Oecologia (2014) 176:637–651
DOI 10.1007/s00442-014-3057-9

SPECIAL TOPIC: ECOPHYSIOLOGICAL EFFECTS OF PREDATION RISK

Diagnosing predation risk effects on demography: can measuring physiology provide the means?

Liana Y. Zanette · Michael Clinchy · Justin P. Suraci

Received: 31 March 2014 / Accepted: 18 August 2014 / Published online: 19 September 2014

Abstract Predators kill prey thereby affecting prey survival and, in the traditional top-down view of predator limitation, that is their sole effect. Bottom-up food limitation alters the physiological condition of individuals affecting both fecundity and survival. Predators of course also scare prey inducing anti-predator defences that may carry physiological costs powerful enough to reduce prey fecundity and survival. Here, we consider whether measuring physiology can be used as a tool to unambiguously diagnose predation risk effects. We begin by providing a review of recent papers reporting physiological effects of predation risk. We then present a conceptual framework describing the pathways by which predators and food can affect prey populations and give an overview of predation risk effects on demography in various taxa. Because scared prey typically eat less the principal challenge we see will be to identify measures that permit us to avoid mistaking predator-induced reductions in food intake for absolute food shortage. To construct an effective diagnostic toolkit we advocate collecting multiple physiological measures and utilizing multivariate statistical procedures. We recommend conducting two-factor predation risk × food manipulations to identify those physiological effects least likely to be mistaken for responses to bottom-up food limitation. We suggest there is a critical need to develop a diagnostic tool that can be used when it is infeasible to experimentally test for predation risk effects on demography, as may often be the case in wildlife conservation, since failing to consider predation risk effects may cause the total impact of predators to be dramatically underestimated.

Keyword Ecology of fear · Food supply · Non-consumptive effects · Non-lethal predator effects · Predator–prey interaction

Communicated by Michael Sheriff.

L. Y. Zanette (✉)
Department of Biology, University of Western Ontario, 1151 Richmond Street, London, ON N6A 5B7, Canada
e-mail: lzanette@uwo.ca

M. Clinchy · J. P. Suraci
Department of Biology, University of Victoria, Station CSC, P.O. Box 1700, Victoria, BC V8W 2Y2, Canada

Introduction

Food and predators are two of the most important factors limiting populations (Fig. 1). Bottom-up food limitation alters the physiological condition of individuals which affects both fecundity (i.e. 'births') and survival. In the traditional top-down view of predator limitation (Fig. 1a), the sole means by which predators affect prey populations is by directly killing prey and so affecting prey survival (Peckarsky et al. 2008). An alternative view is that the total impact that predators have on prey populations is a function of both direct killing and 'predation risk' effects [also called 'non-consumptive effects' (Blaustein 1997), 'non-lethal effects' (Lima 1998), or 'fear effects' (Brown et al. 1999)]. Predators scare prey causing prey to respond with anti-predator defences which may reduce the prey individual's vulnerability to being killed (Lima and Dill 1990; Lima 1998, 2009), but can carry physiological costs that are powerful enough to reduce prey fecundity and increase deaths from other causes, like starvation (Fig. 1b, c). Numerous mesocosm experiments on invertebrate and aquatic species (reviewed in Preisser et al. 2005) and recent manipulations in terrestrial vertebrate systems (e.g. Eggers et al. 2006; Sheriff et al. 2009; Zanette et al. 2011) demonstrate

that predation risk can itself affect demography. Our purpose here is to consider whether physiological measures can be used to help diagnose whether changes in demography are attributable to predation risk effects, which would be exceptionally useful in circumstances where manipulations are not feasible. We focus on physiology rather than behaviour because while behavioural variables may provide evidence that animals are fearful (Brown et al. 1999; Brown and Kotler 2004), it is important to show that such responses are severe enough and sufficiently long lasting to induce physiological costs significant enough to affect fecundity or survival (Clinchy et al. 2004, 2013). The principal challenge, as we see it, in utilizing physiology as an indicator of predation risk effects is distinguishing predation risk effects on physiology from the effects that food limitation has on physiology (Fig. 1). Given that failing to consider predation risk effects may cause the impacts of predators to be dramatically underestimated, providing such a diagnostic tool promises to be of immense benefit to conservation and wildlife management (Creel and Christianson 2008).

Here, we suggest that evaluating multiple measures of physiology will generally provide the best means of identifying the suite of potential measures most diagnostic of predation risk, and we have compiled a list of the most recent studies concerning predation risk effects on physiology (Table 1) to aid in identifying which physiological measures may be good candidates. We build upon a conceptual framework describing some of the most likely pathways through which predators and food can affect prey populations to put the role of physiology into perspective (Fig. 1), and then briefly outline what is known about predation risk effects on populations in a variety of animal taxa. We describe how predator-induced reductions in food intake are one of the most common anti-predator defences animals use. Relying on physiology to identify that predation risk effects are operating will thus generally necessitate distinguishing physiological changes due to predator-induced reductions in food use ('relative food use'; Fig. 2) from physiological changes due to bottom-up food limitation ('absolute food supply'; Fig. 2), which we suggest will require two-factor manipulations of predation risk and food supply to begin the process of diagnosis.

