finally, examining individuals' responses on days the speakers were not actively broadcasting, and across the entire breeding season, allowed us to look at risk perception and carry-over effects on behavior after cue removal. To our knowledge, no other studies have examined the vocal response of birds to predator playbacks on such time scales. In concurrence with the short-term results, we found that areas with increased perceived predation risk had fewer birds vocalizing and fewer songs per bird. Because there was no difference in singing behavior or number of birds vocalizing depending on speaker status, but there was a difference between treatment groups, we can conclude that birds do not interpret predator vocalizations as merely signifying immediate danger. Instead, for at least four days after predator vocalization playback, bird behavior remains altered. This finding is consistent with the notion that cues of avian predators predict future predation risk in this study system. Our study indicates that individuals modify their behavior in response to predation risk, and that the behavior of birds differs depending on the perceived level of risk in the area. Thus, actual or perceived predation risk may influence ecosystems by altering selection pressures.

Additionally, for the short-term experiment we found that birds assigned to the treatment group alarmed less before playback. This difference in initial behavior was unexpected, as individuals were randomly assigned to treatment groups, so we would expect no difference in the before period. Our randomization scheme was such that there was no bias between the treatments due to date or time of day (as all dates received both treatments, and the order of treatments was randomized throughout each day). Therefore, we feel this is merely a sampling artifact that would disappear if the study was repeated, or if sample size was increased. In support of this, we find the same results when analyzing the change in number of songs or alarms (after-before, data not shown). This highlights the importance of using a study design that includes before and after data. In this way we are still able to detect differences between treatments in how individuals change in response to playback, despite initial differences between individuals and groups.

Context-specific aspects of perceived predation risk

We found that detection of a response to perceived predation risk depended on the context in which it was measured. We found a clear vocal response to perceived predation risk when monitoring daily vocalizations, and no response during simulated territory intrusions. Individuals are able to weigh the costs of predation against the benefits of various other behaviors (Lima, 1998a). A study by Greig and Pruett-Jones (2010) in splendid fairy-wrens (*Malurus splendens*), found that females were more attentive after hearing a predator vocalization, and that males sang more after such vocalizations, perhaps to capitalize on the extra attention from females. Conversely, a study examining territory defense in a group living bird species found that groups responded less aggressively in more risky areas (Sorato et al., 2015). However, their focal species have large territories and are cooperative breeders, so the losses they face in an intrusion event would likely only result in partial loss of the territory, and no loss of mate. For great tits, the male suffers high costs associated with territory loss. At a minimum, the male will likely lose the brood for the year but given the hierarchal social system, the male may never regain a territory and therefore lose future breeding opportunities. This study provides further evidence that males may accept increased predation risk if the potential gains in terms of mate guarding, or acquiring, are large enough.

We are aware that the stimuli used to increase perceived predation risk and to simulate territory intrusion represented different timescales of threat and could have influenced our findings. We broadcast calling predator sounds to increase predation risk, however our predators do not call while actively hunting. This means our predator treatment merely indicated that predators are around in the area, but not that there is necessarily an immediate threat of attack. However, for the simulated territory intrusions we used a great tit model and playback. This did represent an immediate threat to the nestbox and territory. Although the predator playbacks were not usually broadcasting during our simulated territory intrusions, indicating that a sparrowhawk could be actively hunting at the time, it is possible that great tits still perceived the territory intrusion stimulus as a more immediate threat than that of the predator. Additionally, the confined space of the simulated territory intrusions (within 15 m of nest box) may decrease the predation risk in comparison with the point count observations. During the simulated territory intrusions, individuals are often observed close to the nest box, which means they are often closer to the ground and inner parts of the tree, perhaps also ameliorating predation exposure. This study was not designed to determine the titration point between the opposing needs of territory defense and predation avoidance.

Interesting future studies could manipulate the levels of predation risk, or intruder male quality, to determine how individuals weigh the relative risk of predation and territory or mate loss.

Conclusion

Our results show that birds are able to recognize predator vocalizations as risky; and that they respond to the increased risk by altering vocalization behavior after playback exposure immediately, and also when predator cues disappear temporarily (carry-over effects). Additionally, these responses were context specific, indicating individuals are able to assess the costs of predation and the benefits of various other behaviors.

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Table 1. Estimated effect sizes and 95% credible intervals around the mean of predictors of number of songs and alarms of individuals immediately after exposure to playback.

	Number of Songs	Number of Alarms
Fixed Effects	β (95% CI)	β (95% CI)
Intercept [a]	-0.52 (-1.72, 0.64)	-0.31 (-1.50, 0.84)
Treatment [b]	1.14 (-0.30, 2.66)	-1.85 (-3.62, -0.09)
Period [c]	0.10 (-0.27, 0.47)	-1.00 (-1.92, -0.07)
Day	0.07 (-0.01, 0.16)	-0.10 (-0.19, -0.02)
Time of day[d]	0.01 (0.00, 0.02)	-0.01 (-0.02, 0.00)
Treatment x Period[e]	-0.54 (-1.00, -0.06)	1.86 (0.44, 3.34)
Random Effects	σ2 (95% CI)	σ2 (95% CI)
Individual	4.84 (4.41, 9.82)	5.13(4.05, 8.54)
Residual	0.03 (0.02, 0.03)	1.32 (0.82, 1.53)
Repeatability	r (95% CI)	r (95% CI)
	0.89 (0.77, 0.95)	0.74 (0.64, 0.83)

[a] Reference category; estimate for blackbird treated birds during the before period

[b] Difference between the treatments (predation – control) during the before period

[c] Difference between the periods (after – before) for blackbird treated birds

[d] Time since sunrise in minutes, mean centered within year

[e] Difference between treatments (predation – control) in the difference between periods (after – before)

Table 2. Estimated effect sizes and 95% credible intervals around the mean of predictors of number of birds vocalizing and number of alarms and songs per bird during long-term exposure to playback.

	Number of Birds	Alarms per Bird	Songs per Bird
Fixed Effects	β (95% CI)	β (95% CI)	β (95% CI)
Intercept[a]	1.02 (0.90, 1.14)	-1.33 (-1.91, -0.72)	2.19 (1.89, 2.50)
Treatment[b]	-0.26 (-0.39, -0.14)	0.24(-0.32, 0.77)	-0.27 (-0.50, -0.05)
Speaker status[c]	-0.12 (-0.24, 0.00)	-0.03 (-0.53, 0.44)	-0.09 (-0.29, 0.12)
Year[d]	-0.14 (-0.26, -0.01)	0.87 (0.25, 1.45)	-0.71 (-1.00, -0.42)
Day	-0.003 (-0.005, -0.002)	0.02 (0.01, 0.02)	-0.02 (-0.02, -0.01)
Time of day[e]	-0.03 (-0.06, -0.00)	-0.03 (-0.12, 0.07)	0.00 (-0.04, 0.04)
Treatment x Speaker status[f]	0.17 (-0.02, 0.35)	0.07 (-0.58, 0.77)	0.11 (-0.18, 0.41)
Random Effects	σ2 (95% CI)	σ2 (95% CI)	σ2 (95% CI)
DayPlotYear	0.00[g]	0.18 (0.16. 0.21)	0.14 (0.12, 0.16)
SpeakerYear	0.00[g]	0.00[g]	0.04 (0.02, 0.04)
PlotYear	0.00[g]	0.14 (0.06, 0.21)	0.02 (0.01, 0.03)
Observer	0.01 (0.00, 0.02)	0.31 (0.19, 0.65)	0.13 (0.09, 0.24)
Residual	0.00[g]	3.94 (3.60, 4.30)	0.51 (0.49, 0.59)

[a] Reference category; estimate for control plots during the off-status for year 2013
[b] Difference between the treatments (predation – control) during the off-status
[c] Difference between speaker status categories (on – off) for control plots
[d] Difference between years (2014 – 2013)
[e] Time since sunrise in minutes, mean centered within year
[f] Difference between treatments (pred – cont) in effect of speaker status (on–off)
[g] At boundary, estimated as zero
[*] The zero estimate for residuals is due to modelling overdispersion parameters, and indicates that this model is not overdispersed, mean and variance are equal.

Table 3. Estimated effect sizes and 95% credible intervals around the mean of predictors of probability to respond, approach distance, and number of songs and alarms of birds during long-term playback and simulated territory intrusions.

	Probability to respond	Songs	Alarms	Approach distance
Fixed Effects	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Intercept [a]	0.34 (-0.11, 0.78)	1.71(1.41, 2.02)	0.14(-0.67, 0.93)	-2.60 (-2.90, -2.28)
Treatment [b]	-0.18 (-0.69, 0.32)	0.12 (-0.25, 0.48)	-0.29 (-1.18, 0.59)	0.14 (-0.20, 0.48)
Speaker status[c]	-0.15 (-0.60, 0.28)	0.07 (-0.22, 0.35)	-0.16 (-0.97, 0.64)	0.02 (-0.24, 0.25)
Breeding context[d]	1.43 (1.14, 1.73)	0.45 (0.26, 0.63)	-1.74 (-2.27, -1.19)	-0.67 (-0.86, -0.49)
Time of day[e]	-0.20 (-0.34, -0.07)	0.09 (0.00, 0.18)	-0.27 (-0.52, 0.00)	0.01 (-0.08, 0.10)
Sequence[f]	0.16 (-0.14, 0.46)	0.12 (-0.07, 0.31)	-0.77 (-1.31, -0.23)	-0.19 (-0.38, 0.00)
Year[g]	-0.26 (-0.62, 0.09)	-0.22 (-0.49, 0.05)	0.46 (-0.162 1.07)	-0.23 (-0.49, 0.02)
Treatment x Speaker status[h]	0.49 (-0.12, 1.13)	-0.16 (-0.56, 0.24)	0.23 (-0.91, 1.41)	-0.05 (-0.46, 0.34)
Random Effects	σ2 (95% CI)	σ2 (95% CI)	σ2 (95% CI)	σ2 (95% CI)
DayPlotYear	0.30 (0.27, 0.38)	0.09 (0.04, 0.06)	0.35 (0.29, 0.42)	0.15 (0.12, 0.17)
Individual	0.63 (0.58, 0.82)	0.09 (0.08, 0.11)	1.61 (1.33, 1.92)	0.34 (0.32, 0.46)
PlotYear	0.03 (0.01, 0.04)	0.05 (0.03, 0.08)	0.00[i]	0.03 (0.01, 0.04)
Residual	1.00[j]	1.63 (1.49, 1.77)	8.00 (7.14, 8.62)	1.17 (1.08, 1.31)
Repeatability	r (95% CI)	r (95% CI)	r (95% CI)	r (95% CI)
	0.20 (0.17, 0.23)	0.05 (0.04, 0.06)	0.15 (0.12, 0.17)	0.24 (0.21, 0.27)

[a] Reference category; control plots during off-status, laying phase, and year 2013
[b] Difference between the treatments (predation – control) during the off-status
[c] Difference between speaker status categories (on – off) for control plots
[d] Difference between breeding context categories (incubation – laying phase)
[e] Time since sunrise in minutes, mean centered within year
[f] Difference between test sequence categories (2nd – 1st test) within breeding context
[g] Difference between years (2014 – 2013)
[h] Difference between treatments (pred – cont) in the effect of speaker status (on – off)
[i] At boundary, estimated as zero
[j] Binomial, residuals fixed at 1

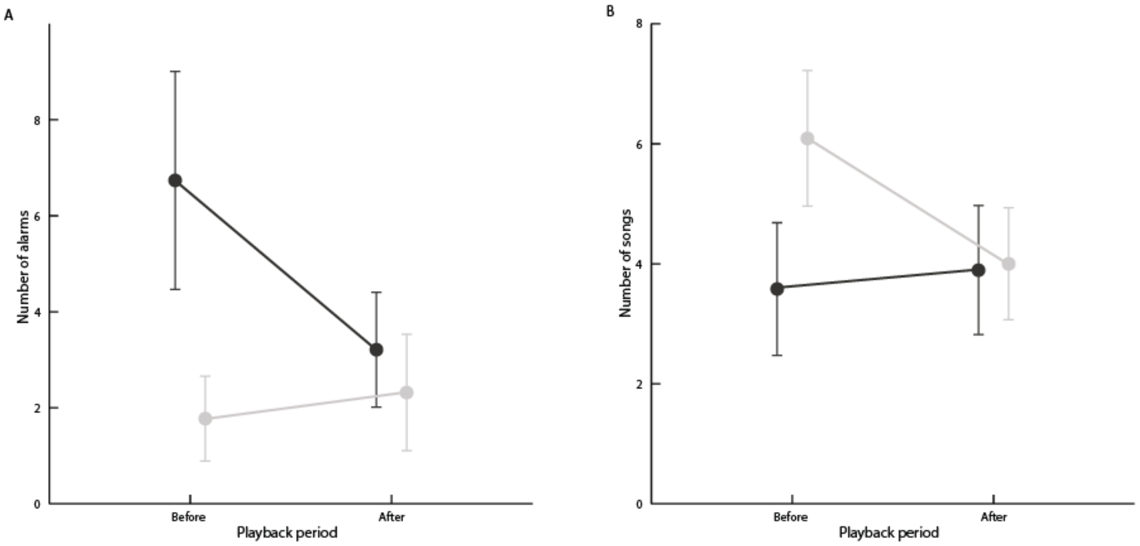


Figure 1. Number of alarms (A) and songs (B) in relation to playback period (before vs. after playback) and perceived predation risk treatment (control (black) vs. predator (grey) sounds). Data are from individuals observed immediately after playback. Points are means with standard error bars.

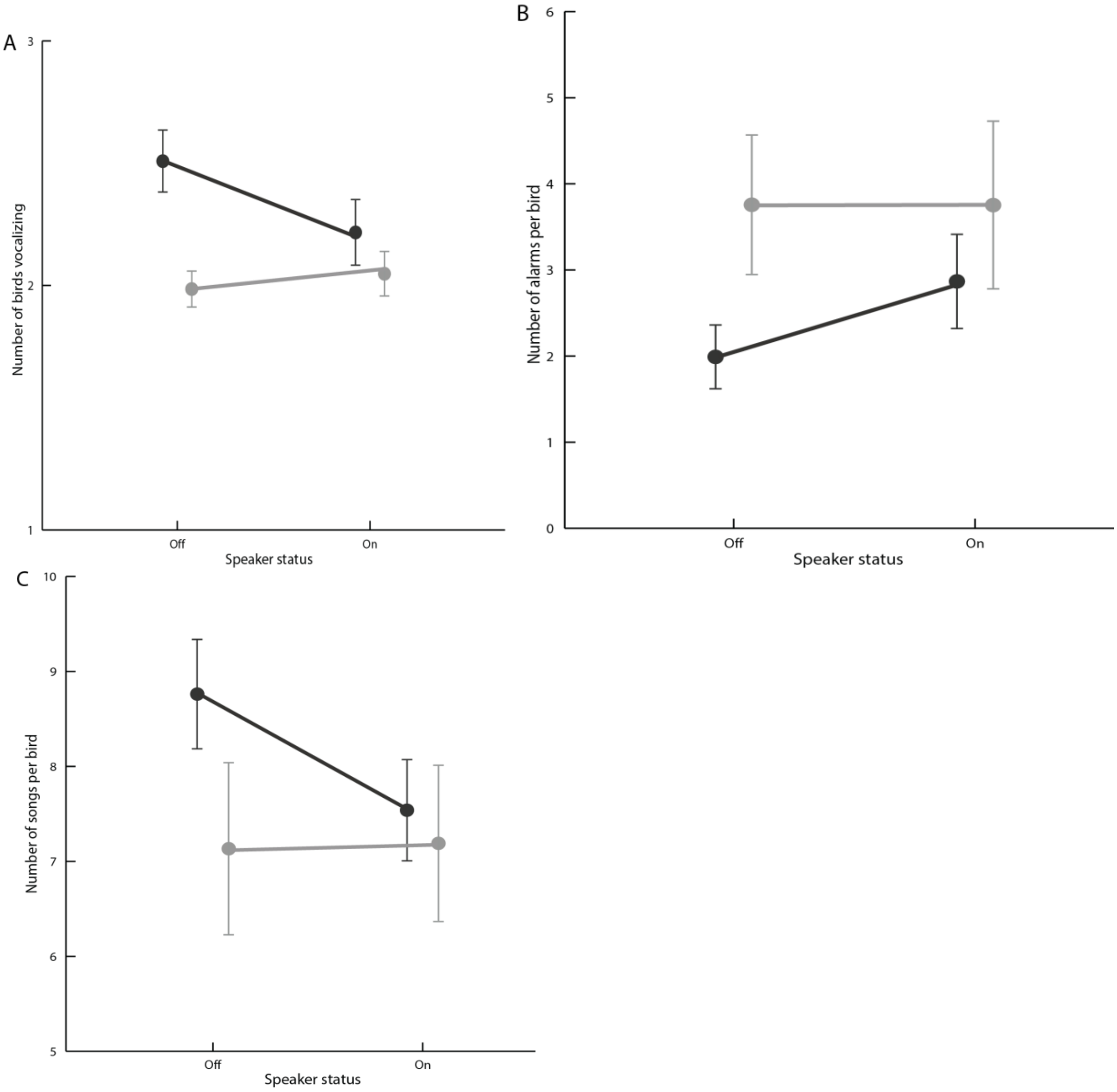


Figure 2. Number of birds vocalizing (A), and number of alarms (B) and songs (C) per bird in relation to speaker status (days speakers are actively broadcasting (on) vs. silent (off)) and perceived predation risk treatment (control (black) vs. predator (grey) sounds). Data are from populations observed during long-term playback. Points are means with standard error bars.

Supplementary Material

Table S1. Means and standard errors for raw data of interest.

	Songs		Alarms		Number of Birds		Approach Distance	
	Control	Predation	Control	Predation	Control	Predation	Control	Predation
Before[a]	3.6 (1.1)	6.1 (1.1)	6.4 (1.8)	1.8 (0.8)	-	-	-	-
After[b]	3.9 (1.1)	4.0 (0.9)	2.9(1.0)	3 (1.2)	-	-	-	-
Carry-over[c]	8.6 (0.3)	7.1 (0.3)	2.8 (0.3)	3.8 (0.4)	2.5 (0.1)	2.0 (0.0)	-	-
STI[d]	12.8 (0.6)	12.8 (0.6)	9.7 (0.9)	9.4 (0.9)	-	-	-3.1 (0.1)	-3.1 (0.1)

[a] Data from Table 1, the period before playback
[b] Data from Table 1, the period after playback
[c] Data from Table 2, songs are the songs per bird, alarms are alarms per bird
[d] Data from Table 3, simulated territory intrusions

Figure S1. Map of the study area for the immediate individual-level manipulations (A) and repeated plot-level manipulations and simulated territory intrusions (B).

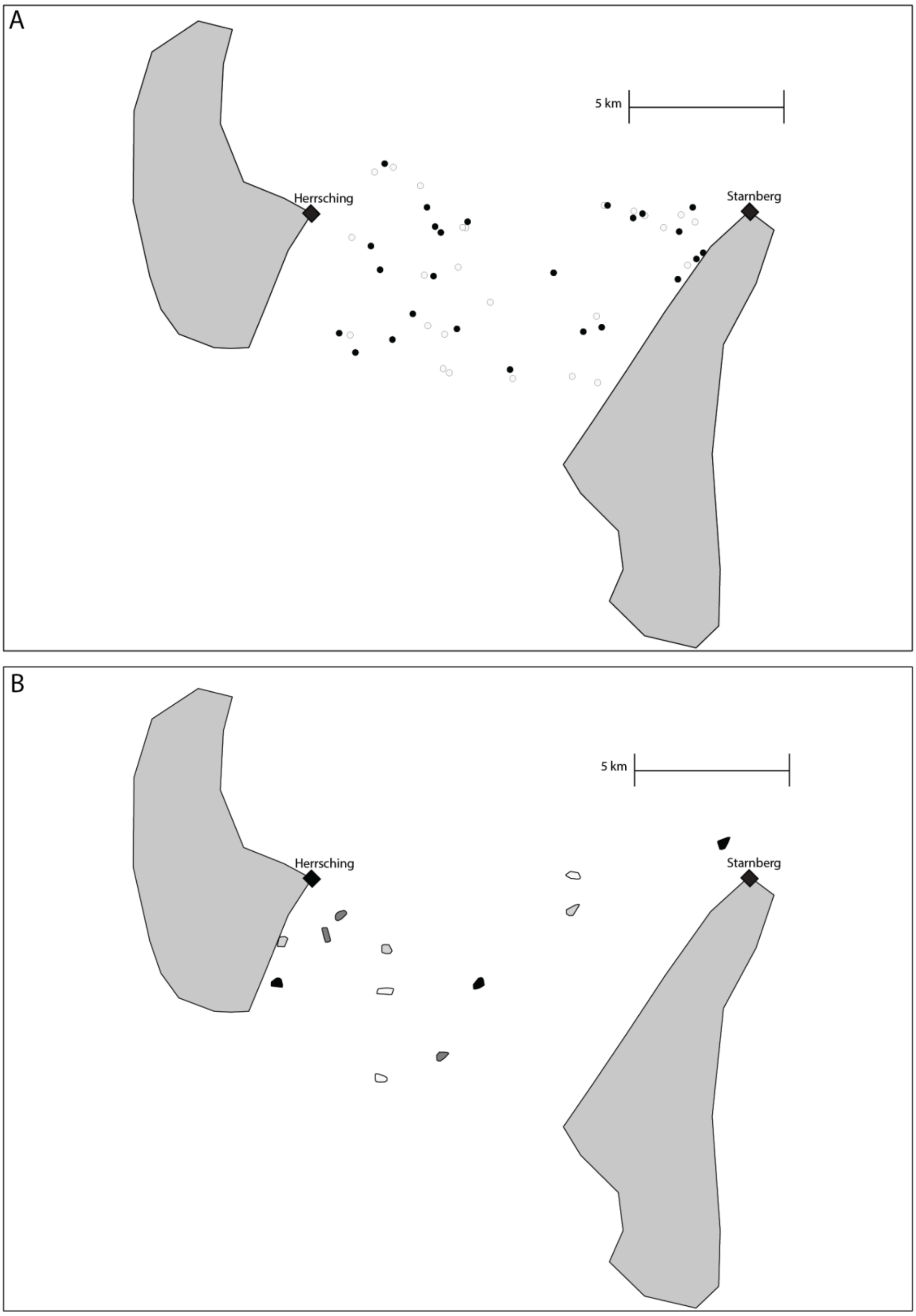
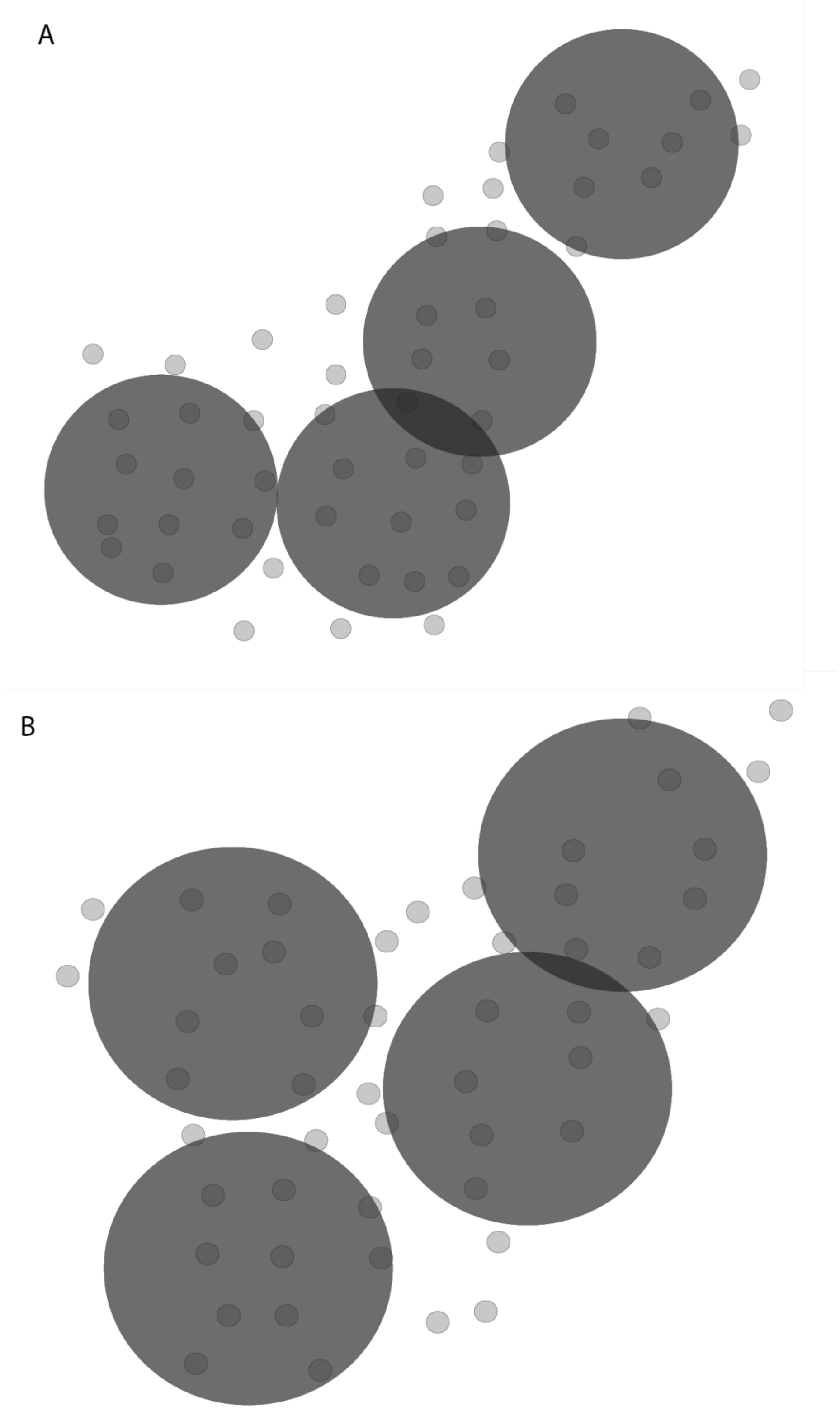


Figure S2. Examples of speaker location of two plots. Points indicate nest boxes and large circles indicate an approximately 100 m radius around each speaker. Speakers were placed to minimize overlap but maximize the number of nest boxes covered.



Chapter 2

Behavioral and morphological responses to perceived predation risk: a field experiment in passerines

Abstract

Predators can affect prey both directly (consumptive effects) and indirectly (nonconsumptive effects), with a growing body of literature showing the latter may have pronounced effects. Prey populations are comprised of individuals that differ in perception of and willingness to take risk; therefore, studying how different types of individuals respond to predation risk is necessary to fully understand prey dynamics. Playbacks were used to experimentally manipulate perceived predation risk in nest-box populations of wild great tits (Parus major) to examine the nonconsumptive effects of avian predators on prey behavior and morphology, and to explore individual differences in prey response. Individuals responded to our treatment, and responses differed depending on both treatment and premanipulation behavioral type. Birds in areas exposed to predator playback tended to decrease in body mass more than birds exposed to nonthreatening (control) playback. Differences between treatment groups were mainly driven by initially fast exploring birds: In the control treatment, fast explorers increased in mass, whereas the initially fast exploring birds in the predation treatment decreased in mass. Furthermore, birds exposed to predator playback decreased exploratory tendency compared with controls. These findings demonstrate that predation risk alters great tit behavior (exploration) and morphology (body mass) and that plasticity in response to risk relates to an individual's willingness to take risks. Our findings suggest that individuals differ in susceptibility to predation risk, causing adaptive individual differences in responsiveness to changes in predation risk. Acknowledging individuality in responses to perceived predation risk has important consequences for understanding prey dynamics.

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Introduction

Predators alter prey demography through direct consumption (Creel and Christanson, 2008; Huffaker, 1958; Lima and Dill, 1990; Nelson et al., 2004; Volterra, 1926), although a growing body of literature demonstrates that non-consumptive effects of predation can also have pronounced effects on prey populations (Brown and Kotler, 2004; Lima, 1998; Lind and Cressell, 2005; Preissier et al, 2005; Zanette et al, 2011). Indeed, perceived risk can cause changes in morphology, behavior, ecology, and social interactions (Werner and Peacor 2003). These phenotypic responses to predation risk suggest that the perception of predation risk alone affects the optimal expression of various phenotypic traits.

Individuals can actively match their phenotype to the environment in an adaptive manner through two mechanisms. Animals may 1) alter their behavior or morphology in response to changes in predation risk (i.e. adaptive phenotypic plasticity) or 2) move to a different (e.g. safer) environment (i.e. habitat matching mediated by selective appearance or disappearance). The evolutionary consequences of predation risk will depend on the relative magnitude of each of these mechanisms (Edelaar et al. 2008; Lima and Dill, 1990; Lind and Cresswell, 2005; Piersma and Drent, 2003).

Phenotypic plasticity can be exhibited in many ways. There is copious evidence that morphology can change when prey are exposed to elevated predation risk. For example, numerous studies have documented that body mass decreases with increased predation risk (e.g. Lilliendahl, 1997; Macleod et al., 2005; Perez-Tris et al., 2004; Witter et al., 1994, but see Pravosudov and Grubb, 1998). Body mass is relatively plastic and can change over short time spans. Decreases in mass may represent an adaptive response because lighter individuals are more maneuverable and can better escape predation (Gosler et al., 1995). Alternatively, decreases in body mass may result as a consequence of other adaptive responses to increased risk, such as decreased time spent foraging due to trade-offs between vigilance and foraging (Brown, 1999; Houston et al, 1993; Lima, 1998; Quinn et al., 2012).

Phenotypic plasticity in response to changes in perceived predation risk has also been described for various behavioral traits (Lima and Dill 1990; Preisser et al. 2005). Animals typically increase their investment in anti-predator vigilance with increasing predation risk (Lima and Dill 1990). Exploration behavior (defined as how much animals move through novel environments) is often linked to an individual's perception of risk, level of risk, and response to risk (e.g. Jones and Godin 2010, van Oers et al., 2004, Quinn et al. 2012). For example, fast exploring great tits (*Parus major*) are more willing to resume foraging following threatening disturbances (van Oers et al., 2004). Furthermore, animals that explore their environment more quickly are expected to be more conspicuous and have higher encounter rates with predators (Brown, 1999; Lima and Dill 1990), making exploration particularly costly in risky environments (e.g. Hedrick and Kortet 2006, Moses and Sih 1998). Consequently, reducing exploration behavior under conditions of elevated predation risk should constitute an adaptive response to manage increased predation risk.

In areas where predation risk varies in space or time, animals can also mitigate risk by moving away from risky habitats (Creel et al., 2005; Heithaus et al., 2009; Werner et al., 1983). Experimental studies have shown that birds select nesting locations in relation to predation risk (e.g. Fontaine and Martin, 2006; Thomson et al. 2006). Because explorative individuals are expected to face higher (mortality) costs when faced with increased predation risk, they might be expected to also be more likely to leave if they are unable to adaptively down-regulate their explorative tendency. This would alter the frequency of types of individuals found in risky versus non-risky areas. Currently, most studies focus on plastic responses (but see Cote et al., 2013). This is because laboratory tests are typically constructed such that individuals cannot remove themselves from the environment. Additionally, in many field studies, within- (i.e. plastic) versus between- (i.e. selective appearance/disappearance) individual effects cannot be disentangled because of insufficient numbers of marked individuals or re-sightings.

Recent research has shown repeatable differences among individuals in boldness and risk perception, which are important for understanding prey dynamics and predator-prey interactions (Bouskila and Blumstein, 1992; Luttbeg and Schmitz, 2000; Luttbeg and Sih 2010; Stankovich and Blumstein, 2005; Wolf and Weissing, 2012). If individuals differ in how they perceive risk, they can be expected to respond to risk differently. Additionally, individuals can differ in their susceptibility to predation, and if predation risk can be reduced by altering components of the phenotype (e.g., by reducing mass or leaving the area) we expect that those types facing most risk should respond most strongly. This would result in adaptive among-individual variation in phenotypic plasticity (Dingemanse & Wolf 2013; Nussey et al. 2007).

Exploration has been found to correlate with boldness and aggression (reviews: Gosling, 2001; Herczeg and Garamszegi, 2012), and individuals have been found to differ consistently in exploration tendency, ranging from “slow” to “fast” explorers in great tits (Dingemanse et al., 2012) and other species (Bell et al. 2009; Réale et al., 2007; 2010). Although exploration behavior is widely used as a proxy for risk-taking behaviors (e.g. Nicolaus et al. 2012), relatively few studies have tested this prediction, particularly under field conditions (but see Cole and Quinn, 2014; Quinn et al. 2012). This study explicitly tests whether the strength of response to manipulations of perceived risk of predation depends on initial exploration tendency.

Assuming that exploration represents a proxy for risk-taking, and that high predation risk favors a reduction in risk-taking, we predict two effects of changes in perceived predation risk. First, if individuals are unable to modify their exploratory behavior over short time periods (e.g., due to limits to rapid phenotype modification; Auld et al. 2010; DeWitt et al. 1998), we would expect birds that face higher risks (i.e., fast exploring birds) to decrease in mass and/or move away in order to mitigate the costs associated with their risk level. If individuals can modify their exploratory behavior, we would expect them to down-regulate exploration in order to decrease their level of risk. In any case, whether individuals respond plastically or move away should depend on the relative costs and benefits associated with phenotypic plasticity and movement (Westneat and Fox, 2010).

This study examines the effects of the risk of predation on mass, exploration behavior, and selective disappearance in a wild population of great tits, using over

100 individually marked birds. Additionally, we explore how these effects may differ depending on initial individual differences in susceptibility to risk. By doing so, this study fills a gap in the literature by examining whether individual differences in vulnerability to predation (inferred from exploration tendency) predict how individuals respond phenotypically to changes in perceived predation risk. Importantly, the design of the experiment allows for distinctions between responses to risk due to behavioral plasticity within individuals versus type-specific selective disappearance. We investigated the response of great tits to experimental manipulations of perceived predation risk using long-term playbacks of avian predators in free-living birds.

Based on the literature reviewed above, four outcomes were assessed after three weeks of treatment exposure. First, we predicted that birds in predator treatment plots would decrease their mass relative to birds in control plots. Also, we expected this decrease in mass to be greatest for initially fast exploring individuals assuming that those individuals effectively experience the greatest increases in perceived predation risk. Second, assuming that fast exploration is particularly costly under high predation risk, we predicted that exploratory tendency would decrease for birds in predator plots relative to birds in control plots. Third, in the event that individuals would be unable to appropriately modify their behavior, or that the cost of phenotype modification would be too high, we predicted that recapture rates would be higher in control plots compared with predator treatment plots because birds would be more likely to abandon risky areas. Additionally, we expected that birds with initially higher mass or exploration tendency would be more likely to leave high risk areas as they were expected to be less well suited to the manipulated environment. However, several studies have found that slow explorers generally exhibit greater behavioral plasticity, presumably due to differences in the way they sample and recognize changes in the environment (reviewed in Mathot et al. 2012). If so, we would expect to see a greater decrease in mass and a lower recapture likelihood for these individuals that are able to better perceive changes in predation risk.

Methods

Data Collection

Experiments were performed in 8 forest plots that were established in 2009, approximately 25 km southwest of Munich, Germany (Araya-Ajoy and Dingemanse 2014; Mathot et al. 2015; Nicolaus et al. 2014; Stuber et al. 2013). Plots were 9-12 ha in size, each with 50 nest-boxes arranged in a regular grid (50 m between adjacent nest-boxes). In order to manipulate perceived predation risk, 4 speakers (Foxpro Shockwave, Foxpro Inc., Lewiston, Pennsylvania) were spaced approximately 150-250 m apart, such that there was good coverage of the entire plot (Figure S1). Assignment of treatments to plots (4 predator plots, 4 control plots) was randomized, with the constraint that there be no initial differences between treatments in average roosting density or body mass based on data from previous years (analyses not shown). In control plots, speakers were programed to play sounds of 2 nonthreatening heterospecifics (Eurasian wren, *Troglodytes troglodytes*, and Common chaffinch, *Fringilla coelebs*). The control sounds were chosen as they come from species found commonly in the study area that sing normally in the months of treatment and do not compete with our focal species for nesting or roosting space. In predator plots, speakers were programmed with calls from the main avian predator (Eurasian sparrowhawk, *Accipiter nisus*) and conspecific (i.e. great tit) mobbing calls. This mix of calls was used because the sparrowhawk does not call frequently enough in these months to be used alone, and because the mix of calls decreases the likelihood of habituation. Using recordings obtained from the Xeno-canto (www.xeno-canto.org/) bird song repository, we created 8 unique sound files (of 3 minutes duration) for each sound type (chaffinch, wren, sparrowhawk, great tit). Each file was normalized with the software program Audacity 2.0.5 and was played at 90 dB (intensity was set to match the normal intensity of bird songs and calls and was measured at 1 m with a sound level meter). In order to keep the playback schemes as similar as possible in the two groups, sound types were paired so that great tits and wrens were used at the same frequency as sparrowhawks and chaffinches. Playbacks were carried out for 3 weeks in each plot and were programmed so that 1 sound file played at each speaker within a plot per hour (12 minutes of sound per plot per hour) between dawn and dusk. The interval between subsequent playbacks was randomized. Sparrowhawk sound files (chaffinch in control plots) were limited to 2 per day per plot; 1 in the hour following sunrise and 1 in the hour preceding sunset. This was done to mimic the natural timing (daylight hours) and frequency of sparrowhawk calls during the months of the experiment, and to minimize habituation effects.

Immediately after speaker placement in January, but before playbacks began, we captured all birds roosting in nest-boxes following standard methods (Dingemanse et al. 2002). Birds were collected from the field after sunset and transported to the laboratory where they were individually housed in holding cages overnight. A subset of birds were held in respirometry chambers overnight (procedure detailed in Mathot et al. 2015) while their basal metabolic rate was scored as part of a complementary study (Mathot et al. in preparation) and were transferred to holding cages the following morning at least 1 hr before exploration behavior was scored. Birds housed individually or in BMR chambers did not differ in exploration behavior (mean pre-manipulation exploration for birds housed in normal cages = 17.76; difference for birds housed in BMR chambers $\beta = -0.40$, 95% CI = -3.65, 2.83; general linear model). Food and water were provided ad libitum in the holding cages.

The following morning, between 08h00 and 10h00, we measured exploratory behavior of each bird in a novel environment room (5.2 L × 2.9 W × 2.3 H m) containing 5 artificial trees (Dingemanse et al. 2002). The exploration score is a count of hops and movements between perches during a 2-min recording period (Dingemanse et al. 2002, 2012) where faster explorers move more and have higher exploration scores than slow explorers (scores ranged from 1 to 56). Following the behavioral test, we also recorded standard morphometric measurements. After processing, all birds were released at the place of capture before 11h00 following standard protocol (Dingemanse et al. 2002, 2012). Playbacks were scheduled to start immediately following release, but due to technical difficulties with the equipment, continuous playback did not begin until 2 weeks later. Playbacks were subsequently carried out for 21 days in each plot after which plots were visited again to collect birds roosting in boxes to acquire post-manipulation behavioral and morphological data.

Data Analysis

Selective disappearance

To determine if there was selective disappearance (recapture: yes/no) based on mass or pre-manipulation exploration score, we constructed a binomial logit-link–generalized linear mixed effects model (package lme4, R 3.1.1). The model included, as fixed effects, plot treatment (control vs. treatment), pre-manipulation mass, pre-manipulation exploration score, the interaction between treatment and initial mass, and the interaction between treatment and pre-manipulation exploration score. Plot was included as a random effect since treatment was applied to the level of the plot. All individuals captured during the first round (January) were used in this analysis (N = 143 birds). Models were not over-dispersed; an over-dispersion parameter was therefore not modelled.