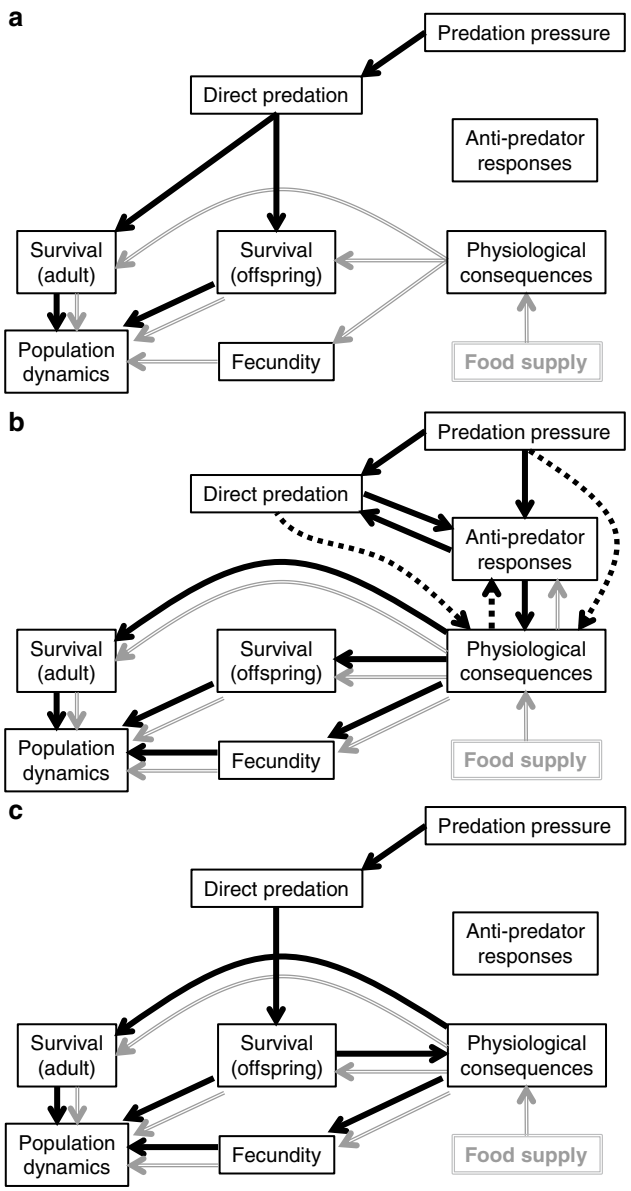

Fig. 1 Hypothetical pathways by which predators (*black arrows*) and food (*grey arrows*) could potentially affect prey population dynamics, showing: **a** the traditional view of top-down predator vs. bottom-up food effects wherein the sole way predators affect prey populations is via direct predation affecting survival while food affects both fecundity and survival through its effects on physiology, **b** predation risk effects on both fecundity and survival mediated by physiological consequences resulting from anti-predator behavioural responses (*black arrows*) or induced physiological changes (e.g. elevated metabolism; *dashed lines*), **c** effects on subsequent fecundity or adult survival resulting from the physiological cost of re-breeding to replace offspring lost to direct predation

Multiple measures of physiology as a tool for diagnosing predation risk effects

The physiology of an animal will reflect the effect of all the stressors it is exposed to and the consequences of all the behaviours it has engaged in. Because physiology is complex, to determine whether an animal is 'stressed', we advocate for an evaluation of the overall physiological profile of an individual using multiple measures at various scales. This could include measures that can be obtained from tissue or other (e.g. faecal) samples [e.g. glucocorticoids, oxidative stress, telomeres, reproductive hormones,

Table 1 Empirical evidence for physiological stress responses to predation risk and their ecological consequences

Taxa	Physiological measure	Response category	Response	Prey	Predator	Risk cue	Source
Acute vs. chronic							
Arthropod							
Chronic	Haemolymph octopamine level	Behaviour, energetics	Reduced weight gain, decreased feeding, increased flight	Long-winged cricket (*Gryllus texenis*)	Robotic hamster (M)	Predator	Adamo and Baker (2011)
Chronic	Metabolic scaling	Energetics, growth	Slower growth at large body size	Amphipod (*Gammarus minus*)	Slimy sculpin (*Cottus cognatus*) (N)	High risk environment	Glazier et al. (2011)
Chronic	Body stoichiometry	Body composition, ecosystem function	Increased grasshopper C:N ratio, decreased soil decomposition rate	Grasshopper (*Melanoplus femurrubrum*)	Spider (*Pisuarina mira*) (M)	Predator	Hawlena et al. (2012)
Chronic	Antioxidant levels	Oxidative stress	Increased oxidative damage	Damselfly larva (*Enallagma cyathigerum*)	Dragonfly larva (*Anax* sp.) (M)	Visual and chemical	Janssens and Stoks (2013)
Chronic	HSP-60, metabolic rate, phenoloxidase	Development, reproduction	Increased maturation rate and reproductive output	Water flea (*Daphnia magna*)	Fish (*Leucisus idus*) (M)	Chemical	Pauwels et al. (2010)
Chronic	Enzyme activity, escape performance	Survival	Predation selects for increased enzyme activity and swimming speed	Damselfly (*Enallagam vesperum*)	Dragonfly larva (*Anax junius*) (M)	Predator	Strobbe et al. (2010)
Chronic	Nutritional physiology, body composition	Energetics	Increased short-term assimilation efficiency and energy storage	Tobacco hornworm (*Manduca sexta*)	Stink bug (*Podisus maculiventris*) (M)	Predator	Thaler et al. (2012)
Mollusc							
Chronic	Contaminant tolerance	Survival	Increased offspring Cd tolerance	Freshwater snail (*Biomphalaria glabrata*)	Crayfish (*Procambarus clarkii*) (M)	Chemical	Plautz et al. (2013)
Fish							
Acute	Cortisol	Behaviour	Increased stress response, increased interaction time	Cleaning goby (*Elacatinus evelynae*)	Piscivorous fish (M)	Predator	Soares et al. (2012)
Chronic	Cortisol release	Stress responsiveness	Reduced response to acute stress	Poeciliid fish (*Brachyrhaphis episcopi*)	Piscivorous fish (N)	High risk environment	Archard et al. (2012)
Chronic	Respiratory rate	Stress responsiveness	Increased response to acute stress	Three-spined stickleback (*Gasterosteus aculeatus*)	Piscivorous fish (N)	High risk environment	Bell et al. (2010)
Chronic	Body stoichiometry	Body composition	Limited effect on %C, C:N and C:P	Guppy (*Poecilia reticulata*)	Piscivorous fish (N)	High risk environment	El-Sabaawi et al. (2012)
Chronic	Egg cortisol	Stress responsiveness, behaviour	Higher egg cortisol, increased offspring anti-predator behaviour	Three-spined stickleback (Gasterosteus aculeatus)	Northern pike (*Esox lucius*) (M)	Model predator	Giesing et al. (2011)
Chronic	Oxygen consumption	Stress responsiveness, behaviour	Lower O_2 consumption following simulated attack, no effect on avoidance behaviour	Smallmouth bass (*Micropterus dolomieu*)	Piscivorous fish (N)	High risk environment	Gravel et al. (2011)