Plasticity

In order to explore patterns of within-individual plasticity, we constructed two linear mixed effects models (package lme4, R 3.1.1) and determined whether within-individual changes in 1) behavioral (exploration score) and 2) morphological (mass) variables were influenced by treatment. Both models included plot treatment (control vs. treatment), catch period (pre- vs. post-manipulation), and the interaction between the two fitted as fixed effects. Only individuals caught both before and after manipulation were used in these analyses (N = 95 birds) since our interest was in estimating within-individual plasticity. The time of measurement was also included as a fixed effect, since body mass in small passerines is known to show marked diurnal variation (Haftorn, 1989; Lilliendahl 2002) and exploration score varies with time of day in some other populations (Dingemanse et al., 2002). Time of mass measurement was mean centered and calculated as time elapsed since sunset (hours), as body mass should be a function of time since last foraging. Time of measuring exploration score (hours) was mean centered and calculated as time since sunrise, since exploration tests occurred in the early morning and standardizing based on sunrise is most biologically relevant. Sex was also included as a fixed effect because great tits are sexually dimorphic for mass. Models that included tarsus (a measure of structural body size) gave similar results to those without, so tarsus was not included. Individual (nested within plot) and plot were included as random effects. Exploration behavior and body mass were normally distributed, and were modelled with Gaussian error distributions.

Personality-related differences in plasticity

In order to determine if pre-manipulation exploration type influenced response strength, we ran the models of individual plasticity using the after value (body mass or exploration score from the February catch period) as our response variable including the initial measure (body mass or exploration score from the January catch period), treatment and sex as fixed effects. The interaction between treatment and the initial measure was also included; plot was added as a random effect. Inclusion of the initial measure in the models means that the main effect of treatment essentially represents the change in that variable across the two measurement periods, and the interaction between treatment and initial measure indicates if change differs between treatment groups. For the model of mass we also included pre-manipulation exploration score and the interaction between treatment and pre-manipulation exploration score, as we were interested in determining if pre-manipulation exploration behavior influenced response strength. Mass and exploration score were both normally distributed and were modeled with Gaussian error distributions. We recognize the non-independent nature of our analyses as a function of the initial value for the same trait, and that a negative covariance between the initial value and the after value would be expected by chance, a statistical pattern called "regression towards the mean" (Bland and Altman, 1994). Therefore, this particular parameter is not biologically meaningful on its own. However, this issue does not apply to the parameters of main interest: the main effect of treatment, and the interaction between pre-manipulation exploration score and treatment.

We used the "sim" function (package arm) to simulate the posterior distribution of the model parameters and values were extracted based on 2000 simulations (Gelman and Hill 2007). Instead of using p-values and drawing dichotomous conclusions (e.g. accept or reject the null hypothesis) based on data which may show a continuous range of support (Cohen 1990; Cumming 2014), we evaluated support for treatment effects based on estimated effects sizes and their 95% credible intervals (Cumming & Finch 2005; Nakagawa & Cuthill 2007). As a guideline for readers less familiar with CIs, a 95% CI that does not overlap zero is roughly equivalent to a significant p-value in the frequentist's sense (Cumming & Finch 2005); we describe such results as showing 'strong support' for predictions. For estimates that are biased away from zero but the 95% CIs slightly overlap zero (up to 85% CI) we instead use the term 'moderate support.' For estimates centered on zero with 95% CIs greatly overlapping zero we use the term 'no support' or the term 'support for lack of effect.' We used visual assessment of the residuals to evaluate model fit.

Results

Selective disappearance

Contrary to our prediction, we found no support for differences in recapture probability between treatment groups (control = 0.64, predator exposed = 0.73; N= 76 vs. 67 respectively). Overall recapture probability was not affected by pre-manipulation exploration score (β= -0.01, 95% CI = -0.07, 0.05) or pre-manipulation body mass (β= -0.39, 95% CI =-1.12, 0.34) (Table 1). There was also no support for treatment-specific recapture probability as a function of pre-manipulation mass or exploration score.

Plasticity

There was strong support for a lack of differences in initial body mass values between birds that subsequently received predator exposed versus control treatment (β= -0.01, 95% CI = -0.37, 0.35; Table 2); males were on average 1.04 (0.71, 1.35) standard deviation units heavier than females. The slight overlap of 95% CIs with zero for the interaction between treatment and catch period implied that there was moderate support for changes in mass as a function of treatment (β= -0.14, 95% CI = -0.36, 0.09) (Table 2, Fig 1a). There was also moderate support for a within-individual decrease in body mass from January to February in control plots (effect of catch period: β= -0.17, 95% CI = -0.35, 0.02; Table 2), whereas there was strong support (owing to 95% CIs not overlapping zero) for birds from predator exposed plots to decrease in mass (β= -0.30, 95% CI = -0.47, -0.13).

There was also strong support for a lack of differences in pre-manipulation exploration scores between birds from plots that subsequently received predator versus control treatments (β= 0.01, 95% CI = -0.04, 0.06; Table 2). There was strong support for changes in exploration differing across treatments (catch period x treatment: β = -0.03, 95% CI = -0.06, 0.00) (Table 2, Fig 1b). There was also strong support for exploration scores increasing within-individuals from January (pre-manipulation) to February (post-manipulation) for birds in control plots (β= 0.03, 95% CI = 0.01, 0.05) and strong support for the absence of such an effect for birds in predator-exposed plots (β= 0.00, 95% CI = -0.02, 0.02).

Personality-related differences in plasticity

There was moderate support for birds from predator-exposed plots to decrease in mass relative to control birds (Treatment: β = -0.15, 95% CI = -0.37, 0.06; Table 3) between January (pre-manipulation) and February (post-manipulation), and there was strong support for an interaction between pre-manipulation exploration score and treatment on mass (β= -0.02, 95% CI = -0.04, 0.00). This interaction occurred because for control areas, there was strong support for mass increasing from January to February with increasing pre-manipulation exploration score (β = 0.01, 95% CI = 0.00, 0.03) while there was instead strong support for an absence of such an effect in predator-exposed plots (β = 0.00, 95% CI = -0.01, 0.01). Graphical inspection showed that this interaction was caused mainly by treatment-specific effects in fast explorers: fast explorers gained mass in control plots and lost mass in predator exposed plots, whereas such treatment-effects did not occur in slow explorers (Fig 2). This finding implies that fast explorers were phenotypically more responsive to experimental changes in perceived predation risk.

We found moderate support for birds from predator-exposed plots to decrease in exploration relative to control birds (Treatment: β = -0.02, 95% CI = -0.06, 0.01; Table 3) between January (pre-manipulation) and February (post-manipulation), however there was no support for the interaction between pre-manipulation exploration score and treatment on exploration score (β= 0.00, 95% CI = -0.003, 0.003) , implying lack of treatment effects on how pre-manipulation behavior affected behavioral change. There was strong support for exploration score increasing from January to February with increasing pre-manipulation exploration score for individuals in control plots ($\beta = 0.01$, 95% CI = 0.00, 0.01) as well as in predator exposed plots ($\beta = 0.01$, 95% CI = 0.00, 0.01), as expected due to regression to the mean (see Methods).

Discussion

In this study, we evaluated morphological and behavioral responses in free-living great tits exposed to an experimental manipulation of perceived predation risk. We found no evidence for predator-induced selective disappearance. Recapture likelihood was not different between the two treatment groups, and was not predicted by initial mass or exploration score. As expected, birds in the predation treatment tended to decrease in mass relative to individuals in the control treatment. Additionally, these mass changes were related to pre-manipulation exploration score: fast explorers responded to the predation treatment by reducing mass more than slow explorers. Birds in control plots increased exploration from pre-manipulation (January) to post-manipulation (February), as expected based on previously reported seasonal and habituation effects (Dingemanse et al. 2002, 2012). However, there was no increase in exploration for birds in predator plots, implying adaptive suppression of exploration. These results imply that individuals decrease in mass when exposed to increased predation risk, and that fast explorers fare worse in high risk contexts and therefore down-regulate their body mass. This supports previous work that shows that perceived predation risk alone can influence prey populations (Fontaine and Martin, 2006b; Zanette et al., 2011), although our study refines this conclusion by showing that behavioral and morphological responses vary between individuals.

Selective disappearance

There are several post hoc explanations for the finding that recapture probability did not differ as a function of treatment. Our prediction for increased dispersal away from risky areas hinged on individuals not being able to modify their behavior, the costs of modification being too high (e.g., Auld et al., 2010; DeWitt et al., 1998), or the costs of re-settlement not outweighing its benefits. Previous research strongly suggests that the social system of great tits in winter (Elkman, 1989; Hinde, 1952; Saitou, 1978) imposes great costs on dispersal, which may have limited the willingness of individual birds to leave risky areas in late winter. Great tits form social foraging flocks with dominance hierarchies, thus moving to a new area requires investment in re-acquiring dominance. Dispersal-related decreases in status and associated necessary investment in acquiring a new territory might thus not outweigh the costs of staying in a risky area. Also, since individuals begin rigorously defending breeding territories during the end of our treatment period (Drent, 1984; 1987; Krebs, 1971; 1982), they face a seasonal tradeoff between the risk of predation and the ability to reproduce. An important direction for future research is thus to repeat the experiment described in this paper at other times of year to determine if predator-induced costs and benefits associated with dispersal and re-settlement decisions indeed change as a function of season. For example, in late summer and early fall there are presumably lower costs to leaving an area and a similar study in this time period may find lower recapture probabilities for great tits exposed to perceived predation risk.

Plasticity

We found moderate support for decreases in mass from January to February for birds exposed to control sounds whereas we found strong support for decreases in mass for birds exposed to predator sounds. Similar decreases in mass have been observed following manipulations of perceived predation risk in numerous other studies (e.g. Lilliendahl, 1997; Macleod et al., 2005; Perez-Tris et al., 2004; Witter et al., 1994) including other studies in great tits (Gentle and Gosler, 2001). These results demonstrate that great tits respond plastically by decreasing mass more when exposed to increased predation risk. In the context of this study, the reduction in mass was consistent with adaptive phenotypic plasticity; weight did not drop so low that it increased mortality risk (because there was no evidence for lower recapture probabilities for birds from predator treatment plots). If the predation risk experiment had lasted longer, other responses may have become prominent, including relocation. Future research could thus vary the duration of predation risk treatments to determine if responses to risk accumulate over time.

We found that birds in control areas increased in exploration from January to February. Such increases in exploration with both Julian date and repeat testing exist in all sampled West-European populations of this species (Dingemanse et al., 2012). However, in the predation areas there was no within-individual change in exploration behavior, supporting our prediction that this plastic seasonal change would be dampened in the predation treatment. This suppression of exploration is thought to be adaptive because decreasing exploration corresponds to decreases in activity and therefore the encounter rates with predators (Brown, 1999; Lima and Dill 1990). In addition to varying in behavioral type, individuals can also vary in the repeatability of their behavior (intra-individual variability) (Stamps et al., 2012). Some individuals are more predictable in behavior than others, and it has been suggested that high predation risk may favor reduced predictability (Briffa, 2013). Recently, Briffa (2013) found that intra-individual variability in hermit crabs (*Pagurus bernhardus*) was greater in the presence of a predator. This suggests that unpredictable behavior may help ameliorate predation risk. Our study was not designed to test this prediction (we do not have repeated measures of behavior following treatments), however, this is an interesting avenue of research for future studies of individuality in a predation risk context.

Personality-related differences in plasticity

Fast exploring birds may be at greater risk due to their increased visibility and more frequent encounters with predators (Biro and Stamps 2008; Niemelä et al., 2015; Smith and Blumstein, 2008), and it is in these birds that we found the largest reductions in mass. This is especially interesting because the fast exploring birds showed the largest increases in mass in the control areas. This finding suggests that normally fast exploring birds gain mass during winter, perhaps because they are better able to find and exploit high quality food patches (Dingemanse and De Goede, 2004). However, in response to the increase in perceived risk they decreased mass more prominently than slow explorers. This decrease in response to predation risk is likely because they must mitigate the extra risk they experience relative to slow explorers, and the decrease in mass allows them to ameliorate the increase in risk either directly to be better able to out maneuver predators, or indirectly through decreases in activity and subsequent changes to habitat use or foraging patterns.

Conclusion

In summary, birds exposed to increases in perceived predation risk tended to decrease in mass more than individuals exposed to control conditions. Additionally, we found that fast explorers showed a stronger response to increased risk than slow explorers. This study provides evidence that behavioral types experience changes in perceived predation risk differently, and that individuals respond adaptively based on their individual level of perceived risk. Therefore, certain types of individuals must change more to reduce the threat of predation, leading to differential selection pressures on behavioral types. A key question is whether the predator-induced phenotypic adjustments in body weights shown largely in fast explorers come with (delayed) costs. This is interesting because it would imply that predators may non-consumptively alter selection pressures on 'personality' types. In our case, such effects could come about by predators inducing selection pressures favoring slow explorers since those types do not have to carry the costs of predator-induced phenotypic modification. Our findings thus imply that individuality in predation risk should be considered in the study of non-consumptive effects of predators in micro-evolutionary processes.

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Table 1. Estimated effect sizes and 95% credible intervals around the mean of predictors of recapture probability.

Fixed effects	β (95% CI)
Intercept [a]	0.76 (-0.12, 1.67)
Treatment [b]	0.44 (-0.91, 1.72)
Pre-manipulation mass [c]	-0.39 (-1.12, 0.34)
Pre-manipulation exploration score [d]	-0.01 (-0.07, 0.05)
Pre-manipulation mass[c] x treatment[b, e]	-0.05 (-1.17, 1.13)
Pre-manipulation exploration score[d] x treatment[b, f]	0.03 (-0.05, 0.11)
Random effects	σ^2 (95% CI)
Plot	0.58 (0.22, 1.29)
Residual	1

[a]Reference category; estimate for control plots for individuals with average pre-manipulation mass and exploration scores
[b]Difference between the treatments (predation – control) for individuals with average pre-manipulation mass and exploration scores
[c]Mean-centered within sex
[d]Mean-centered within treatment
[e]Difference between treatments (predation – control) in effect of pre-manipulation mass
[f]Difference between treatments (predation – control) in effect of pre-manipulation exploration score

Table 2. Estimated effect sizes and 95% credible intervals around the mean of predictors of standardized mass and exploration score.

	Body mass (g)	Exploration score
Fixed effects	β (95% CI)	β (95% CI)
Intercept[a]	-0.41 (-0.71, -0.12)	-0.02 (-0.05, 0.02)
Catch period [b]	-0.17 (-0.35, 0.02)	0.03 (0.01, 0.05)
Treatment [c]	-0.01 (-0.37, 0.35)	0.01 (-0.04, 0.06)
Measurement time[d]	-0.11 (-0.38, 0.16)	0.00 (-0.02, 0.02)
Sex[e]	1.04 (0.71, 1.35)	0.01 (-0.02, 0.04)
Catch period[b] x treatment[c,f]	-0.14 (-0.36, 0.09)	-0.03 (-0.06, 0.00)
Random effects	σ^2 (95% CI)	σ^2 (95% CI)
Plot	Non-estimable	Non-estimable
Individual	0.66 (0.59, 0.83)	0.004 (0.003, 0.006)
Residual	0.16 (0.13, 0.19)	0.003(0.002, 0.004)

[a] Reference category; estimate for females in control plots before manipulation
[b] Difference between the periods (post-manipulation – pre-manipulation) in control plots
[c] Difference between the treatments (predation – control) during the pre-manipulation period
[d] Mean-centered
[e] Difference between the sexes (male – female)
[f] Difference between catch periods (after-before) in effect of treatment (predation – control)

Table 3. Estimated effect sizes and 95% credible intervals around the mean of predictors of post-manipulation (February) standardized mass and exploration score

Fixed effects	Body Mass (g) β (95% CI)	Exploration Score β (95% CI)
Intercept[a]	-0.62 (-0.80, -0.44)	0.01 (-0.01, 0.04)
Pre-manipulation mass [b]	1.06 (0.84, 1.26)	-
Pre-manipulation exploration score [c]	0.01 (0.00, 0.03)	0.01 (0.00, 0.01)
Treatment [d]	-0.15 (-0.37, 0.06)	-0.02 (-0.06, 0.01)
Sex[e]	1.46 (1.25, 1.68)	0.01 (-0.02, 0.04)
Pre-manipulation mass[b] x treatment[d, f]	-0.01 (-0.32, 0.30)	-
Pre-manipulation exploration score[c] x treatment[d, g]	-0.02 (-0.04, 0.00)	0.00 (-0.003, 0.003)
Random effects	σ^2 (95% CI)	σ^2 (95% CI)
Plot	Non-estimable	Non-estimable
Residual	0.25 (0.20, 0.36)	0.006 (0.005, 0.009)

[a] Reference category; estimate for females of average mass and exploration in control plots

[b] Mean-centered within sex

[c] Mean-centered

[d] Difference between the treatments (predation – control)

[e] Difference between the sexes (male – female)

[f] Difference between treatments (predation-control) in effect of pre-manipulation mass

[g] Difference between treatments (predation-control) in effect of pre-manipulation exploration

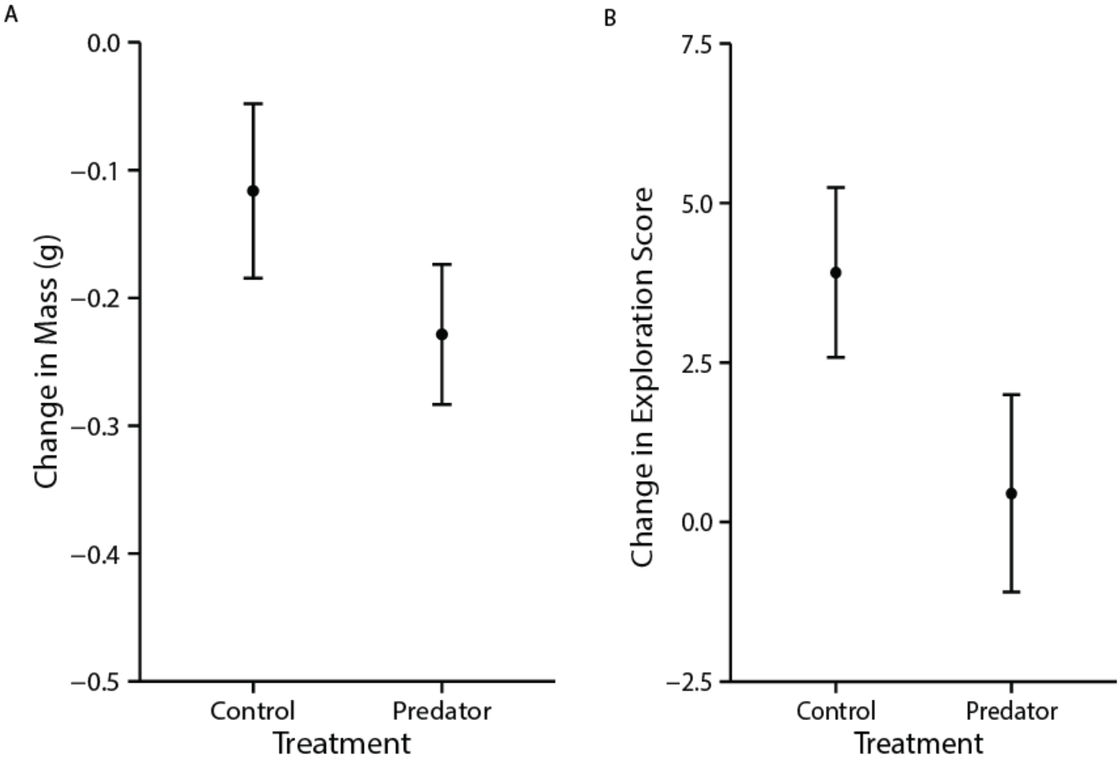


Figure 1. Change in body mass (A) and exploration score (B) (post-manipulation (February) - pre-manipulation (January)) in relation to perceived predation risk treatment (control vs. predator sounds). Points are means with standard errors based on raw data.

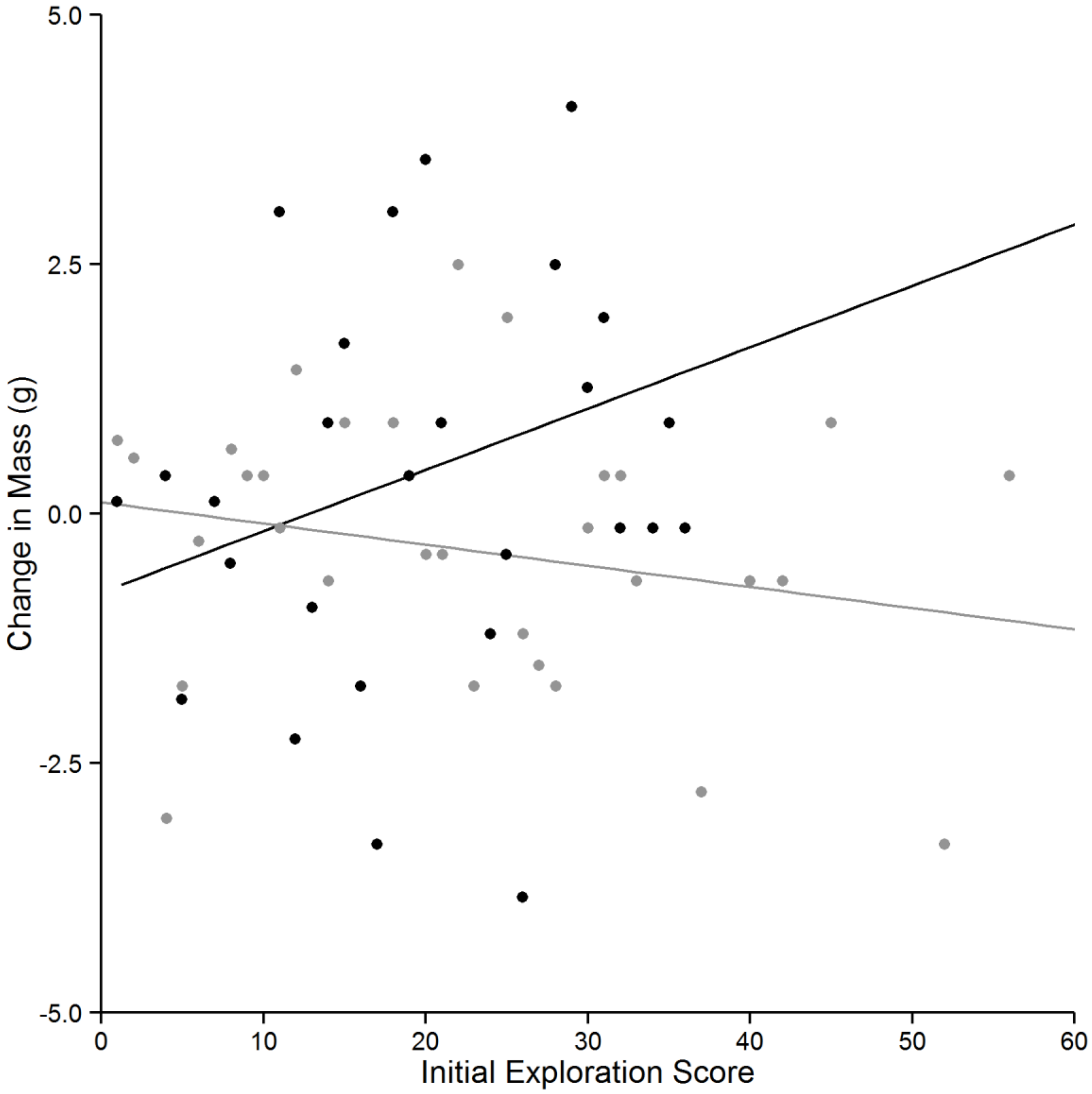
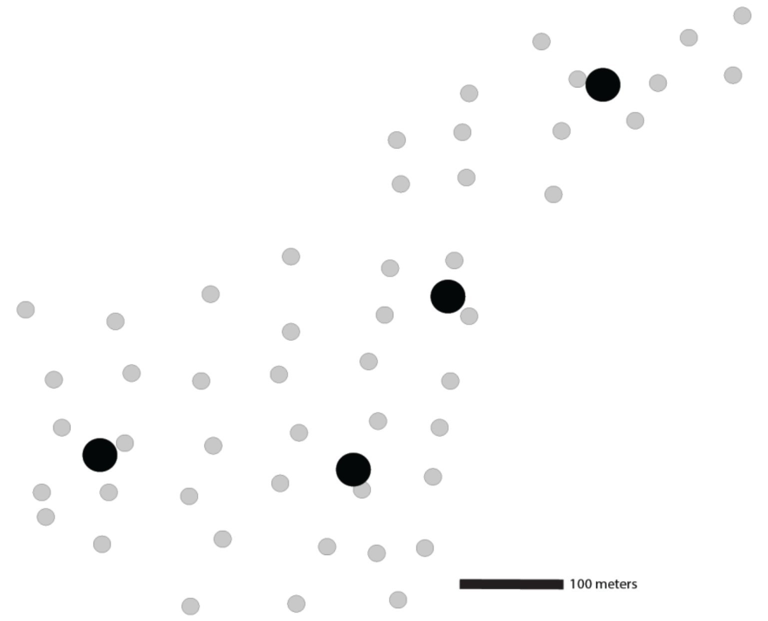
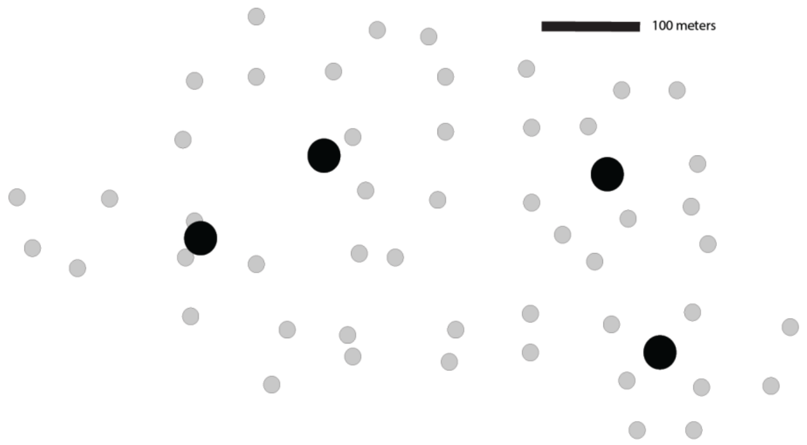


Figure 2. Change in body mass (post-manipulation (February) - pre-manipulation (January)) as a function of pre-treatment exploration score and treatment (control vs. predator sounds). Control plots are in black, plots with increased perceived predation risk are in gray.

Supplementary Material

Figure S1. Examples of speaker location of two of our 12 plots (Sonnau, Perchting). Gray points indicate nest boxes and black points indicate speakers. Speakers were placed to minimize overlap and maximize the number of nest boxes covered.





Chapter 3

Do great tits (*Parus major*) suppress basal metabolic rate in response to increased perceived predation danger? A field experiment

Abstract

Several studies have shown that individuals with higher metabolic rates (MRs) feed at higher rates and are more willing to forage in the presence of predators. This increases the acquisition of resources, which in turn, may help to sustain a higher MR. Elevated predation danger may be expected to result in reduced MRs, either as a means of allowing for reduced feeding and risk-taking, or as a consequence of adaptively reducing intake rates via reduced feeding and/or risk-taking. We tested this prediction in free-living great tits (Parus major) using a playback experiment to manipulate perceived predation danger. There was evidence that changes in body mass and BMR differed as a function of treatment. In predator treatment plots, great tits tended to reduce their body mass, a commonly observed response in birds to increased predation danger. In contrast, birds from control treatment plots showed no overall changes in body mass. There was also evidence that great tits from control treatment plots increased their basal metabolic rate (BMR) over the course of the experiment, presumably due to decreasing ambient temperatures over the study period. However, there was no evidence for changes in BMR for birds from predator treatment plots. Although the directions of these results are consistent with the predicted directions of effects, the effects sizes and confidence intervals yield inconclusive support for the hypothesis that great tits would adaptively supress BMR in response to increased perceived predation risk. The effect size observed in the present study was small (~ 1%) and would not be expected to result in substantive reductions in feeding rate and/or risk-taking. Whether or not ecological conditions that generate greater energetic stress (e.g. lower food availability, lower ambient temperatures) could produce an effect that produces biologically meaningful reductions in feeding activity and/or risk-taking remains an open question.

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Introduction

Among-individual differences in resting or basal metabolic rates (MRs) have recently received increasing attention as potentially important correlates of among-individual differences in behaviour (Biro and Stamps, 2010; Careau et al., 2008; Mathot and Dingemanse, 2015). In some cases, higher MRs may favour higher expression of behaviours associated with resource acquisition (Mathot and Dall, 2013; Mathot et al., 2015). At the same time, a higher expression of such behaviours may facilitate the maintenance of costly metabolic machinery (Biro and Stamps, 2010 and references therein; Careau and Garland, 2012). Indeed, several studies have found that individuals with higher MRs spend more time foraging (Cutts et al., 2001; Mathot et al., 2015), behave more boldly in the face of predators (Cutts et al., 1998; Finstad et al., 2007; Krams et al., 2013a; Krams et al., 2013b) or are more constrained in their behavioural responses to predation danger (Mathot et al., 2015).

All else being equal, higher feeding rates and greater risk-taking expose individuals to a higher risk of mortality due to predation, and consequently, individuals with lower MRs may be expected to have an advantage under conditions of high predation danger. Indeed, mealworm beetles (*Tenebrio molitor*) with higher MRs suffer higher mortality due to predation (Krams et al., 2013a; Krams et al., 2013b). Adaptive suppression of feeding rates and risk-taking behaviours are well documented in animals under conditions of elevated predation danger (Lima and Dill, 1990). If higher feeding rates and/or greater risk-taking facilitate the maintenance of costly metabolic machinery (Biro and Stamps, 2010 and references therein; Careau and Garland, 2012), then adaptive reductions in these behaviours under conditions of elevated predation danger may result in reduced MRs (Figure 1).

Here, we report on an experiment that manipulated perceived predation danger in a free-living population of great tits (*Parus major*). The aim of our study was to test the prediction that increased predation danger leads to within-individual reductions in basal metabolic rate (BMR). Great tits are a good study system in which to test this prediction, because previous work in this population showed that higher BMR was associated with higher feeding rates and constrained behavioural responses to increased perceived predation danger (Mathot et al., 2015). This suggests that there should be a cost to high BMR under conditions of high predation danger. Furthermore, great tits are able to adjust their BMR to current ecological conditions (Bouwhuis et al., 2011; e.g. ambient temperature, Broggi et al., 2007) suggesting that within-individual changes in BMR in response to temporal variation in predation danger is physiologically possible. Finally, because great tits readily roost in artificial nest boxes, marked individuals can be recaptured with ease, facilitating the study of within-individual variation in free-living populations (Abbey-Lee et al., 2016b).

Materials and Methods

Playback experiment

The experiment was carried out in 8 forest plots located in Bavaria, Germany (48°N, 11°E) in the winter of 2014 (Figure 2) under Regierung von Oberbayern permit no. 55.2-1-54-2532-140-11. Each plot consists of 50 nest boxes hung in a regular grid, with 50 meters between adjacent nest boxes. Perceived predation danger was manipulated at the plot level (4 predator plots and 4 control plots, see below). Assignment of treatment to plots was randomized, while ensuring that there were no initial differences between predator and control plots in roosting densities or body mass based on data from the previous winter. Treatments were also stratified with respect to perceived predation danger treatments that were performed the previous breeding season as part of a separate experiment (Abbey-Lee et al., 2016a, see Supplementary Table S1).

Perceived predation danger was manipulated using playbacks. In early January 2013, 4 speakers were placed in each plot such that there was good auditory coverage of the entire plot. Sound files used to experimentally increase perceived predation danger consisted of either Eurasian sparrowhawk (*Accipiter nisus*) calls (a natural predator of great tits) or great tit mobbing calls (typically produced in response to predator encounters). For the control playbacks we used common chaffinch (*Fringilla coelebs*) calls and Eurasian wren (*Troglodytes troglodytes*) songs. These control sounds were chosen because both species are common in the study area, call and sing regularly during the period of the experiments (January through February), and do not compete with the focal species. In total, 8 unique sounds files were created for each sound type using recordings obtained from the Xeno-canto (www.xeno-canto.org) bird song repository. Each sound file was 3 minutes long. Sound files of Eurasian wren songs and sparrowhawk calls were comprised of alternating bouts of sounds and silences of (5 to 15 seconds of sound followed by 5 to 15 seconds of silence, on repeat for 3 minutes), while chaffinch call and great tit mobbing call sound files were made up of continuous vocalizations (i.e. no prolonged bouts of silence). This was done to mimic the vocalisation patterns normally heard for each of these song and call types.

Playbacks were programmed so that 1 sound file played at each speaker within a plot per hour (4 different sound files per plot per hour) between dawn and dusk. The exact interval between subsequent playbacks was randomized. Sparrowhawk calls (or chaffinch songs for control plots) were limited to 2 per day per plot; 1 in the hour following sunrise and 1 in the hour preceding sunset. This was done to mimic the natural timing and frequency of sparrowhawk calls during the months of the experiment, and to minimize habituation effects. Due to technical difficulties with the speakers, playbacks did not commence until 2 weeks after the first roosting inspections, and were then carried out for 3 weeks in each plot.

Roosting inspections and BMR measurements

Immediately after speaker placement in early January, but before playbacks began, roosting inspections were performed after sunset in each of the plots following standard protocols (Dingemanse et al., 2002). During roosting inspections, all birds were marked with aluminum rings if not already marked and brought to the laboratory for behavioural and morphological measurements as part of the general data collection for this study population (details provided in Abbey-Lee et al., 2016b) (January, N = 143; February, N = 115). Predator and control treatment plots were sampled alternately, to avoid confounding treatment and date. The roosting inspection of a given plot ended either when all 50 nestboxes had been checked, or when 24 roosting birds had been collected (N = 6 occasions of 16), as this was the maximum number of birds that could be held in the laboratory overnight. In these cases, the remaining nestboxes were checked the following evening. Our equipment allowed measuring BMR for up to 9 individuals per night. Thus, nine individuals were randomly selected from the total number of birds that were brought into the lab on any given night. In total, we measured BMR of 111 individuals during the pre-treatment period (January) and 65 individuals during the post-treatment period (February), with a total of 56 repeated measures.

A detailed description of the respirometry setup is provided in the Electronic Supplementary Material (ESM Text S1). Briefly, BMR was measured as O_2 consumption rates using three identical setups, each measuring up to 3 birds per night. Upon arrival in the laboratory (between circa 19h00 and 22h30), great tits were weighed then placed individually in airtight, 1 L metabolic chambers that were housed in darkened environmental cabinets. The environmental cabinets were kept at 25 ± 0.1°C, which is within the thermoneutral zone of great tits (range 15°C to 30°C, Broggi et al., 2005). Dry, CO_2-free air was pumped through each metabolic chamber at a rate of 200 mL per minute, and the concentrations of O_2, CO_2 and H_2O in effluent air streams were measured using a water vapour analyser (Sable Systems, Las Vegas, Nevada, USA) and O_2 and CO_2 analysers (FoxBox, Sable Systems, Las Vegas, Nevada, USA). The rate of O_2 consumption was calculated following Lighton (2008). The following morning, great tits were removed from the metabolic chambers approximately one hour before sunrise and weighed before being placed in individual cages (40 x 60 x 50 cm) with solid bottom, top, side and rear walls, and with ad libitum access to food (meal worms and sunflower seeds) and water and later scored for exploration behaviour as part of a complementary study (Abbey-Lee et al., 2016b).

Roosting inspections were repeated following the same protocol in February, immediately following completion of the 3 week playback experiment. Protocols were identical to those from the January roosting inspections, with the exception that when more than 9 birds were obtained in a given night, BMR measurements were preferentially taken for birds from which we had obtained BMR measurements in January.

Statistical analyses

Analyses were performed in two steps. First, we investigated sources of variation in initial BMR and body mass. This was done to verify that there were no BMR- or mass-related biases in the assignment of treatments to plots, and to identify important covariates (e.g. sex, body mass) to control for in subsequent analyses. To do this, we constructed linear mixed effects models with either BMR or body mass fitted as response variables. We included sex (female = 0, male = 1) and future treatment (control or predator) as fixed effects. Plot was included as a random effect. Body mass taken during the evening immediately prior to BMR measurements strongly correlated with body mass measurements taken the morning after measurements ($r^2 = 0.89$). We used evening body mass in the analyses presented here, although analyses using evening, morning, or average body mass yielded quantitatively similar results (analyses not shown). Results are also similar to those from an analysis on a larger sample of birds (birds included in the present study as well as birds that were not measured for BMR) using body mass taken at time of capture during the evening roosting inspections also produced quantitatively similar results (Abbey-Lee et al., 2016b).

There were no differences in either initial BMR or initial body mass among plots assigned to predator or control treatments. We subsequently estimated changes in BMR and body mass within each treatment level (control and predator) as well as the difference across treatments (i.e. treatment x observation period interaction).

Our sample sizes did not allow for meaningful tests for selective disappearance of birds as a function of BMR and body mass. For example, if individuals with higher BMR were more likely to disperse out of predator treatment plots, BMR could decrease in predator treatment plots in the absence of within-individual changes. Therefore, we present analyses including only individuals for which we have repeated measurements in the main text. However, analyses including individuals without repeated measures yielded qualitatively similar results (see Supplementary Table S2). Additionally, analyses of for selective disappearance in a larger sample of birds (including birds for which we did not obtain BMR measurements) and using body mass taken at time of capture found no evidence for selective disappearance by body mass (Abbey-Lee et al., 2016b).

Analyses of body mass (taken at the time of BMR measurements) were carried out in addition to analyses of BMR for two reasons. First, decreasing body mass is a well-documented response to increased predation danger in birds (Gosler et al., 1995). Therefore, estimating treatment effects on changes in body mass provided a means of assessing whether playback experiments effectively manipulated perceived predation danger. Additionally, body mass and BMR are typically strongly correlated (Burton et al., 2011). By analyzing the effect of treatment on both BMR and body mass, we were able to evaluate whether any observed metabolic response to experimental manipulation of perceived predation danger could have been an indirect response to predation danger mediated via the direct effect of predation danger on body mass.

In order to directly compare the effects of predator manipulation on BMR and mass, and to control for sex-related differences in BMR and body mass, BMR and mass data were centered within sex and standardized to one unit variance prior to analyses (Gelman, 2008; Vittinghoff et al., 2005). All models were constructed using the MCMCglmm package in R. Details of the parameter estimation method are provided in Supplementary Text S2. We evaluated support for treatment effects based on estimated effects sizes and their 95% credible intervals (Cumming and Finch, 2005; Nakagawa and Cuthill, 2007). This approach is advocated to avoid drawing dichotomous conclusions (e.g. accept or reject the null hypothesis) based on data which can show a continuous range of support (or lack of support) for a given interpretation (Cohen, 1990). A 95% CI that does not overlap zero is roughly equivalent to p-value < 0.05 in the frequentist's sense (Cumming and Finch, 2005). We describe such results as showing 'strong support' for predictions. For estimates that are biased away from zero but the 95% CIs slightly overlap zero we instead use the term 'moderate support'. For estimates centered on zero with 95% CIs greatly overlapping zero we use the term 'no support' or the term 'support for lack of effect'.

We used visual assessment of the residuals to evaluate model fit. All data used in this study are provided in the Electronic Supplementary Material (Appendix S1).

Results

Prior to applying the treatment, there was no evidence for treatment-related differences in either BMR (both mass-dependent and controlling for body mass) or body mass (estimates centered on zero, Table 1). There was strong support for a sex effect on both body mass and BMR (Table 1): males were heavier and had higher BMR compared with females, and BMR increased with increasing body mass.

There was evidence for treatment-specific effects on both body mass and BMR. Body mass did not change in control plots, but there was moderate support for a decrease in body mass in predator plots (Table 2, Figure 3). There was also moderate support for the interpretation that these effects differed between the two treatments (estimated difference: β= -0.12, 95% CI = -0.36, 0.10). There was moderate support for an increase in BMR for birds from control plots following the playback experiment, but no evidence for a change in BMR for birds from predator treatment plots (Table 2, Figure 4). There was moderate support for the interpretation that the effects on BMR differed between the treatments (estimated difference in mass-dependent BMR: β= -0.21, 95% CI = -0.48, 0.08). However, this estimated difference amounted to only an approximately 1% difference in BMR between predator and control plots post manipulation. The point estimate of the difference was qualitatively similar when mass was included as a covariate, but the CI showed greater overlap with zero (β= -0.16, 95% CI = -0.42, 0.14). These analyses also revealed that higher BMR was associated with high body mass both at the between- and within-individual level, but the effect of between-individual variation in body mass on BMR was much greater.