Table 1 continued

Taxa	Physiological measure	Response category	Response	Prey	Predator	Risk cue	Source
Amphibian							
Acute	Whole-body CORT	Stress responsiveness	Greater stress response at higher latitudes	Common frog (*Rana temporaria*)	Dragonfly larva (*Aeshna* sp.) (N)	Visual and chemical	Dahl et al. (2012)
Chronic	Metabolic rate	Energetics	Increased energetic cost of ultraviolet-B exposure	Striped marsh frog (*Limnodynastes peronii*)	Freshwater shrimp (*Macrobranchium australiense*) (M)	Chemical	Alton et al. (2012)
Chronic	Respiration rate	Energetics	Reduced respiration may offset energetic cost of reduced activity	Arabian toad (*Bufo arabicus*)	Dragonfly larva (multiple species) (M)	Chemical	Barry and Syal (2013)
Chronic	Body stoichiometry	Body composition	Increased %C and %N	Eastern gray tree frog (*Hyla versicolor*)	Diving water beetle (*Dytiscus verticalis*) (M)	Chemical	Costello and Michel (2013)
Chronic	Immune response	Survival, immune response	Increased adult fungal resistance	Wood frog (*Rana sylvatica*)	Dragonfly larva (*Anax junius*) (M)	Chemical	Groner et al. (2013)
Chronic	Immune response	Survival, immune response	No effect	Larval anurans (multiple species)	Multiple invertebrate predators (M)	Chemical	Haislip et al. (2012)
Chronic	Whole-body CORT	Morphology, development	Expression of defence morphology	Wood frog (*Rana sylvatica*)	Dragonfly larva (*Anax* sp.) (M)	Chemical	Maher et al. (2013)
Chronic	Whole-body CORT	Immune response	No effect	Wood frog (*Rana sylvatica*)	Various invertebrate predators (M)	Chemical	Reeve et al. (2013)
Chronic	Immune response (T-cell proliferation)	Immune response, development	Decreased immune response, delayed development	Wood frog (*Rana sylvatica*)	Dragonfly larva (*Anax* sp.) (M)	Chemical	Seiter (2011)
Reptile							
Acute	Aversive learning	Learning	Inhibiting CORT decreases anti-predator learning	Fence lizard (*Sceloporus undulatus*)	Novel attacker (red square box) (M)	Model attacker	Thaker et al. (2010)
Bird							
Acute	Plasma CORT and testosterone	Stress responsiveness	Decreased CORT, increased testosterone	Common blackbird (*Turdus merula*)	Magpie (*Pica pica*) (M)	Acoustic	Ibáñez-Álamo et al. (2011)
Acute	Plasma CORT	Stress responsiveness	Increased stress response relative to capture-restraint protocol	Rock dove (*Columba livia*)	Multiple avian predators (N)	Predator	Pakkala et al. (2013)
Acute	Total CORT	Stress responsiveness	No effect	White-crowned sparrow (*Zonotrichia leucophrys*)	Parental alarm call (predator proxy) (M)	Acoustic	Rivers et al. (2011)
Chronic	Glucocorticoid response (multiple measures)	Stress responsiveness	Increased baseline glucocorticoid	Song sparrow (*Melospiza melodia*)	Various mammalian and avian predators (N)	High risk environment	Clinchy et al. (2011a, b)
Chronic	Steroid hormones (testosterone, androstenedione, progesterone)	Reproduction	Lower testosterone concentration in eggs	Great tit (*Parus major*)	Sparrowhawk (*Accipiter nisus*) (M)	Model predator, acoustic	Coslovsky et al. (2012)