Discussion

Several studies have suggested that individuals with higher MR may be more vulnerable to predators (Finstad et al., 2007; Krams et al., 2013a; Krams et al., 2013b; Mathot et al., 2015), and therefore, increased predation danger may lead to reductions in MR. We used a playback experiment in a population of free-living great tits to test this prediction. Birds from predator plots tended to decrease in body mass (Abbey-Lee et al., 2016b), as expected if the playbacks in predator plots increased perceived predation danger (Gosler et al., 1995), while birds from control plots showed no changes in body mass. Consistent with the prediction that higher predation danger favours reduced BMR, great tits from control plots tended to increase their BMR, but birds from predator plots showed no changes in BMR over the course of the experiment. However, these results are inconclusive because the estimated effect of changes in both body mass and BMR as a function of treatment, although in the predicted direction, both overlapped zero. Furthermore, the estimated effect size observed under the present experimental conditions for changes in BMR as a function of treatment was small (approximately 1%) and it is unclear how such a small effect would mitigate increased predation risk.

Although BMR and body mass are typically strongly correlated (Burton et al., 2011), treatment-related changes in BMR occurred independently of treatment related-differences in body mass; body mass tended to decrease in predator plots with no concomitant changes in BMR, and BMR tended to increase in control plots with no concomitant changes in body mass. The average decrease in body mass observed in predator plots was 0.17g. Again, analyses on a less restrictive sample of birds and using mass taken at time of capture produced quantitatively similar results, with an estimated decrease of 0.14 g in predator treatment plots relative to control treatment plots (Abbey-Lee et al., 2016b). These estimated effects on body mass are smaller compared with two previous studies in great tits which observed decreases in body mass of approximately 0.55 g in response to manipulations of perceived predation risk (Gentle and Gosler, 2001; Senar et al., 2002). However, the effect sizes reported in both of these studies was based on mass data from great tits caught during the day. In contrast, our body mass data were obtained during evening roosting inspections. Previous studies in great tits have shown that perceived predation danger influences diurnal patterns of mass gain, with birds experiencing a high perceived predation danger delaying mass gain until the latter part of the day (MacLeod et al., 2005). In other words, predation danger related differences in body mass tend to be greatest early in the day, and smallest late in the day. Thus, the body mass measure used in the present study (taken in the evening) provides a conservative estimate of the mass differences that exist during the active foraging periods of these birds.

In contrast, there was no evidence for changes in BMR in predator treatment plots, but there was evidence for an increase in BMR in control plots post-manipulation (Table 2 and Figure 3). Within-individual changes in BMR across repeated metabolic measurements have previously been shown in budgerigars (*Melopsittacus undulatus*). When measured 5 times over the course of approximately

6 weeks, budgerigars showed reductions in BMR of approximately 30% (Jacobs and McKechnie, 2014). This result was interpreted as a habituation effect, with an attenuation of the stress response to handling for metabolic measurements across repeated measurements. If the patterns of BMR change observed in the present study represent habituation or sensitization effects, the increased BMR in birds from control plots could indicate heightened stress responsiveness, while the lack of change in BMR for birds from predator treatment plots could indicate lack of sensitization and habituation. This seems unlikely, as the predator stimulus would be expected to have a greater effect on stress responsiveness compared to the control treatment (Zanette et al., 2014). Furthermore, the potential for carry-over effects between repeated measurements is expected to be low given that our protocol involved handling each bird a maximum of 2 times with an interval of 5 weeks between the two measurements.

Alternatively, the observed increase in BMR in control birds may have occurred in response to decreasing temperatures over the course of the experiment. The average minimum temperature from the preceding 5 -7 days is a significant predictor of variation in BMR in great tits, presumably because higher BMR generates more heat for maintaining body temperature at low ambient temperatures. Previous studies estimate that BMR decreases by an average of 0.006 mL O_2 per min per 1°C increase in temperature in great tits (Bouwhuis et al., 2011; Broggi et al., 2007). Indeed, 5 day average minimum temperatures were 1.1°C higher (95% CI = 0.93, 1.26) during the first BMR measurement session (pre-treatment) compared with the later BMR measurement session (post-treatment) (N = 21 measurement days). Thus, our observed increase in BMR among control birds (0.008 mL O_2 per min) is in line with expected temperature related changes in BMR.

Another possibility is that changes in BMR in control birds may not have been driven by temperature but instead reflect a seasonal change (independent of temperature) in phenotype (e.g. associated with the approaching breeding season and the need for territory defense). For example, several studies have shown that great tits show seasonal increases in exploration behaviour (Abbey-Lee et al., 2016b; Dingemanse et al., 2002; Dingemanse et al., 2012), and higher BMR may be a physiological mechanism underpinning such a change (Réale et al., 2010). Our study design does not allow us to disentangle seasonal effects (pre- versus post-treatment) on BMR from temperature effects, as season and temperature were confounded in this study. However, two previous studies in great tits that measured winter BMR continuously, and could therefore disentangle temperature effects from seasonal effects, found that BMR decreased towards the end of winter when controlling for seasonal changes in temperature (Bouwhuis et al., 2011; Broggi et al., 2007). We therefore suggest that temperature remains the most likely explanation for the observed increase in BMR among control birds over the course of the study.

Great tits from the predator treatment plots showed no change in BMR between pre- and post-treatment measurements despite experiencing the same decrease in temperatures across measurement sessions. This apparent suppression of the normal temperature-related increase in BMR is consistent with the hypothesized relationships between MR, feeding rate, risk-taking and predation danger outlined in Figure 1. A potential criticism of our experimental design is that our manipulations of perceived predation danger may have simultaneously manipulated perceived local densities of birds. Specifically, the use of conspecific mobbing calls in predator plots may have suggested a higher local density of great tits. MR generally declines with increasing conspecific density (reviewed in DeLong et al., 2014), and therefore, we cannot exclude the possibility that the suppression of BMR in predator plots was a response to an increase in perceived local density. However, under natural conditions, all else being equal, we expect that higher predation danger is also associated with a higher frequency of conspecific mobbing calls, and therefore, that the combination of sparrowhawk and great tit mobbing calls provide meaningful cues of predation danger. Indeed, previous work in the same population of great tits has shown that increased perceived predation danger results in increased alarm calling compared with birds exposed to control playbacks (Abbey-Lee et al., 2016a).

Several earlier laboratory studies have shown that increased perceived predation danger can result in suppression of MRs (Handelsman et al., 2013; Pauwels et al., 2010; Steiner and Van Buskirk, 2009; Stibor and Machacek, 1998). Here, we present, to our knowledge, the first field experiment to test for adaptive suppression of MR in response to experimental manipulations of perceived predation danger. Our study yielded inconclusive support: estimated effects were in the predicted direction, but credible intervals around the estimates showed some overlap with zero. Furthermore, the estimated magnitude of metabolic adjustment in the current experiment was small (<1%), corresponding to an energy saving of only 0.25 kJ per day. Such differences in energy requirements (equivalent to the energetic content of 1 sunflower seed) would not be expected to have meaningful effects on feeding rates or risk-taking behaviour, and therefore, it is unclear how such a small reduction in BMR could offset any perceived increase in predation danger (Figure 1). At least one previous study documenting adaptive suppression of MR in response to increased perceived predation danger found that the effect of predation danger on MR was greatest under conditions of low food availability (Pauwels et al., 2010). Although our study did not investigate the interacting effects of food and predation danger on metabolic suppression, our study suggests that predator-induced suppression of MR may occur in great tits. Whether or not ecological conditions that generate greater energetic stress (e.g. lower food availability, lower ambient temperatures) produce a large enough effect for such a suppression to mitigate predation risk by allowing for reduced feeding activity remains an open question.

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Author contributions

KJM conceived of the study question. All authors jointly designed the study, KJM and RNA performed the experiments and collected the data. KJM performed the statistical analyses with input from NJD. KJM wrote the manuscript, and all coauthors contributed to revisions.

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Table 1: Sources of variation in BMR (mL O_2 per minute) and body mass (g) prior to commencement of the playback experiments.

	BMR	**Body mass**	**BMR controlling for mass**
Fixed effects	**β ± 95 % CI**	**β ± 95 % CI**	**β ± 95 % CI**
Intercept	0.91 (0.87, 0.95)	17.20 (16.90, 17.47)	0.92 (0.88, 0.95)
Sex[1]	0.06 (0.04, 0.09)	1.46 (1.19, 1.72)	0.06 (0.03, 0.08)
Mass[2]	NA	NA	0.06 (0.03, 0.08)
Treatment[3]	0.01 (-0.04, 0.06)	-0.01 (-0.39, 0.30)	0.00 (-0.03, 0.08)
Random effects	**σ^2 ± 95 % CI**	**σ^2 ± 95 % CI**	**σ^2 ± 95 % CI**
Plot	0.0009 (0.0001, 0.0023)	0.03 (0.00, 0.11)	0.0009 (0.0001, 0.0024)
Residual	0.005 (0.004, 0.006)	0.53 (0.39, 0.68)	0.004 (0.003, 0.005)

1. Coded as female = 0, male = 1
2. Centered within sex, and standardized to 1 unit variation.
3. Coded as control = 0, predator = 1.

Table 2: Standardized coefficients for variation in BMR (mL O_2 per minute) and body mass (g) of free-living great tits as a function of treatment.

	BMR[5]	**Body mass**[6]	**BMR**[7] **controlling for mass**
Fixed effects	**β ± 95 % CI**	**β ± 95 % CI**	**β ± 95 % CI**
Control[1]	-0.31 (-0.96, 0.30)	0.02 (-0.52, 0.67)	-0.37 (-1.04, 0.19)
Period[2]	0.18 (-0.02, 0.40)	0.01 (-0.15, 0.20	0.18 (-0.03, 0.36)
Predator[1]	0.003 (-0.64, 0.60)	0.13 (-0.45, 0.77)	-0.03 (-0.59, 0.56)
Period[2]	-0.03 (-0.22, 0.16)	-0.12 (-0.27, 0.03)	0.03 (-0.18, 0.19)
$\text{Mass}_{\text{within}}$[3]	NA	NA	0.12 (0.03, 0.25)
$\text{Mass}_{\text{between}}$[4]	NA	NA	0.49 (0.26, 0.69)
Random effects	**σ^2 ± 95 % CI**	**σ^2 ± 95 % CI**	**σ^2 ± 95 % CI**
Individual	0.13 (0.07, 0.21)	0.19 (0.10, 0.28)	0.10 (0.05, 0.15)
Plot	0.28 (0.06, 0.66)	0.27 (0.05, 0.66)	0.26 (0.05, 0.62)
Residual	0.15 (0.10, 0.21)	0.10 (0.07, 0.14)	0.13 (0.09, 0.18)

1. Reference category “pre-manipulation”
2. Post-manipulation change
3. Body mass centered within individual and standardized to 1 unit variation.
4. Body mass centered among individuals within sexes and standardized to 1 unit variation.
5. BMR centered within sex and standardized to 1 unit variation.
6. Body mass centered within sex and standardized to 1 unit variation.

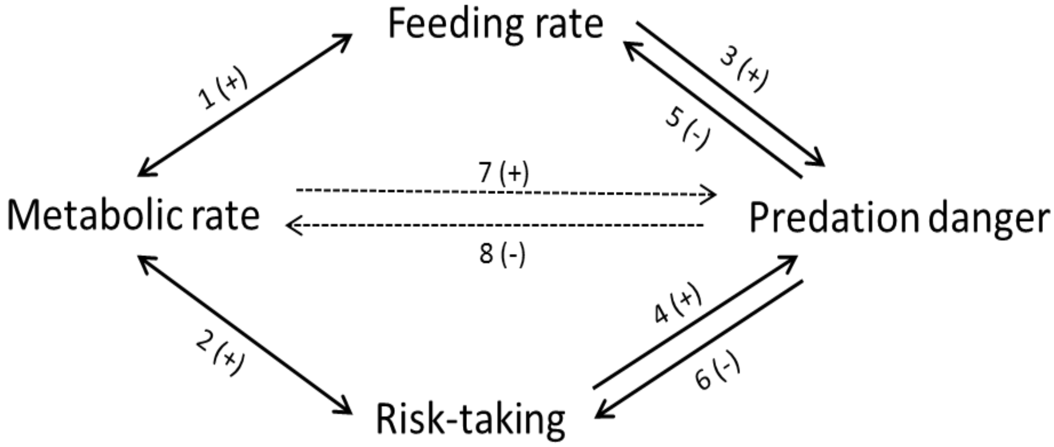

Figure 1: Illustration of hypothesized relationships between MR, behaviour and predation danger. Direct (causal) relationships are illustrated with solid arrows, indirect relationships are illustrated with dotted arrows. The nature of the relationship (positive or negative) is indicated in parentheses. If higher MR requires greater total energy intake, it may favour higher feeding rates (1) and greater risk-taking (2). The greater resource acquisition conferred by these behaviours may in turn facilitate the maintenance of higher MRs (illustrated by doubled sided arrows in (1) and (2)). All else being equal, higher feeding rates (3) and greater risk-taking (4) expose animals to greater risk of predation, resulting in a positive indirect relationship between MR and predation danger (7). However, because higher predation danger favours adaptive suppression of feeding rate (5) and risk-taking (6), increasing predation danger may indirectly result in reduced MR (8).

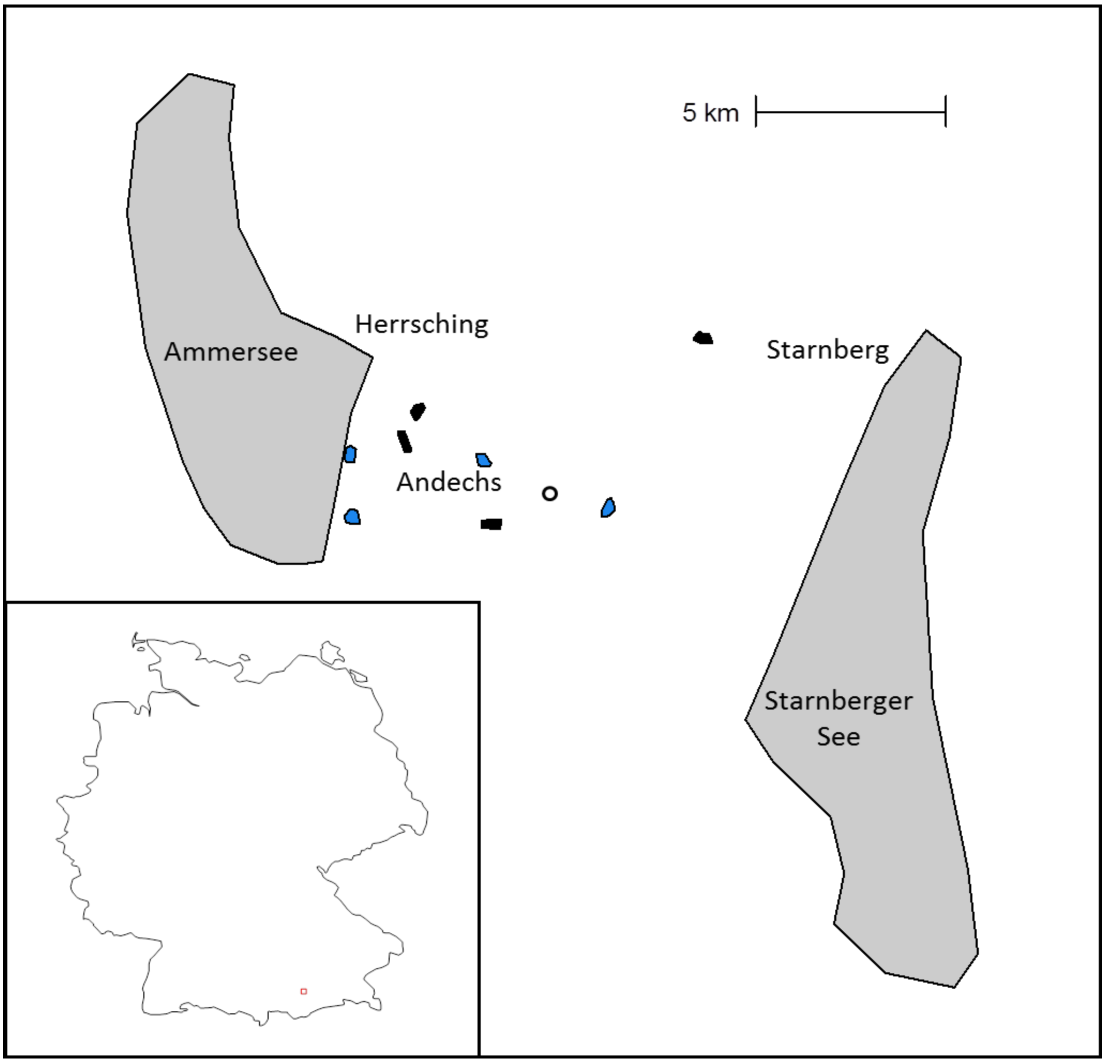

Figure 2: Map of study area in Bavaria, Germany (48°N, 11°E). The region is indicated by the red box in the inset map of Germany. Plots that received predator playbacks are shown in blue, plots that received control playbacks are shown in black. The weather station from which daily temperature data were obtained is shown with an open circle, and lakes are indicated in light grey.

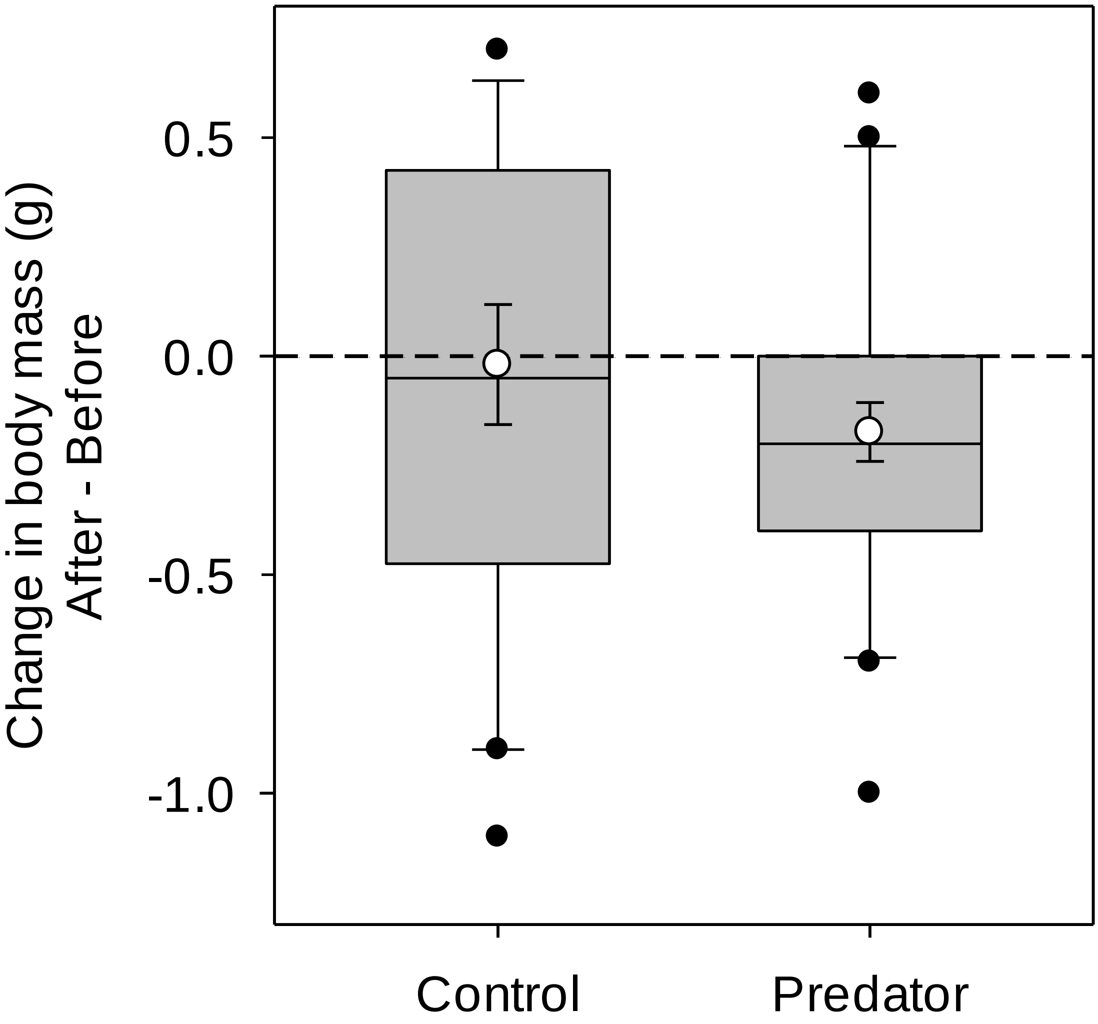

Figure 3: Illustration of the effect of the experimental manipulation of perceived predation danger on changes in body mass (grams) as a function of treatment (control shown on left, predator on right). The 10th and 90th percentiles are shown with whiskers, while the 25th and 75th percentiles are shown by the boundaries of the box. The line within each box denotes the median value, and outliers are shown with black circles. The white circle and errors bars inside each box plot denote means ± 1 s.e. Data shown are differences calculated from raw data for individual great tits that were measured both before and after the treatment (N = 26 Control, N = 31 Predator). The horizontal dashed line at zero represents no overall change in measurements following treatment.

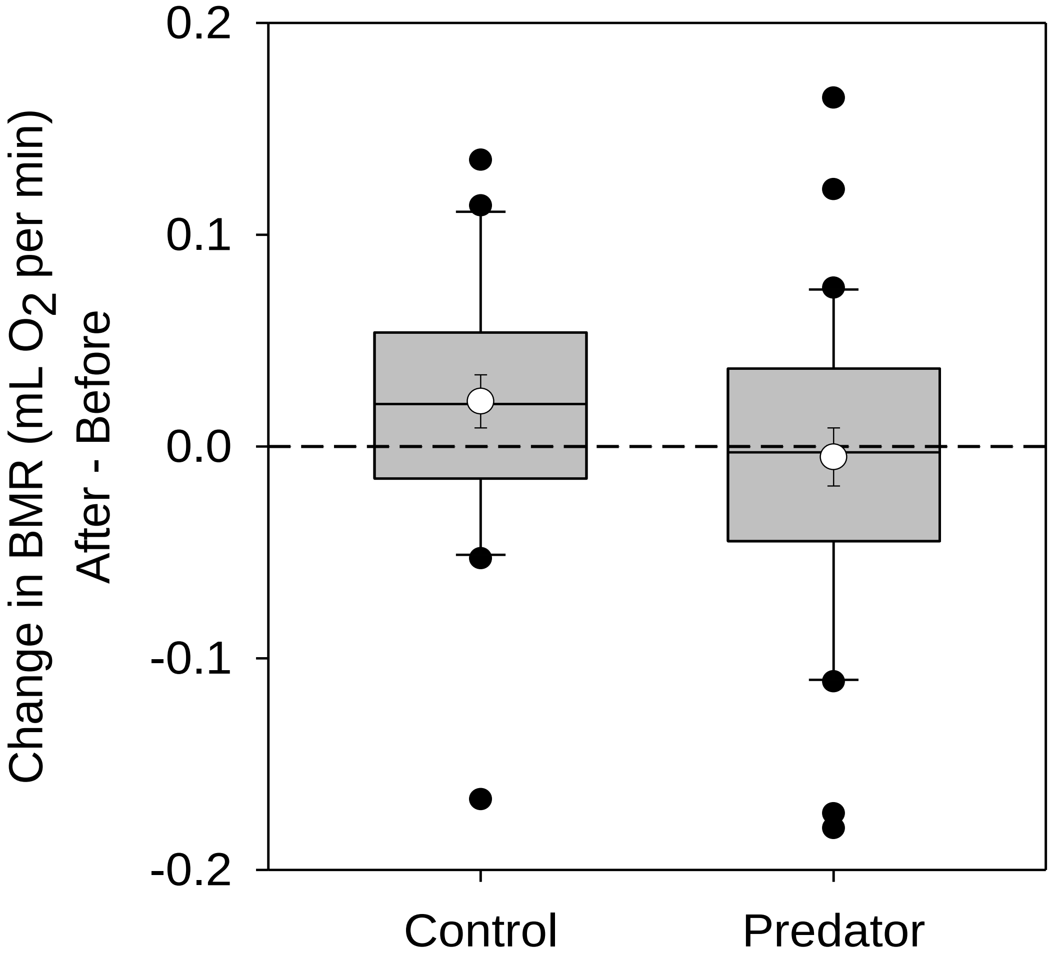

Figure 4: Illustration of the effect of the experimental manipulation of perceived predation danger on changes in BMR (mL O_2 per min) as a function of treatment (control shown on left, predator on right). The 10th and 90th percentiles are shown with whiskers, while the 25th and 75th percentiles are shown by the boundaries of the box. The line within each box denotes the median value, and outliers are shown with black circles. The white circle and errors bars inside each box plot denote means ± 1 s.e. Data shown are differences calculated from raw data for individual great tits that were measured both before and after the treatment (N = 26 Control, N = 31 Predator). The horizontal dashed line at zero represents no overall change in measurements following treatment.

Supplementary Materials

Supplementary Text S1: Respirometry setup

BMR was measured as O_2 consumption rates using three identical setups. Each set up consisted of a four-channel open flow respirometry system with water vapour, CO_2 and O_2 analysers. H_2O and CO_2 were removed from influent air using Drierite and Ascarite, and the air was then pumped through three metabolic chambers made from 1-L metal cylinders with airtight lids. The chambers were kept in an environmental chamber (Binder KB53 Refrigerated Incubator, Binder GmbH, Tuttlingen, Germany), which maintained the chambers at a constant temperature of 25.0 ± 0.1˚C, which is within the thermoneutral zone of great tits (Broggi et al., 2009). A constant air-flow rate into the chambers of 200 ml min^{-1} (following Broggi et al., 2009) was maintained using mass-flow controllers (Sable Systems, Las Vegas, NV, USA). The O_2, H_2O and CO_2 concentrations in effluent air streams were measured using a water vapour analyser (Sable Systems) and oxygen and CO_2 analysers (FoxBox; Sable Systems). An additional stream of dry, CO_2 free air was used as a baseline throughout recordings. An automatic valve switched between streams, so that 10 min of baseline O_2 concentrations were recorded between every 30 min recording of a zebra finch. Thus, on each of the three identical respirometry set-ups, 130 min of recording were made for each complete cycle (four 10 min baseline recordings and three 30 min great tit recordings), and an average of five complete cycles were recorded for each set-up per night (that is, five 30 min recordings per bird).

Analogue outputs from the H_2O, O_2 and CO_2 analysers were fed to a computer via a 16-bit A/D converter card. H_2O, O_2 and CO_2 concentrations were recorded at 1 s intervals. The rate of O_2 consumption (VO_2) was calculated following Lighton (2008). Briefly, VO_2 was calculated as:

$$VO_2 = FR \times ((F_iO_2 - F_eO'_2) - F_eO'_2 \times (F_eCO'_2))/(1-F_eO'_2) \qquad \text{Eq. 1}$$

where FR is the flow rate of dry, CO_2 free air into the metabolic chambers (in ml min^{-1}), F_iO_2 is the partial pressure of O_2 in the influent air, $F_eO'_2$ is the partial pressure of O_2 in the effluent air after correcting for dilution due to water vapour pressure and $F_eCO'_2$is the partial pressure of CO_2 in the effluent air after correcting for water vapour pressure dilution. Water vapour pressure dilution corrections were performed as follows:

$$F_eO'_2 = F_eO_2 \times BP / (BP - WVP) \qquad \text{Eq. 2}$$

$$F_eCO'_2 = F_eCO_2 \times BP / (BP - WVP) \qquad \text{Eq. 3}$$

where F_eO_2 and F_eCO_2 are the uncorrected values of O_2 and CO_2 concentrations measured in the effluent air, BP is the barometric pressure (kPa) and WVP is the water vapour pressure (kPa). We used ExpeDataPro (Sable Systems) to select and calculate the lowest 10min average VO_2, and this was used to represent the BMR. Minimum O_2 consumption rates occurred in the early morning hours (between circa 02h00 and 05h00), which was between 8 and 12 hours after the last feeding opportunity from the previous day. Hence, birds were assumed to be post-absorptive at the time of minimum O_2 consumption.

Analyzers were calibrated daily. The O_2 analyzer had a fuel cell sensor type, and therefore only required span calibrations (not zero calibrations). The O_2

analyzer was spanned to 20.95% using dry, CO_2-free air (outdoor air scrubbed using Drierite and Ascarite) as the reference gas. Nitrogen was used for zero calibrations of the O_2 and CO_2 analyzers. The CO_2 analyzer was spanned to 0.977% using a stock gas. The H_2O analyzer was spanned based on the dilution observed in the O_2 analyzer when switching from dry CO_2-free air to un-scrubbed outside air using the following equation:

$$WVP = BP\,(F'_iO_2 - F_iO_2)/F'_iO_2 \qquad \text{Eq. 4}$$

Where F'_iO_2 is the partial pressure of oxygen in the chemically dried air-stream (20.95%) and F_iO_2 is the partial pressure of O_2 in the airstream without chemical drying.

Supplementary Text S2: Parameter estimation methods

Analyses presented in the main text used an inverse wishart prior. Parameter estimates for “Plot” were sensitive to nu (degree of belief in the prior), and we therefore present estimates obtained from models where nu = 0.002 (i.e. a non-informative prior). Fixed effects estimates were robust across different prior settings and were also identical when estimates were obtained using the ‘sim’ function of the ‘arm’ package to simulate the posterior distribution of model parameters for models constructed using the ‘lmer’ function (analyses not shown).

Models were run for 16000 iterations, with a burn-in period of 6000 and thinning interval of 10. This produced a sample of 1000 estimates for each model. These estimates were used to calculated the most likely value for each parameter (the mode of the distribution), as well as its 95% credible interval.

Supplementary References

Broggi J, Hohtola E, Koivula K, Orell M, Nilsson J-Å, 2009. Long-term repeatability of winter basal metabolic rate and mass in a wild passerine. Funct Ecol 23:768-773.

Lighton JRB, 2008. Measuring metabolic rates: a manual for scientists. New York: Oxford University Press.

Supplementary Table S1: Summary of roosting densities, body mass and previous treatment for plots used the present study

Plot ID	**Winter 2013 roosting count**	**Average mass (SD)**	**Spring 2013 treatment**	**Winter 2014 treatment**
10	11	18.95 (0.94)	Predator	Predator
11	15	18.09 (1.24)	Control	Predator
12	16	18.58 (1.12)	Predator	Control
13	21	18.83 (0.94)	Predator	Control
14	14	18.29 (1.31)	Control	Predator
15	11	18.57 (0.81)	Predator	Predator
16	15	18.35 (0.76)	Control	Control
20	17	18.83 (1.12)	Control	Control

Supplementary Table S2: Standardized coefficients for variation in BMR (mL O_2 per minute) and body mass as a function of treatment. Analyses include all individuals, including those without repeated measures. Analyses on a restricted data set (including only individuals with repeated measures) are provided in the main text.

	BMR[5]	**Body mass**[6]	**BMR**[5] **controlling for mass**
Fixed effects	**β ± 95 % CI**	**β ± 95 % CI**	**β ± 95 % CI**
Control[1]	-0.23 (-0.70, 0.29)	0.62 (-0.09, 1.17)	-0.21 (-0.86, 0.29)
Period[2]	0.17 (0.01, 0.35)	0.00 (-0.18, 0.18)	0.14 (-0.06, 0.30)
Predator[1]	0.05 (-0.48, 0.59)	0.73 (0.11, 1.37)	-0.02 (-0.52, 0.52)
Period[2]	-0.05 (-0.23, 0.13)	-0.14 (-0.27, 0.05)	0.02 (-0.16, 0.18)
$\mathrm{Mass}_{\mathrm{within}}$[3]	NA	NA	0.12 (0.01, 0.22)
$\mathrm{Mass}_{\mathrm{between}}$[4]	NA	NA	0.42 (0.27, 0.58)
Random effects	**σ^2 ± 95 % CI**	**σ^2 ± 95 % CI**	**σ^2 ± 95 % CI**
Individual	0.13 (0.07, 0.19)	0.16 (0.10, 0.22)	0.04 (0.00, 0.09)
Plot	0.008 (0.000, 0.029)	0.01 (0.00, 0.04)	0.006 (0.000, 0.022)
Residual	0.15 (0.11, 0.20)	0.09 (0.06, 0.12)	0.17 (0.11, 0.22)

1. Reference category “pre-manipulation”
2. Post-manipulation change
3. Body mass centered within individual and standardized to 1 unit variation.
4. Body mass centered among individuals within sexes and standardized to 1 unit variation.
5. BMR centered within sex and standardized to 1 unit variation.
6. Body mass centered within sex and standardized to 1 unit variation.

Chapter 4

Perceived predation risk influences behavior and life-history: a variance partitioning approach

Abstract

Predation induces strong selection on phenotypic traits, affecting prey via consumptive and non-consumptive effects. Environmental stress can affect the variance components that shape the repeatability of such traits and thus their environment-specific evolutionary potential. We manipulated perceived predation risk using audio playback in wild great tits (Parus major) for 2 years for a 5-month period (March–July) in 12 nest box populations; monitoring behavioral and life history responses. Of the 12 populations, 3 received the control treatment both years, 3 the predator treatment both years, 3 first control then predator, and 3 predator and then control. This allowed us to examine correlations between traits and plasticity within and among individuals exposed to the same treatment across the years, to test if repeatability or correlations differed in the different predation regimes. Additionally, using the individuals that were exposed to a different treatment in each year, we could determine if traits were repeatable across contexts and if different types of individuals responded differently to predation risk. We found higher variation among individuals in lay date for birds exposed to increased perceived predation risk, and consequently higher repeatability. Exploration behavior shows tight cross-context correlation over treatments and lay date did not; the relationship between exploration and lay date changed with treatment. These findings suggest that predation risk can influence heritability of traits and correlations between traits, and thus has the potential to alter evolutionary processes.

Prepared as: Abbey-Lee RN, NJ Dingemanse. Perceived predation risk influences behavior and life-history: a variance partitioning approach

Introduction

Environmental stress can potentially greatly affect the expression of variance components (Hoffmann and Merila 1999, Charmantier and Garant 2005). Predation pressure is an environmental stressor, and prey have been shown to adjust a large range of phenotypic traits in response to changes in risk including morphology (Relyea and Werner 2000), life history (Eggers et al. 2006, Zanette et al. 2011), foraging (Macleod et al. 2005), vigilance (Brown 1999), and parental care (Tilgar et al. 2011, Zanette et al. 2011, Bonnington et al. 2013, Ghalambor et al. 2013). Few studies test how exposure to predators affects the variance components that shape the repeatability (r), or even heritability (h^2), of ecologically relevant traits, i.e. the among and within individual variance (Relyea 2005, Kraft et al. 2006, Dingemanse et al. 2009, Izhar and Eilam 2010, Dammhahn and Almeling 2012, Niemelä et al. 2012, Stein and Bell 2012, Briffa 2013, Furtbauer et al. 2015, Brown and Robinson 2016). These variance differences alone can influence selection because evolution depends on narrow-sense heritability, the fraction of phenotypic variance (V_P) owing to additive genetic variance (V_A) (Falconer and Mackay 1996). However, determining additive genetic variance requires pedigree data that is not available for all study populations, so often among-individual variance (V_I) (and its standardized metric repeatability) is used as a proxy for V_A (Dochtermann et al. 2015). The few studies exploring this topic have shown that predators can induce short-term effects on prey populations in the lab by influencing the expression of V_I (Quinn 2005, Dingemanse et al. 2009, Izhar and Eilam 2010, Stein and Bell 2012, Brown and Robinson 2016).

This is due to different phenotypes within the same population responding differently to predators (called individual × environment interaction (I × E). Additionally, predators can also affect repeatability/heritability if they influence V_R, i.e. the sum of environmental (V_E) and error (e) variances (Falconer and Mackay 1996). Specifically, predators may increase V_E (and hence V_R), consequently decreasing heritability (Briffa 2013). This means that regardless of the direction or strength of selection induced by predation, predation exposure can also importantly influence population dynamics because changes in heritability alter the response to selection. Thus, these more cryptic response of changes in the magnitude of variance components as a function of perceived predation risk are important in order to understand predator-induced evolution (Brommer et al. 2008).

Both the mean and variance responses described above examine population level responses to predation risk. However, phenotypic variation among individuals has recently gained interest in behavioral ecology research (Dall et al. 2004, Réale et al. 2007, Westneat et al. 2015) and individuals have been shown to differ consistently in boldness and risk perception (Bouskila and Blumstein 1992, Luttbeg and Schmitz 2000, Stankowich and Blumstein 2005, Luttbeg and Sih 2010, Wolf and Weissing 2012). If individuals differ in their perception or sensitivity to risk, they can be expected to respond to risk differently. Specifically, those facing most risk should respond more strongly and pay higher (fitness) costs, resulting in adaptive among-individual variation in phenotypic plasticity (i.e. greater plasticity within certain individuals) (Nussey et al. 2007, Dingemanse and Wolf 2013).

Recently, the concept of reaction norms has been developed to describe the variation among individuals (e.g. Dall et al. 2004, Sih et al. 2004, Dingemanse and Réale 2005, Nussey et al. 2005, Wilson et al. 2005, Dingemanse et al. 2010, Kluen and Brommer 2013). Specifically, the reaction norm framework allows researchers to determine if individuals respond the same or differently to an environmental gradient. All individuals may alter their behavior similarly across the environment, leading to parallel reaction norms and repeatable behavior both within and between contexts (Figure 1). Conversely, individuals may alter their behavior differently across the environment, leading to crossing reaction norms and differences in repeatability across contexts. Therefore, predation risk may also affect the correlations among traits within each environment and within traits across environments. Thus, variances can change because genotypes differ in plasticity (G × E). This can manifest as changes in variance only, while individual rank-order differences are maintained. Also, more cryptic manifestation occurs if genotypes change rank-order. This latter effect is only visible when studying if the cross-context correlation for a single trait differs from 1 (Falconer and Mackay 1996).

This study was designed to determine if predation risk affected the expression of individual variance and covariance components of prey behavioral and life history traits in the field. We used playback to manipulate the perceived predation risk in multiple populations of wild birds across two years. Half of the populations received the same treatment both years, allowing us to assess treatment specific repeatabilities, and half alternated treatments in order to assess cross context correlations and crossing of reaction norm slopes (Figure 1). To comprehensively evaluate the effects of predator risk, our analyses were threefold: 1) we evaluated whether there were mean differences in trait expression between predator and control exposed individuals; 2) we determined if predator exposure affected V_I and V_R by comparing V_I and V_R between the two treatment groups; 3) we tested correlations within traits across treatments and among traits within a treatment. As detailed above, the latter two tests matter for evolutionary processes even though they have not yet been studied in natural populations.

First, we predict that birds exposed to an increased perceived predation risk will show mean differences for traits compared to control birds. Specifically, we predict that on average birds exposed to increased perceived predation risk will have lower body mass (e.g. Witter et al. 1994, Lilliendahl 1997, Perez-Tris et al. 2004, Macleod et al. 2005, Abbey-Lee et al. 2016b); and exploration behavior (Abbey-Lee et al. 2016b). Previous work showed no differences in aggression behavior between birds exposed to predator and control treatments (Abbey-Lee et al. 2016a). In terms of life history, we expect that birds exposed to the predator treatment will invest less in reproduction, and therefore have later lay dates and fewer eggs per clutch (Perrins and McCleery 1989, Travers et al. 2010). Second, in terms of variance changes, we predict that there will be higher V_R (more variation within individuals) and lower V_I (less variation between individuals) in areas exposed to increased perceived predation risk. Deviations from the optimal phenotype in a given environment will lead to greater fitness costs when conditions are tough, hence we expect individuals to modify their own behavior to converge upon a more optimal phenotype when exposed to increased predation pressure

(Nicolaus et al. 2013). Finally, we predict that the behavioral traits exploration and aggression show tight cross-context correlations as they represent a component of an individual's personality (Dingemanse et al. 2009). Owing to evidence suggesting that explorative animals are willing to shift their investment towards current reproduction when given the opportunity (Nicolaus et al. 2015), we predict that individuals differ in their plastic adjustment of life-history decisions (e.g. lay date, clutch size), that consequently cross-context correlations are weaker for life-history traits, and that they show treatment-specific correlations with personality.