Table 1 continued

Taxa	Physiological measure	Response category	Response	Prey	Predator	Risk cue	Source
Chronic	Baseline CORT	Stress responsiveness	Increased parental CORT across species	Various small passerines	Various mammalian and avian predators (N)	High risk environment	Fontaine et al. (2011)
Chronic	Immune factors transferred to eggs	Immune response	Increased immunoglobulin transfer to eggs	Pied flycatcher (*Ficedula hypoleuca*)	Pygmy owl (*Glaucidium passerinum*), weasel (*Mustela nivalis*) (M)	Predator, chemical	Morosinotto et al. (2013)
Chronic	Body mass, HSP60, HSP70, plasma immunoglobulin	Stress responsiveness, behaviour	Increased stress levels, increased provisioning behaviour	Pied flycatcher (*Ficedula hypoleuca*)	Sparrowhawk (*Accipiter nisus*) (M)	Proximity to predator	Thomson et al. (2010)
Chronic	HSP60, HSP70, body condition	Stress responsiveness, reproduction	No effect on stress response, decreased offspring size	Pied flycatcher (*Ficedula hypoleuca*)	Sparrowhawk (*Accipiter nisus*) (N)	Proximity to predator	Thomson et al. (2012)
Chronic	15 Indices of physiological condition	Reproduction	Physiological dysregulation, reduced 'birth' rate	Song sparrow (*Melospiza melodia*)	Simulated (M)	Simulated nest predation	Travers et al. (2010)
Mammal							
Acute	Blood cortisol concentration	Stress responsiveness	Increased cortisol concentration	Belding's ground squirrel (*Urocitellus beldingi*)	Conspecific alarm call (predator proxy) (M)	Acoustic	Mateo (2010)
Chronic	FCM	Behaviour	Increased vigilance, altered burrow architecture	Common vole (*Microtus arvalis*)	White-toothed shrew (*Crocidura russula*) (M)	Predator	Liesenjohann et al. (2013)
Chronic	FCM	Reproduction, behaviour	Interactive effects with maternal age on litter size and offspring behaviour	Yellow-bellied marmot (*Marmota flaviventris*)	Unspecified (N)	High risk environment	Monclús et al. (2011)
Chronic	FCM, blood cortisol, free fatty acids, white blood cell count	Stress responsiveness	Increased offspring stress response	Snowshoe hare (*Lepus americanus*)	Lynx (*Lynx canadensis*) (N)	Predator	Sheriff et al. (2010)
Chronic	Cortisol, metabolism, leukocyte levels, body condition	Stress responsiveness, body condition	Increased stress during decline phase of population cycle	Snowshoe hare (*Lepus americanus*)	Lynx (*Lynx canadensis*) (N)	Predator	Sheriff et al. (2011)

HSP Heat shock protein, *CORT* corticosterone, *FCM* faecal corticosteroid metabolites, *M* manipulations, *N* non-manipulations

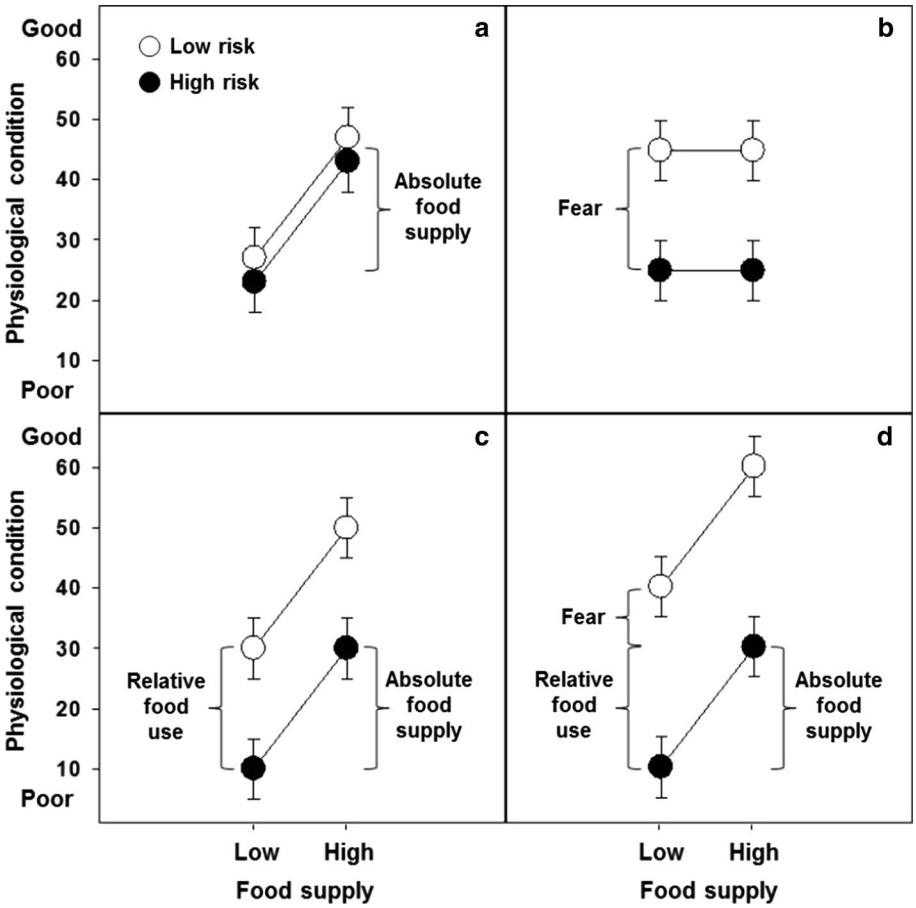

Fig. 2 Potential main effects on a given physiological measure (or suite of measures) in a two-factor experiment manipulating predation risk (high risk and low risk) and food supply (*x*-axis), showing: **a** main effect of food supply (*Absolute food supply*) with no predation risk effect, **b** main effect of predation risk independent of food supply due to what we here term *Fear* to distinguish from predation risk effects resulting from predator-induced reductions in food intake (*Relative food use*), **c** main effects of predation risk and food supply with no interaction wherein the magnitudes of the effect sizes are comparable suggesting the predation risk effect could be solely due to predator-induced reductions in food intake (*Relative food use*), **d** main effects of predation risk and food supply with no interaction wherein the predation risk effect is larger indicating that some added facet of predation risk (*Fear*) is affecting physiology beyond that which can be accounted for by predator-induced reductions in food intake (*Relative food use*). Units on the *y*-axis are arbitrary and are simply intended to assist in gauging relative effect sizes

plasma metabolites, haematology, immune function (Boonstra et al. 1998; Clinchy et al. 2004, 2011b; Travers et al. 2010; Breuner et al. 2013)] in addition to measures of gross physiological condition such as mass and fat scores (e.g. Boonstra et al. 1998; Clinchy et al. 2004; Williams 2005; Harshman and Zera 2007; Travers et al. 2010; Dickens and Romero 2013), and other traits like body temperature, energy or energy expenditure (reviewed in Wikelski and Cooke 2006).