Materials and Methods

Data collection

Data were collected in 2013 and 2014 in 12 forest plots that were established in a 15 km^2 area around the Max-Planck Institute for Ornithology, Seewiesen, Germany (Stuber et al. 2013, Araya-Ajoy and Dingemanse 2014, Nicolaus et al. 2015). Plots consisted of 50 nest boxes arranged in a regular grid spanning approximately 9-12 ha. We checked nest-boxes bi-weekly beginning April 1st each year. Lay date, clutch size, parental identity, nestling growth and fledging success were monitored using standard methods (detailed in Nicolaus et al. 2009). Simulated territorial intrusions (i.e., "aggression tests") were applied to each breeding male during egg laying (1and 3 days after the first was laid) and incubation (1 and 3 days following the onset of incubation) (for exact procedure, see Araya-Ajoy and Dingemanse 2014; briefly described below). Based on incubation onset and clutch size, shortly before the expected hatch date we checked nest-boxes daily to determine hatch date (day 0). At day 6, nestlings were weighed, bled and given an aluminium ring with a unique identifying number. At day 7, parents were caught using a spring trap in the nest-box, tested for exploratory behavior (detailed below), weighed, bled, measured and given an aluminium ring with a unique identifying number (if necessary). If we failed to catch parents on day 7, another catch attempt was made on day 9. At day 14, standard body measurements (body mass ± 0.1 g, tarsus ± 0.1 mm, wing length ± 0.5 mm) and behavioral measurements (breathing rate, number of breaths per minute; docility, number of struggles per minute; as part of another study) of nestlings was recorded. From day 19 onwards, boxes were checked every second day to determine fledge date.

Exploratory behavior was measured for all breeding birds when their nestlings were 7 or 9 days old using a cage test adapted from the classic 'novel environment' test (Verbeek et al. 1994, Dingemanse et al. 2002, Nicolaus et al. 2015). See Stuber et al. (2013) for a full description of the procedure. Briefly, each individual was initially kept in a small compartment connected to an exploration cage for 1 min for acclimatization. Birds were then flushed into the exploration cage, a solid plastic box with 3 perches and 1 barred side (61 L × 39 W × 40 H cm). Each individual was recorded for two minutes and their movements between perches, walls, and floor were scored later from the recording. The sum of movements between different locations (scores ranged from 2 to 130) was used as a proxy of exploratory behavior as is done with the classic winter exploration test (e.g. Dingemanse et al. 2002), because active animals differ in willingness to take risk (Stuber et al. 2013) and act in a more proactive way (Nicolaus et al. 2014). Before scoring videos, observers (N = 5) were trained using 10 randomly selected sample exploration videos until the between-observer correlation coefficient was greater than 0.85. We performed 607 tests on 497 unique [ringed] birds. Of these, 387 were tested in only 1 year and 110 were tested in both years. Of the 110 birds with repeat measures, 29 individuals received the predator treatment both years, 32 received control both years, and 49 received both treatments.

Aggression tests were done by simulating territory intrusions by placing one of 18 taxidermic mounts of a male great tit as a visual stimulus with one of 21 playback songs as an acoustic stimulus 1 m in front of a nest box. Each breeding male was subjected to 4 aggression tests: 2 during egg laying (1 and 3 days after the first was laid) and 2 during incubation (1 and 3 days following the onset of incubation). Tests were performed for first breeding attempts only and were conducted between 07:00 and 12:00 h with the specific time for each nest semi-randomly assigned. One of 18 observers performed the observation at a distance of 15 m. Taxidermic mount, song identity, and observer were all randomly assigned. Following the arrival of a focal male within a 15-m radius of the nest box, we recorded the following behaviors within a 3-min period: number of calls, number of songs, and minimum distance from the mount. Subjects that did not arrive within 15 min were scored as nonresponsive. In the 2 years included in this study, we performed 1612 tests. Only tests where male identity was known (n = 1197 tests [71%], n = 247 unique [ringed] males) were used for statistical analysis; in 803 (67%) of these tests, the male responded. Of the 55 males sampled in both years, 13 received the predator treatment both years, 17 received the control treatment both years, and 25 received both treatments.

Manipulation of perceived predation risk

We conducted a playback experiment in order to manipulate perceived predation risk (Abbey-Lee et al. 2016a). Four speakers (Shockwave, Foxpro, Pennsylvania, USA) were spaced approximately 150-250 m apart, such that there was good coverage of the entire plot. Speakers were place in February and removed in July after all first broods had fledged. Assignment of treatments to plots was randomized, with the constraint that there be no initial differences between treatments in average breeding density, lay date, latitude, or longitude based on data from previous years (analyses not shown). Each year, six plots received a control treatment and six plots received a predator treatment, half of the plots switched treatment for the second year. This created four distinct treatment groups, three plots that received the control treatment both years, three that received the predator treatment both years, three that received control one year, predator the next, and finally three that received predator one year and control the second. This design allowed us to examine individual repeatability within and across treatments, within- and among-individual correlations within treatment, as well as individual-level cross-context correlations. In control plots, speakers were programed to play songs of the Eurasian blackbird (*Turdus merula*; a sympatric, avian non-predator species). In predator plots, speakers were programmed with calls from sparrowhawks (*Accipter nisus*; a sympatric, avian predator species). Bird sounds were acquired from the Xeno-Canto database (www.xeno-canto.org) or provided by Hans Heiner Bergmann. Eight unique files were made for each sound type; each file was normalized with the software program Audacity 2.0.5 and edited to last 6 minutes.

All speakers were programed following the same scheme, matching the normal timing and frequency of vocalization of our playback species. That is, for the first 3 hours after dawn, and for the last 3 hours before dusk, speakers broadcast, on average, six 6-minute song/call bouts per hour (i.e., 60% of the time). During the daylight hours between these two intensive periods, speakers broadcast approximately 1.5 bouts per hour (i.e. 15% of the time). The amount of silence between playbacks was determined randomly to avoid habituation. Playback was broadcast at 90 dB (intensity was set to match the normal intensity of bird songs and calls and was measured at 1 m with a sound level meter). Playback was given for 4 consecutive days ("on"), followed by 4 consecutive days of non-playback (speakers were off), after which the cycle was repeated, throughout the season; this design is known to prevent habituation and investigation of immediate and carry-over effects of predator cues was explored in an earlier publication (Abbey-Lee et al.2016a).

Statistical analyses

Mean comparisons

As a first step, we determined if perceived predation risk influenced mean differences in life history or behavior, using univariate mixed effects models. Linear models were created for all response variables: lay date (date first egg was laid, one measure per year, attributed to female), clutch size (one measure per year, attributed to female), aggression (male minimum distance to mount, four measures per year, attributed to male), exploration (one measure per individual, per year, attributed to male and female separately), and adult body mass (one measure per individual per year, attributed to male and female separately). Response variables were normally distributed and modeled with Gaussian error distributions; all variance components were, for this analysis, assumed to be equal across treatments (for validation, see below). All models were fit using restricted maximum likelihood. Treatment, a categorical variable with two levels (control, predator) was included as a fixed effect in all models. All models included the random intercepts for individual identity and the unique combination of plot and year (PlotYear, n = 24 levels) because the treatments varied at this level (see also Abbey-Lee et al. 2a). Additionally, some response variables required additional fixed effects. The model of aggression included nest stage (categorical with two levels: incubation or laying), sequence (categorical with two levels), and time of measurement (as time since sunrise in hours, expressed as the deviation from the average time of all tests) because aggression has been shown to vary at these levels (Araya-Ajoy and Dingemanse 2014); exploration included sex (categorical with two levels: male or female) and measurement time because exploration score varies with time of day in some other populations (Dingemanse et al. 2002); body mass included sex and time of measurement because great tits are sexually dimorphic for mass and body mass in small passerines is known to show marked diurnal variation (Haftorn 1989, Lilliendahl 2002). The random intercepts for individual identity and PlotYear enabled us to partition the total variance into variance attributable to individual, spatio-temporal location (i.e. PlotYear effects), and residual. Individual repeatability was calculated as the among-individual variance component divided by the total phenotypic variance not attributable to fixed effects (i.e. "adjusted" repeatability;

Nakagawa and Schielzeth 2010). The significance of all fixed effects was based on the F-statistic and numerator and denumerator degrees of freedom from the algebraic algorithm in ASReml 3.0 (Gilmour et al. 2009). Statistical significance of focal random effects was calculated using a likelihood ratio test where the χ^2 distributed test statistic was twice the difference in log likelihood between the full model and a model with the random effect removed (Shaw 1991, Meyer 1992, Wilson et al. 2010). We used 0.5 degrees of freedom because variances are bound to be positive and we assumed an equal mixture of $\chi^2(0)$ and $\chi^2(1)$ (Self and Liang 1987, Pinheiro and Bates 2000, Visscher 2006).

Variance and cross-context correlations

As a second step, we determined if perceived predation risk influenced the variance components within single traits. To determine if variances differed across treatments we used bivariate mixed-effects models to partition the variation for each measured trait into within- and among-individual variance (Table 2). All models were fit using restricted maximum likelihood. We created separate bivariate models for each of the response variables above (aggression, exploration, body mass, lay date, clutch size, offspring quality, and offspring quantity). However, this time instead of fitting treatment as a fixed effect, each response variable was split into two unique y-variables based on treatment (e.g. aggressiveness expressed in the control, and aggressiveness expressed in the predator treatment). Otherwise, these models used the same fixed and random effects structure detailed for univariate models above. We ran 2 versions of the model: 1) the base model where the variance across treatments was unconstrained (i.e. separate estimates for each treatment) and 2) a model where variance was constrained to be equal (i.e. the same estimate for both treatments). Comparisons of the log-likelihood values from these models allowed us to determine if the variance components in the predator and control treatments differed significantly from each other (i.e. if the model where variance was allowed to differ fit better than the model where variance was equal). Statistical significance was calculated using the likelihood ratio test detailed above. The χ^2-distributed test statistic was calculated over 2 degrees of freedom.

Importantly, because some birds were exposed to both control and predator treatments, we were able to use the same base model to determine the correlation of a trait across treatments by estimating patterns of cross-treatment trait covariance. Statistical significance of the correlation was calculated by comparing the fit of the base model with one where the correlation (depending on the specific question) was constrained to either 0 or 1 using a likelihood ratio test as detailed above (with df = 1 or df=0.5, respectively). We used 0.5 degrees of freedom for the comparison with 1 because correlations have an upper-bound of 1 and we therefore assumed an equal mixture of $\chi^2(0)$ and $\chi^2(1)$ (Self and Liang 1987, Pinheiro and Bates 2000, Visscher 2006). If correlations were significantly different from 0, this indicates the existence of significant rank-order differences across environments (treatments); similarly, if correlations differed from 1, this indicates significant crossing of ranks across environments (Roff 1996).

Correlations between traits

As a final step, we estimated patterns of among-trait covariance to determine if perceived predation risk altered correlations between behavior and life history. We estimated patterns of trait covariance using a multivariate mixed-effects model. All models were fit using restricted maximum likelihood. Lay date, clutch size, and body mass were chosen because there was evidence for their cross-context correlations deviating from 1 (see Results below).We used only exploration as our focal behavioral trait in our models because our life history variables are female attributes, and aggression was not measured for females. We created one model to determine the among- and within- individual covariances and correlations between exploration and either lay date, clutch size, or body mass. Similar to the cross-context models described above, the among- and within-individual correlations between behaviors were estimated by means of a multivariate mixed-effects model where all traits were treated as two treatment-specific traits (see above) (Table 3). These models used the same fixed and random effects structure detailed for models above. Statistical significance of a focal covariance was calculated by comparing the fit of the full model with one where the focal covariance was constrained to be equal in the two treatments (using the likelihood ratio test detailed above).

Results

Mean comparisons

We found that body mass tended to be lower in predator treated plots but differences in means were not detected for any other measured traits (Table 1). There was significant among-individual variance for aggression, exploration, body mass, clutch size, and lay date ($p < 0.001$ for all), with individual repeatability ranging from 0.10 to 0.65.

Variance and cross-context correlations

We found that variances and repeatability of lay date differed between treatments (Table 2). There was higher among-individual variation and lower within-individual variation in the predator treated plots, and, consequently higher repeatability. There were no differences between treatments on the (relative) magnitudes of these variance components for any of the other measured traits.

We found that exploration ($r = 0.93$), body mass ($r = 0.50$), and clutch size ($r = 0.84$) were significantly correlated across treatments at the among-individual level, indicating that females that had relatively high values in the control treatment also had relatively high values in the predator treatment (Figure 1b). The other measured traits did not show significant among-individual correlations across treatments (Figure 1a).

We found that the among-individual cross-treatment correlations for aggression, body mass and lay date were significantly different from 1, and that clutch size also tended to differ from 1 (Table 3). This indicates that treatment influenced the expression of these traits by altering the rank order of individuals across treatments (Figure 1c). For traits where correlations did not differ from 1, rank orders of individuals were either maintained across contexts or we had insufficient statistical power to detect crossing of reaction norms. Some traits were correlated across treatments and some were not, forcefully demonstrated the potential for among-individual correlations between traits to also change across treatments.

Correlations between traits

We found that the among-individual correlation between exploration and lay date tended to differ across treatments. In control plots there was a positive relationship; more exploratory birds initiated nests later than less exploratory birds (r = 0.32). Conversely, in the predator plots the relationship was negative; more exploratory birds initiated nests earlier than less exploratory birds (r = -0.21).

There were no significant differences across treatments for the correlations between exploration and mass or exploration and clutch size. The relationship between exploration and mass was slightly negative in both treatments. The relationship between exploration and clutch size tended to be positive.

Discussion

This study evaluated life history and behavioral responses of free-living great tits exposed to an experimental manipulation of perceived predation risk. We found that great tits did not differ in average trait expression when exposed to predator vocalizations versus control sounds. Contrary to our predictions, we found that there was lower variation among individuals (and a trend for higher within-individual variance) in lay date for birds exposed to increased perceived predation risk. These variance differences led to a higher repeatability of lay date in the predator treated areas. Finally, as predicted, we found that exploration was highly consistent across contexts (i.e. levels of perceived predation risk), and that the relationship between exploration and lay date changed with treatment. These findings imply that behavioral types differed in how they changed this major life-history decision with perceived predation risk. Specifically, fast explorers bred earlier in response to increased perceived predation risk while slow explorers delayed breeding.

It was previously unknown if increases in perceived predation risk would reduce or enhance differences among individuals within a wild population. We tested this important question by exploring how variance between and within individuals differed within and across treatments in order to determine if individuals responded similarly or differently to the predation treatment. Variances differed between the treatment groups, but for only one of our measured traits, lay date. We found that the among-individual variance was higher, and that the within individual variance tended to be lower in birds exposed to the predator treatment. This contradicts our hypothesis that individuals would become more homogeneous when exposed to increased risk. The observed pattern may have occurred because of individual differences in plasticity: change in lay date depended on an individuals' type, increasing the among-individual variance in this trait (see discussion below). These differences in variance lead to higher repeatability of lay date for birds exposed to the predator treatment. This difference in repeatability may indicate that historical selection pressures differed between the two environments (Relyea 2005, Kraft et al. 2006, Dingemanse et al. 2009, Briffa 2013), causing variance components to differ between these selective environments at the current time. Specifically, such differences in among-individual variance suggest that past selection pressures may have depended upon predation levels and inappropriate reaction norms were removed (i.e. inappropriate plastic responses were removed). Additionally, in terms of current response to selection, this implies that now evolution in response to the same selection pressures is likely different between the two conditions; lay date should evolve more quickly in the presence of predators.

We also expected within-individual responses to covary with behavioral type. We found that exploration behavior was highly consistent across treatments, supporting the common assumption that an individual's exploration behavior represents a rather stable characteristic that does not change with context. Therefore, we were able to explore how the relationship between exploration and life history changed with treatment. We found that in the control areas, among-individual levels of exploration and lay date were positively correlated, meaning that faster exploring birds initiated broods later in the season. We found the opposite relationship in the predator exposed areas: faster exploring birds initiated broods earlier in the season and slower exploring birds delayed broods. Lay date is a key life history variable for birds. Earlier laying birds have larger clutches, higher offspring quality, and higher recruitment (Perrins 1970, Perrins and McCleery 1989, van Noordwijk et al. 1995). Our study indicates that predation risk increases individual differences in lay date, and alters the relationship between behavioral type and lay date.

Recently, "pace of life" concepts have been applied to individuals within a population to explain relationships between behavior and life history (Réale et al. 2010). Some individuals might be more plastic in their investment in current reproduction to ensure that they do not pay costs in terms of survival (Nicolaus et al.

2015). For example, slower explorers are thought to have a slower pace of life so they should invest less in current reproduction in order to survive to the next reproductive period. Conversely, fast explorers are thought to have a fast pace of life, and previous work in this population has indeed shown that fast explorers are also more susceptible to risk (Abbey-Lee et al. 2016b). Therefore, in response to our predator treatment, fast explorers are likely to benefit from terminal investment in reproduction and increased investment in current broods, whereas slow explorers should benefit from decreased investment in current broods in favor of future reproductive opportunities. We found support for our prediction that the relationship between behavior and life history differed across treatments. Specifically, in the control exposed areas slow exploring birds had the earliest lay dates, whereas in the predator exposed areas the fast explorers had the earliest lay dates. This indicates that the fast exploring individuals in the predator plots were investing more in the current reproductive effort than both the slow explorers in the same treatment and the fast explorers in the control treatment, potentially due to their fast pace of life and a terminal investment strategy. Conversely, the slow explorers that are thought to have a slower pace of life and favor lower current investment in reproduction showed a delay in brood initiation compared to their slow exploring counterparts in the control treatment.

Conclusion

We found that increased perceived predation risk affected the structure of among-individual correlations between behavior and life-history. This implies that predation risk can potentially affect evolutionary processes over and above the selection pressures induced by predation, which represents an overlooked mechanism. Specifically, we found that predation risk altered the expression of variation among and within individuals, altered the repeatability of traits, and affected correlations among traits. Our study design and statistical approach (that incorporated individual level differences in response) allowed us to determine that the reason we did not detect mean differences in traits is that individual birds responded, but in different ways. This highlights the importance of incorporating individual differences into the study of perceived predation risk and its consequences for micro-evolution in natural populations.

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Table 1. Sources of variation in mean of Aggression, Exploration, Body Mass, Lay Date, and Clutch Size

	Aggression			Exploration			Body Mass		
Fixed effects	β(SE)	$F_{NUMdf, DENdf}$	*P*	β(SE)	$F_{NUMdf, DENdf}$)	*P*	β(SE)	$F_{NUMdf, DENdf}$	*P*
Intercept[a]	9.74 (0.74)	684.22 (1, 23.8)	<0.001	60.52 (1.80)	3317.1 (1, 30.2)	<0.001	17.15 (0.18)	120000 (1, 25.6)	<0.001
Treatment[b]	-0.66 (0.88)	0.57 (1, 21.3)	0.46	1.27 (2.06)	0.38 (1, 19.8)	0.54	-0.16 (0.10)	2.84 (1, 21.4)	0.11
Nest Stage	3.00 (0.51)	35.11 (1, 666.1)	<0.001	-	-	-	-	-	-
Sequence	0.81 (0.50)	2.63 (1, 632.3)	0.11	-	-	-	-	-	-
Time of day[c]	-0.07 (0.24)	0.09 (1, 709.0)	0.76	0.77 (0.49)	2.44 (1, 212.9)	0.12	0.04 (0.01)	8.26 (1, 468.0)	0.004
Sex[d]	-	-	-	6.80 (1.97)	11.94 (1, 459.6)	<0.001	-0.33 (0.07)	21.30 (1, 466.7)	<0.001

Random effects	σ² (SE)	R (SE)	$\chi^2_{0.5}$	*P*	σ² (SE)	R (SE)	$\chi^2_{0.5}$	*P*	σ² (SE)	R (SE)	$\chi^2_{0.5}$	*P*
PlotYear	2.21 (1.34)	0.04 (0.02)	6.54	0.004	6.37 (7.57)	0.01 (0.01)	0.98	0.16	0.03 (0.02)	0.04 (0.02)	15.32	<0.001
Among Individuals	9.05 (2.33)	0.16 (0.04)	26.38	<0.001	264.65 (40.68)	0.52 (0.07)	33.8	<0.001	0.31 (0.05)	0.44 (0.07)	461.61	<0.001
Within Individual	46.36 (2.75)	0.80 (0.04)	-	-	237.89 (32.00)	0.47 (0.06)	-	-	0.36 (0.05)	0.51 (0.07)	-	-

	Lay Date			Clutch Size		
Fixed effects	β(SE)	$F_{NUMdf, DENdf}$	*P*	β(SE)	$F_{NUMdf, DENdf}$	*P*
Intercept[a]	20.60 (2.75)	110.55 (1, 22.3)	<0.001	8.60 (0.22)	2843.65 (1, 29.8)	<0.001
Treatment[b]	-0.20 (3.89)	0.00 (1, 22.1)	0.96	0.03 (0.30)	0.01 (1, 22.4)	0.92

Random effects	σ² (SE)	R (SE)	$\chi^2_{0.5}$	*P*	σ² (SE)	R (SE)	$\chi^2_{0.5}$	*P*
PlotYear	89.42 (27.34)	0.82 (0.05)	463.42	<0.001	0.39 (0.16)	0.13 (0.05)	31.77	<0.001
Among Individuals	10.99 (2.22)	0.10 (0.03)	15.91	<0.001	1.90 (0.24)	0.65 (0.06)	50.07	<0.001
Within Individual	8.87 (1.65)	0.08 (0.02)	-	-	0.62 (0.12)	0.21 (0.04)	-	-

[a] reference category; estimate is for males in the control plots during the laying stage

[b] difference between the treatments (predation – control)

[c] in hours since sunrise, mean centered

[d] difference between the sexes (females – males)

Table 2. Summary of models of variance and repeatability

	Variance				Repeatability			
	Control	Predator			Control	Predator		
Aggression	σ² (SE)	σ² (SE)	χ^2_1	*P*	R (SE)	R (SE)	χ^2_1	*P*
PlotYear	3.01 (2.29)	1.16 (1.47)	0.52	0.47	0.05 (0.03)	0.02 (0.03)	0.33	0.57
Among Individuals	9.85 (3.52)	10.86 (3.47)	0.04	0.84	0.16 (0.05)	0.21 (0.06)	0.34	0.56
Within Individuals	49.69 (4.15)	40.25 (3.58)	2.98	0.08	0.78 (0.06)	0.77 (0.06)	0.06	0.81
Exploration	σ² (SE)	σ² (SE)	χ^2_1	*P*	R (SE)	R (SE)	χ^2_1	*P*
PlotYear	10.83 (13.22)	13.81 (14.44)	0.02	0.89	0.02 (0.02)	0.03 (0.03)	0.02	0.89
Among Individuals	285.51 (70.43)	257.02 (65.59)	0.08	0.78	0.55 (0.12)	0.52 (0.12)	0.03	0.86
Within Individuals	226.71 (59.71)	227.29 (56.44)	0	1.00	0.43 (0.12)	0.46 (0.11)	0.02	0.89
Body Mass	σ² (SE)	σ² (SE)	χ^2_1	*P*	R (SE)	R (SE)	χ^2_1	*P*
PlotYear	0.03 (0.02)	0.02 (0.02)	0.24	0.62	0.05 (0.03)	0.02 (0.03)	0.34	0.56
Among Individuals	0.34 (0.09)	0.49 (0.08)	1.43	0.23	0.52 (0.12)	0.66 (0.09)	0.66	0.42
Within Individuals	0.28 (0.07)	0.23 (0.06)	0.29	0.59	0.43 (0.11)	0.32 (0.08)	0.7	0.40
Lay Date	σ² (SE)	σ² (SE)	χ^2_1	*P*	R (SE)	R (SE)	χ^2_1	*P*
PlotYear	91.40 (39.44)	87.19 (37.78)	0.002	0.96	0.83 (0.06)	0.80 (0.07)	0	1.00
Among Individuals	11.73 (2.84)	19.45 (2.96)	3.86	0.05	0.11 (0.05)	0.18 (0.07)	4.03	0.04
Within Individuals	6.38 (2.13)	2.80 (1.01)	2.68	0.10	0.06 (0.03)	0.03 (0.01)	2.62	0.11
Clutch Size	σ² (SE)	σ² (SE)	χ^2_1	*P*	R (SE)	R (SE)	χ^2_1	*P*
PlotYear	0.29 (0.18)	0.42 (0.25)	0.04	0.84	0.10 (0.06)	0.14 (0.07)	0	1.00
Among Individuals	2.04 (0.31)	2.07 (0.36)	0.05	0.82	0.74 (0.08)	0.69 (0.09)	0	1.00
Within Individuals	0.42 (0.14)	0.52 (0.18)	0.2	0.65	0.15 (0.05)	0.17 (0.06)	0.14	0.71

Table 3. Cross-context correlations within single traits. Correlations [r_I (SE)] across the control versus predator exposed plots were tested against zero and one, where deviations from one represent evidence for individual by environment interaction (I×E).

		Test of $r_A = 1$		test of $r_A = 0$	
	r_A (SE)	$\chi^2_{0.5}$	*P*	χ^2_1	*P*
Aggression	0.22 (0.55)	2.28	0.05	0.16	0.69
Exploration	0.93 (0.22)	0.1	0.48	14.62	<0.001
Body Mass	0.50 (0.21)	3.75	0.02	5.54	0.02
Lay Date	0.42 (0.24)	5.53	0.007	1.87	0.17
Clutch Size	0.84 (0.12)	1.97	0.07	14.74	<0.001

Table 4. Within-context correlations between traits. Correlations [r_I (SE)] between behavior (exploration) and life history (mass, lay date, clutch size) in the control versus predator exposed plots.

	Control	Predator		
	r (SE)	*r* (SE)	χ^2_1	*P*
exploration - mass	-0.19 (0.17)	-0.03 (0.15)	0.38	0.54
exploration - lay date	0.32 (0.20)	-0.21 (0.18)	3.68	0.05
exploration - clutch size	0.22 (0.14)	0.26 (0.19)	0.02	0.89

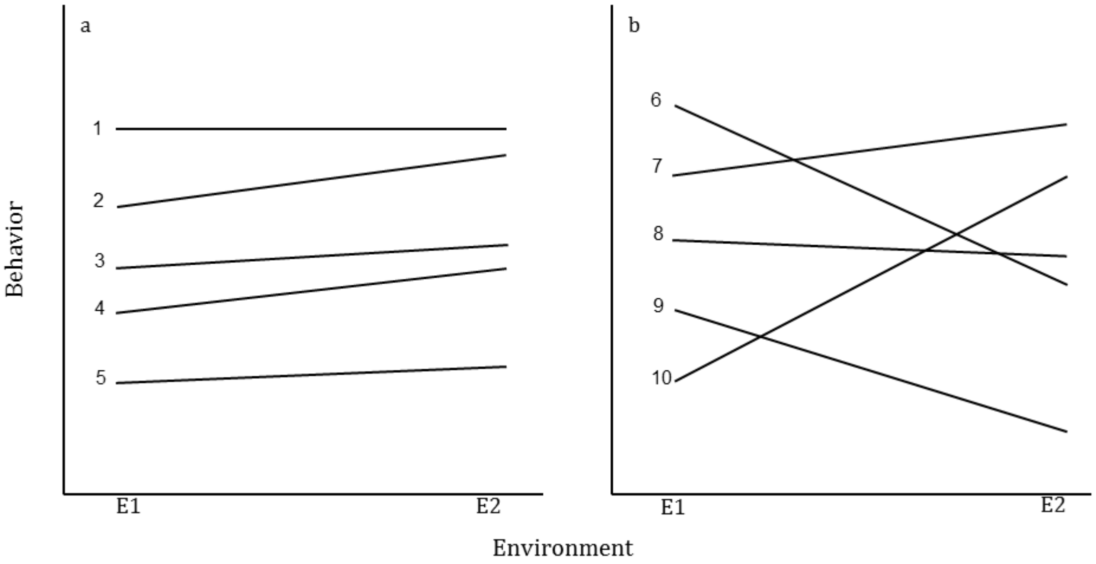

Figure 1. Illustration of variation viewed from the reaction norm approach, showing expressed values of the same behavior in two different environments. Modified from (Dingemanse (2007)). Each individual (number) is represented by a line. Each environment (E1, E2) represents a repeated measure within an individual. (a) Depicts a situation where all individuals respond the same to the environment and consistent individual differences exist. (b) Depicts a situation where individuals respond differently to the environment. The elevation of each line represents an individual's average behavior and the slope of each line represents the degree of phenotypic plasticity.

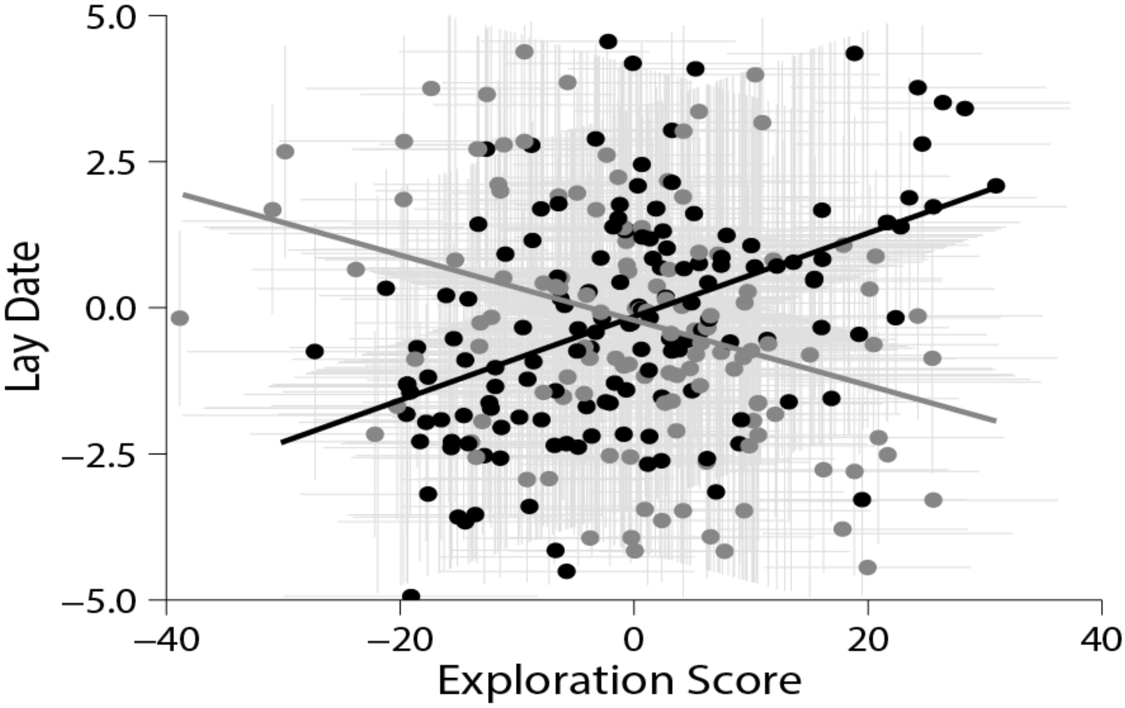

Figure 2. Graph of the relationship between exploration score and lay date for control (black) and predator (gray) exposed plots. Each point represents the best linear unbiased predictor (BLUP) of an individual based on our multivariate mixed – effects model results.

General Discussion

Predators are a major cause of mortality for prey and current research shows they also influence prey via non-consumptive predator effects (Lima 1998, Brown and Kotler 2004, Lind and Cresswell 2005, Preisser et al. 2005, Zanette et al. 2011). These more nuanced effects of perceived predation risk are poorly understood, partially because the effects are harder to observe and manipulations in the field are difficult. In this dissertation, I describe large scale field manipulations with wild birds to help address gaps in the study of perceived predation risk. Specifically, we manipulated predation risk at large spatial scales for long time periods in order to quantify how changes in perceived predation risk also influenced less labile traits such as life history decisions. This dissertation, and other studies like it, are key missing pieces in our understanding of predator effects. Research to date clearly demonstrates that prey perceive and respond to predators. However, few studies explore prey response in the wild, thus they miss key aspects of how prey evaluate predation risk relative to other needs. Additionally, long term manipulations using an on/off cue scheme, provide important details as to how prey deal with chronic stress, how they interpret predator cues, and how the trade-off between predation risk and other behaviors changes with predator duration. Finally, studying life history decisions gives insight as to how non-consumptive effects alone can influence prey demography and selection pressures.

Very few studies have explored the effects of perceived predation risk on long time scales, and none of those have explored individual differences in response.

Individuals of many taxa have been shown to differ consistently for many traits (Bell et al. 2009). If individuals differ in how they perceive risk, or if certain behavioral types are at greater risk, they should respond to risk differently. Studies examining individual level responses to perceived predation risk are few and studies in the field are even fewer. By analyzing data at an individual level, we can explore how the relative costs and benefits of anti-predator responses differ among individuals. Additionally, we can better understand population level responses by viewing the population as the sum of unique individuals, as opposed to only examining population mean responses to perceived predation risk. Thus, this dissertation begins to fill a large gap in knowledge by examining the interacting effects of perceived predation risk and individual behavioral type.

Persistence of risk

In chapter 1, we looked at the effects of perceived predation risk on a long time scale in detail. We exposed birds to playback for approximately 5 months using a 4 day on- 4 day off scheme. To our knowledge, no other studies have examined the vocal response of birds to predator playbacks on such long time scales. We found that birds responded vocally to our treatment by decreasing singing and increasing alarming on average in predator exposed areas. This provides evidence that our treatment manipulations were effective and that great tits responded to the change in threat. Additionally, the predator species, Eurasian sparrowhawk, that we used for playback cues is a territorial hunting species, thus we expected that if great tits were aware of the predator's hunting style, they should respond to the cue as if predation were possible even after cessation of the predator cue. There was no difference in vocal response depending on speaker status (days speakers were actively broadcasting or not), thus birds responded to the possibility of risk for a number of days after cessation of predator cues. Consequently, we concluded that birds do not interpret predator vocalizations as merely signifying immediate danger. Instead, for at least 4 days after predator vocalization playback, bird behavior remains altered. These long term, or carry-over, effects indicate that predators can have lasting effects on prey populations.

Trade-offs

Anti-predator responses are costly because they require time and energy, and often lead to missed opportunities, as they often preclude other behaviors. Theory predicts that the decision regarding which option to pursue will depend on the costs of predation and the benefits associated with the other option (Lima 1998). Thus, predators should only influence prey when the costs of predation outweigh the benefits.

In chapter 1, we looked at the effects of perceived predation risk on vocal behavior across two contexts. We found that birds responded to manipulations of predation generally, but not during simulated territory intrusions. This highlights the important tradeoffs that great tits face. Territory loss is a high cost for male great tits. At best, the male will only lose the current brood (no reproduction that year). However, the hierarchal social system of great tits means the male may never regain a territory and therefore lose all future breeding opportunities. This study provides further evidence that, although predators are a major threat, they are not the only threat, and birds asses the relative importance of each response. This adaptive and plastic level of response highlights the effects predators can have on ecological time scales.

In chapters 2 and 3, we looked at the trade-off between predation and starvation by examining exploration, body mass, and metabolic rate. These traits are interconnected such that individuals that move more also weigh more and have higher metabolic rates. Such types of individual thus have higher energetic demands, as well as increased susceptibility to risk, making the starvation-predation trade-off especially pertinent for them. We found that individuals exposed to increased perceived predation risk decreased in mass and suppressed metabolic rate and exploration behavior, demonstrating that they responded to predation risk despite the risk of starvation. Certain birds responded more strongly than others, and we interpreted this as adaptive because there was no difference in mortality in the two treatment groups.

In chapters 2 and 4, we investigated individual level responses to predation risk. Individuals have been shown to differ in boldness and risk perception (Luttbeg and Schmitz 2000, Luttbeg and Sih 2010, Wolf and Weissing 2012). If individuals differ in how they perceive risk, they can be expected to respond to risk differently. Additionally, individuals can differ in their susceptibility to predation depending on their phenotype (e.g., more active individuals are more likely to encounter predators), thus those types facing most risk should respond most strongly. Fast exploring birds are likely to be at greater risk due to their increased visibility and more frequent encounters with predators (Biro and Stamps 2008, Smith and Blumstein 2008, Niemelä et al. 2015). We found slow exploring individuals behaved the same in both treatments, but that fast exploring individuals showed different responses depending on treatment group. Specifically, fast explorers showed the greatest reductions in mass (chapter 2) and greater differences in lay date (chapter 4). Thus, in addition to individuals assessing differences in risk depending on context, individuals can also assess differences in risk depending on their own state. These greater responses in certain types of individuals likely enable them to mitigate the extra risk they experience.

Individuals have many needs and must constantly face decisions as to where to allocate their time and energy. This dissertation shows that such trade-offs vary depending on environmental factors, such as an aggressive neighbor or food availability, but also that individuals' inherent differences alter the relative costs and benefits. Thus, perceived predation risk is an important factor affecting variation in prey behavior because it adds costs that are individual specific.

Individual level responses

As discussed above, in chapters 2 and 4, we explored the relationship between an individual's behavioral type and its response to perceived predation risk. In addition to influencing how certain types of individuals respond, predation risk can also potentially alter variation within and among individuals. In chapter 4, we explicitly tested the effects of perceived predation risk on the expression of individual variance and covariance components of prey behavioral and life history traits in the field. We found that perceived predation risk increased variance among individuals for certain traits, that some traits were highly consistent across contexts (while others were not), and thusly, the correlations among traits varied with treatment.

Meta-analyses of behavior have shown that behavioral repeatability and individual-level correlations predict heritability (Dochtermann et al. 2015) and genetic correlations (Dochtermann 2011), the two parameters upon which evolution depends. Specifically, among individual variation, and consequently repeatability and heritability, measures the relative amount of variability that can be attributed to additive gene action (Roff 1996) and traits with higher repeatabilities (greater among individual variation) can evolve more rapidly (Houle 1992). Thus our results indicate that certain traits in populations exposed to increased perceived predation risk can potentially evolve more rapidly than in populations with less risk. Additionally, correlations between traits represent the amount of shared genes, and thus the extent to which a trait changes in response to selection acting upon the other trait with which it shares genes (Roff 1996). Therefore, genetic correlations influence how selection can change the distribution of phenotypes in a population and can limit the evolutionary trajectories available to populations (Dochtermann 2011). Our finding that perceived predation risk alters correlations between traits indicates that populations exposed to perceived predation risk have altered evolutionary trajectories, and consequently have different phenotypic options than populations exposed to less risk. Thus, our finding that perceived predation risk influenced these traits indicates that perceived predation risk alone may be sufficient to induce evolution; a result which cannot be detected using current methods focusing on population means alone.

Life history decisions are complex and individuals must evaluate many aspects of the environment to make an optimal choice. Individuals must consider external environmental conditions as well as internal conditions. Individuals differing in age or body condition should invest differently in current versus future reproduction, and thus choose different life history strategies. Such trade-offs in current versus future reproduction are well known in research focusing on pace of life (POL). Species that die sooner consequently evolve to mature faster and invest more heavily in early reproduction (Stearns 1976). Recently, it has been proposed that individuals within a species or population may also show different paces of life (Réale et al. 2010). Individuals may differ in survival likelihood depending on individual specific conditions (i.e. body condition, parasite load), and therefore also differ in investment in current versus future reproduction. These differences in state, and consequently investment strategy should alter their life history. Our findings support the application of pace of life theory to individuals within populations; fast explorers invested more in current reproduction when exposed to increased perceived predation risk. Thus, our results show that when certain types of individuals face reduced life expectancies, they invest more in current reproduction leading to different life history strategies within a population.

General conclusions

My work on great tits expanded our knowledge of perceived predation risk by using free-living individuals, manipulating risk on long time scales, including individuality in analyses, and measuring effects other than population mean responses; all of which have not been previously studied. I investigated the vocal, behavioral, physiological, and life history response of great tits to perceived predation risk. My dissertation presents critical first evidence that individuals differ in their response to risk, likely due to individual differences in relative costs and benefits of responding to risk, highlighting the importance of incorporating individual differences into studies of perceived predation risk. Future studies can expand upon this groundwork to examine the specific mechanisms involved in creating and maintaining individual differences and their consequences for micro-evolution in natural populations.

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Statutory declaration and statement

Ehrenwörtliche Versicherung

Ich versichere hiermit an Eides statt, dass die von mir vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt ist.

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