Our goal here is not only to determine whether an animal is 'stressed' but to identify the aspects of physiological 'stress' that are imposed by predation risk. Given the complexity of physiology, it would not possible to evaluate every conceivable measure, so measures must be chosen carefully and based on what the literature suggests may be associated with the stressor(s) of interest. Hawlena and Schmitz (2010b, Tables 2, 3) reviewed the literature concerning predation risk effects on physiology published up to and including 2009, identifying 42 studies, the earliest of which was published in 1987. We conducted a review of the literature published since 2009, the results of which are presented in Table 1. Our review identified 39 studies published in just the past 4 years, which is almost as many as Hawlena and Schmitz (2010b) identified were published in the preceding decades, testifying to the rapid growth in research on predation risk (i.e. fear) effects. Also of interest is an evident shift in focus from acute predation risk effects to chronic predation risk effects. Whereas fewer than half (45 %) of the studies identified by Hawlena and Schmitz (2010b) considered chronic predation risk effects this is the focus of more than three-quarters (82 %) of the studies published since 2009. Assessing multiple different

physiological measures rather than just glucocorticoid levels also appears to be becoming more common given that three-quarters of the papers on chronic predation risk in vertebrates identified by Hawlena and Schmitz (2010b) evaluated the effect on glucocorticoid levels whereas only half of the more recent papers we reviewed did so.

To conduct our review we performed forward searches in Web of Science for 'predat* stress' and 'predat* physiolog*' in March 2014. We supplemented these searches by further examining all papers that cited Boonstra et al. (1998), Scheuerlein et al. (2001) and Clinchy et al. (2004, 2013), which examined physiology, predation risk, and some measure of demography in wildlife. We also included papers that cited the review by Hawlena and Schmitz 2010b. These various searches yielded 231 publications which were carefully read through to identify papers in which a change in some physiological trait was measured in response to varying levels of predation risk. A total of 39 recent studies fit these criteria and are reported in Table 1. Following Hawlena and Schmitz (2010b), we categorized studies as addressing acute or chronic predation stress, where acute stress results from exposure to a single stressor of short duration, and chronic stress results from exposure to a lasting stressor (e.g. occupying habitat with a high abundance of predators) or repeated exposure to an acute stressor [e.g. frequent predator attack (Hawlena and Schmitz 2010b)]. Most studies reviewed in Table 1 measured the effect of predation stress on some ecologically relevant variable in addition to changes in physiology. These variables are listed in the response category column of Table 1, and include effects on survival, reproduction, immune function, body elemental composition and several other responses. We also classified studies by the type of cue used to stimulate the stress response (risk cue column, Table 1), which included manipulations ('M') and non-manipulations ('N') ranging from experimental presentation of a live predator ('predator') to living in areas of high predator abundance ('high risk environment').

Once a suite of candidate physiological variables have been identified from the literature and subsequently measured in the species of interest, multivariate techniques [e.g. multivariate analysis of variance (MANOVA) or discriminant function analysis] could be used to determine whether the overall physiological profiles of animals under high predation risk are distinct from those experiencing low risk. If between-group differences do occur for physiology in addition to demography, then this would be compelling evidence that predation risk affects both. One could also use each individual's multivariate score to test for a more direct relationship between the animal's physiological profile and demography. In addition to examining whether two or more groups differ in their overall physiological profile, the direction of the effect could also be established using a physiological dysregulation score as done in biomedical research (e.g. Seeman et al. 2001, 2004). Here, each physiological variable is divided into quartiles with the highest quartile representing an individual considered to be in relatively poor condition compared to an individual in the lowest quartile as assessed by the researcher [e.g. a human with a cholesterol reading in the highest quartile would be considered in worse condition than one in the lowest (Seeman et al. 2001)]. The quartile values for each individual are summed across all physiological variables to give one score per individual [for an example, see Travers et al. (2010)], and the relationship between an individual's score and their fecundity and/or survival could also be assessed. To get a better idea of which particular variables may be most important in explaining how predation risk affects physiology one could also, for example, examine the canonical scores from a multivariate model (e.g. Travers et al. 2010). The specific variables that load most heavily into the multivariable model would presumably be those that best explain the variance in predation risk effects on physiology. One could then relate each score back to demography using various statistical techniques such as step-wise regression.

Pathways by which predators and food affect prey populations

Some of the pathways through which predators and food may affect prey populations are presented in Fig. 1, which is adapted from Creel and Christianson (2008, Fig. 2) whose focus was on the possible predator pathways only. Figure 1a describes the traditional top-down predator vs. bottom-up food effects on populations. Here, predation directly affects the survival of adults or offspring and is the sole means by which predators can affect prey populations. Food supply, on the other hand, can alter the physiological condition of prey to such an extent that it can affect both fecundity and survival leading to possible changes in population growth rates. Figure 1b shows the potential pathways through which predation risk and food supply can alter demography. In this case, both predation risk and food supply alter physiological condition, which could carry costs affecting population dynamics. Figure 1c outlines how direct predation on offspring could affect physiological condition through a cost of reproduction on the adult that may affect fecundity in subsequent re-breeding attempts within a season as well as annual survival.

In addition to adding the role of food limitation, we have made several other substantive changes to Creel and Christianson's (2008) conceptual framework. The first is that, rather than separating 'energetic' from 'physiological' consequences, as done by Creel and Christianson, we include 'energetic consequences' under the rubric of 'physiological

consequences' since we consider energetic balance to be a physiological trait. Second, we have divided 'reproduction' into 'fecundity' and 'survival'. Reproduction is sometimes used to refer to the total number of offspring that parents produce in a year, which is a function of both fecundity (the number of propagules) and the survival of those propagules. Either or both of these demographic parameters could be affected by predation risk. Regarding alterations to the pathways themselves, our third major change is that 'predation pressure' no longer affects 'anti-predator responses' solely through 'direct predation' (Fig. 1b). While prey may perceive the ambient level of predation risk in the environment as being a function of the rate at which individuals in the population are killed ['direct predation' e.g. Zanette et al. (2013)], it is quite clear that other cues (e.g. sounds, odours) signifying the presence of predators (predation pressure) can themselves induce chronic anti-predator responses powerful enough to affect prey fecundity and survival (e.g. Zanette et al. 2011). Fourth, there has been a suggestion in the literature that predator cues (including those from direct predation) may lead to physiological changes in prey [e.g. changes in metabolism (Hawlena and Schmitz 2010a, b; Hawlena et al. 2012)] above and beyond those associated with predation risk effects on anti-predator responses (these include the 'fear' effects that we describe in Fig. 2d). These pathways are indicated by dashed lines in Fig. 1b. Finally, in Fig. 1c, we illustrate a possible pathway whereby in species that typically have more than one breeding attempt per year (e.g. insects, fish, small birds and mammals), demography may be affected by the direct killing of offspring through a physiological cost of reproduction imposed by relatively high levels of re-breeding, which may affect fecundity within a season (Travers et al. 2010) and have either a positive or negative effect on annual adult survival rates.

Regardless of how one draws the predator pathways, it is clear that if the suite of anti-predator defences that prey exhibit carry costs, the cumulative effect should be evident in the prey's physiological condition. Thus, we suggest that physiology may permit us to diagnose the level of fear a prey individual has been experiencing, in addition to the total costs of predation risk, which should then allow us to correlate with—and so allow us to predict—the effect on demography. In addition to being powerful, the need for such a diagnostic tool is critical in cases where it is logistically (or financially) infeasible to experimentally test for predation risk effects on demography.

Predation risk effects on demography across animal taxa: the role of manipulations

Predation risk effects on demography are well documented in invertebrate and aquatic vertebrate (e.g. tadpoles and fishes) systems (Preisser et al. 2005; Creel and Christianson 2008). Part of the reason that this body of work is so convincing is that it is based on elegant manipulations. Because predators can affect the survival of prey by both killing and scaring them, eliminating direct killing, manipulating risk, and examining effects on populations will unambiguously reveal predation risk effects. Direct killing by predators has often been actively eliminated by gluing shut (e.g. Peckarsky et al. 1993; Schmitz et al. 1997) or partially amputating (e.g. Nelson et al. 2004) the mouthparts of predators (e.g. stoneflies, spiders and damsel bugs). These risky predators (Schmitz et al. 1997), which can intimidate but not kill (Preisser et al. 2005), are then placed together with prey (e.g. mayfly larvae, grasshopper nymphs, pea aphids). Predation risk effects are then gauged by comparing prey populations in enclosures with and without predation risk. Other studies have used caged predators or predator odour (Kats and Dill 1998; Paterson et al. 2013) to intimidate prey, again eliminating direct killing. Though most of these manipulations have been done in artificial enclosures, there is some evidence that they translate to the field as well (e.g. Peckarsky et al. 1993; Peacor et al. 2012).

Due to the logistical challenges of conducting manipulations at a large enough spatial scale over a long enough duration to affect the demography of free-living terrestrial vertebrates, few analogous manipulations have yet been conducted in terrestrial vertebrate systems. Nonetheless, the evidence to date does appear to point to predation risk effects on demography being as important in these systems as in invertebrate and aquatic vertebrate systems. Zanette et al. (2011) used protective measures at the nests of song sparrows to eliminate the direct killing of offspring. Predation risk was then manipulated throughout the breeding season using predator vocalizations broadcast for some populations, while others heard non-threatening sounds. The researchers found significant effects on fecundity, because female song sparrows laid fewer eggs, in addition to effects on offspring survival, wherein a lower proportion of both eggs and nestlings survived in the predator vocalization treatment. Continuous video surveillance at the nest confirmed that no deaths were due to direct killing, thereby unambiguously demonstrating predation risk effects. The net effect of predation risk was a 40 % reduction in the total number of young produced per year. The effects on survival found in this manipulation were comparable to those found in song sparrow populations that exhibited naturally high and low levels of risk (Zanette et al. 2011), even when these populations had access to an unlimited, high-quality, supplemental food source (Zanette et al. 2006). Thus, even though the predator playback manipulation relied on cues from a single modality only (i.e. sound), it appeared to have simulated some of the demographic consequences that animals face

when under naturally high levels of risk where predator cues would be relatively intense and multi-modal including sound, sight, smell, and attacks. In another study, Eggers et al. (2006) provided convincing evidence that predator call playbacks caused Siberian jays to lay fewer eggs in the first clutch of the season, though direct killing was not actively eliminated. In a captive study on snowshoe hares (Sheriff et al. 2009), pregnant females exposed to a trained dog 1–2 min every other day before parturition were significantly less likely to give birth to young compared to control females.

Challenges of identifying predation risk effects on the demography of wildlife

Across taxa and biological systems, all animals, with few exceptions, respond to predators with some type of defence be it morphological, behavioural or physiological (Fig. 1). It makes logical sense, therefore, that predation risk effects on populations are likely to be pervasive in the animal world. However, whether or not, or the extent to which predation risk effects operate in wildlife populations is a contentious issue, where predator effects on prey demography have traditionally been ascribed solely to direct killing while food limitation is the factor best known to affect fecundity fecundity and survival through physiology. For example, one of the most high-profile and hotly debated issues, far from being resolved, is whether or not the reintroduction of wolves to Yellowstone National Park in 1995 and 1996 has caused reductions in the population sizes of elk which are larger than can be accounted for through direct killing, and represent predation risk effects (Creel et al. 2007, 2011; White et al. 2011; Middleton et al. 2013; see also Vucetich et al. 2005; Beschta and Ripple 2013; Kauffman et al. 2013). Manipulations involving risky but non-lethal predators could help resolve the debate in this very high-profile case, but such manipulations are unlikely to ever be commonplace in studies of wildlife populations. Free-living wildlife of even modest size often roam over large areas making it logistically difficult, though certainly not impossible, to conduct manipulations on entire populations with sufficient spatial replication. Moreover, it is a challenge to design a manipulation in which free-ranging, terrestrial vertebrate predators can intimidate but not kill their wildlife prey; disabling mouthparts, for example, is not an option. The extent to which predation risk effects influence prey demography across taxa and biological systems may be in danger of being poorly understood if we are unable to provide convincing evidence that identify these effects in wildlife populations, in cases where such predation risk effects actually do exist.

Like any process of diagnosis the challenge is to identify the measure, or set of measures, that most clearly signify predation-risk effects. We anticipate that the main issue here will be disentangling predation risk effects from bottom-up food limitation (Figs. 1b, 2). One of the most well-established consequences of anti-predator behaviour, exhibited by animals across taxa, is that increased vigilance leads to predator-induced reductions in food intake (relative food use, Fig. 2; Lima and Dill 1990). There is increasing evidence (discussed below) that predation risk can induce chronic, long-term reductions in food intake which are powerful enough to affect both fecundity and survival. If so, then any effect between high- and low-predation risk populations in a natural contrast could easily be ascribed to differences in the absolute food supply. Measures of demography on their own will be ineffective in teasing apart reductions in food intake that are due to predators vs. absolute food. If physiological measures can help do this, then misdiagnoses can be better avoided (e.g. Macleod et al. 2014).

Animals commonly lose body mass when exposed to predators or predator cues (Lima 1986; Gosler et al. 1995; Pérez-Tris et al. 2004; MacLeod et al. 2007, 2014) and some studies in terrestrial vertebrate systems have examined whether such nutritional deficits may be strong enough to lead to demographic costs (e.g. MacLeod et al. 2014). Further, Travers et al. (2010) manipulated predation risk for song sparrow adults such that some birds experienced frequent nest predation (direct predation of offspring; Fig. 1c) while others experienced none. They found an effect on fecundity because song sparrows laid fewer eggs in each subsequent clutch in the high-risk treatment. Fifteen different physiological measures representing six scales (oxidative stress, glucocorticoids, immune function, haematology, plasma metabolites, and visible condition—mass and fat scores) were combined together into a multivariate discriminant function analysis which indicated that birds in the two treatments were physiologically distinct, while 'physiological dysregulation' scores demonstrated that females that experienced frequent nest predation were in poorer condition. Critically, all of the birds in this experiment were supplied with high-quality, ad libitum food, eliminating the possibility that these effects could be attributed to bottom-up food supply (absolute food supply, Fig. 2) and thereby clarifying that the observed effects on physiology and fecundity were due to predation risk. In a subsequent study using data collected during the same experiment, Zanette et al. (2013) evaluated whether the predation risk effects demonstrated in Travers et al. (2010) were due to predator-induced reductions in food intake (relative food use, Fig. 2). Zanette et al. (2013) were able to track whether predation risk chronically altered food-intake by testing for the distinctive stable isotope ($\delta^{13}C$) signature of the

supplemental feed in the blood of the birds. They reported that females in the high risk treatment definitely ate significantly less food, thereby conclusively demonstrating that predation risk can chronically reduce food-intake even when the absolute food supply is unlimited. With regards to the effects of predation risk on physiology, Zanette et al. (2013) further found that the $\delta^{13}C$ signatures of individuals were strongly correlated with their physiological dysregulation score indicating that females that ate less food were in relatively poor condition, and that the effects on physiological condition reported in Travers et al. (2010) were thus in part due to predator-induced reductions in food intake (relative food use, Fig. 2).

In a separate field experiment on free-living song sparrows Zanette et al. (2011) demonstrated that predation risk can chronically alter food use to such an extent as to affect survival, as well as fecundity. Adults exposed to relatively high, manipulated levels of risk, fed their nestlings less (lower relative food use, Fig. 2), their nestlings were consequently lighter (in poorer condition; Fig. 2) and a greater proportion died (Zanette et al. 2011). Critically, continuous video surveillance established that these deaths were definitely not due to direct predation but to the effects of predator-induced reductions in food acquisition (Fig. 1b).

The reintroduction of wolves to Yellowstone was followed by a decline in the birth rate of the elk, which a large body of work suggests may be due to 'intimidation' of the elk by the wolves (Creel et al. 2007, 2011, 2013; Creel and Christianson 2008; but see Hamlin et al. 2009; White et al. 2011; Middleton et al. 2013). Furthermore, behavioural and dietary analyses were consistent with the idea that the reduction in the birth rate of elk could be due to predator-induced reductions in food intake (Creel et al. 2009; Christianson and Creel 2010).

Disentangling physiological effects of predation risk vs. food supply

In biological systems where predation risk effects largely operate through predator-induced reductions in food intake, the physiological profile of an animal may be expected to reflect this on the one hand (relative food use, Fig. 2), in addition to the bottom-up food supply on the other (absolute food supply, Fig. 2), making it difficult to distinguish food and predator effects (Fig. 2c) in cases where predation risk is not manipulated. This could make using physiology as an indicator of predation risk effects in such cases problematic because, in the end, the physiological profile of an individual may reflect food intake, but whether the mechanism driving differences in food intake is due to predators or food supply would not be clear. To determine the extent to which physiology can be used to diagnose predation risk effects per se, we suggest that the best way forward will be to conduct a series of two-factor predator × food manipulative experiments on several model species of wildlife.

In the process of diagnosis, we would start by looking for the traits that best describe predation risk at all levels of food supply. For simplicity, we illustrate some of the conceptual outcomes based on a single physiological trait (see Fig. 2), though the concept would apply to a multivariate analysis of multiple measures as well. In a two-factor manipulation, finding that a physiological trait shows a main effect of food supply and no effect of predation risk (Fig. 2a) would indicate that this trait is of little value in diagnosing predation risk effects. At the other extreme, finding a physiological variable that shows a main effect of predation risk and no effect of food (Fig. 2b) would be diagnostic of predation risk, and therefore, could be of immense use. We suggest it unlikely that we will find many physiological measures that vary with just one of these key limiting factors or the other (Fig. 1a or b). For example, finding a main effect of predation risk and no effect of food (Fig. 2b) could mean that the physiological consequences of reduced food intake due to predation risk differ from those due to low food supply. Though possible and worth investigating, this is based on no theoretical or empirical evidence that we know of. A main effect of predation risk only also could mean that predation risk effects operate completely independently of food intake whether generated by predators or food supply. Such measures are likely rare but some do potentially exist. Recently, Janssens and Stoks (2013) demonstrated that predation risk led to more oxidative damage (i.e. higher lipid peroxidation, lower levels of an antioxidant enzyme—superoxide dismutase, and higher concentrations of a reactive oxygen species—superoxide anion) of damselfly larvae that were reared in the lab under ad libitum food, which the authors suggested resulted from elevated metabolism as a response to predation risk (sensu Hawlena and Schmitz 2010a, b; Hawlena et al. 2012). Oxidative stress has been investigated primarily as a cost of reproduction (Speakman and Garratt 2014). Several authors have suggested that oxidative stress levels may reflect non-resource based physiological mechanisms related to reproduction that will be unaffected by food supply [see Williams (2005) for a discussion of non-resource based physiological mechanisms; Harshman and Zera (2007); Speakman and Garratt (2014)].

We expect that in most cases predation risk and food supply together will affect the physiological profile of an individual. The task is to determine precisely how they affect physiology, i.e. to what degree a particular physiological variable is more affected by one factor than the other. We suggest that physiological variables with main effects of both predation risk and food supply, with no interactions, may provide the greatest potential as

diagnostic tools because they would indicate that predation risk effects do not attenuate at a given level of food but instead, remain strong (Fig. 2c, d). The magnitude of the effect sizes of predation risk relative to food supply could also be useful in helping us understand the degree to which predation risk effects on physiology are due to reductions in food intake vs. predation 'stress' per se. If the magnitude of the predation risk effect is similar to that of food supply (Fig. 2c), then this would suggest that predation risk effects operate mainly through reductions in food intake. If, on the other hand, the effect size due to predation risk exceeds that of food supply (Fig. 2d), then this could be due to some added facet of predation risk (which we have identified as fear in Fig. 2d and by dashed lines in Fig. 1b), not associated with food intake. Such effects of fear or predator stress likely do exist and we have already outlined the possibility of non-resource based mechanisms. Further to this, in vertebrates, chronic predation risk can lead to an increase in the demand for food due to changes in respiration, heart rate, and metabolism, reductions in heat shock proteins inhibiting repair of molecular and cellular structures, in addition to impaired digestive and assimilation efficiency (see Table 1; Hawlena and Schmitz 2010a, b, Hawlena et al. 2012). Such effects could compound those due to behaviourally mediated predator-induced reductions in food intake, rendering animals under high predation risk physiologically identifiable irrespective of food supply.

To clarify, we are not suggesting that the outcomes depicted in Fig. 2 are the only ones that could result from the proposed two-factor food × predator manipulative experiments. Our focus is on those outcomes that would provide the clearest identifiers of predation risk effects on physiology for future non-manipulative studies. It is probable that many variables may be found to show interactive food and predator effects. Theory (Houston et al. 1993; MacLeod et al. 2014) and empirical evidence [reviewed in Bolnick and Preisser (2005) for invertebrates and aquatic species; see also Pauwels et al. (2010); MacLeod et al. (2014)] indicate that the physiological consequences of predation risk may attenuate, and hence be less detectable, in either low-food or high-food environments, pointing to the existence of interactive effects. Nonetheless, if the physiological profile of animals is assayed using multiple measures at various scales, then combining even a fairly weak set of variables together into multivariate models can still produce powerful results. We suggest that any physiological measure that is associated with predation risk (regardless of the impact of food supply) should be examined in different combinations in multivariate models to determine if there is a model that best describes effects due predominantly to predation risk. To illustrate this we use data from Clinchy et al. (2004), which is the only study on free-living wildlife we are aware of that has examined the effects on physiology of simultaneously varying food and predation risk. Clinchy et al. (2004) employed a 2 × 2 design crossing a food-supplementation manipulation with a natural contrast in predation risk and examined the physiological effects on song sparrows at four separate scales: hormonal, energetic, haematological and immunological. Predator pressure and food supplementation together or separately affected six of the ten variables measured. Significant main effects of both predator pressure and food were found in the case of four variables {maximum plasma corticosterone [max CORT; effect size predator (ESP, low minus high) = −26.5 ng ml^{-1}; effect size food (ESF, supplemented vs. not) = −22.4 ng ml^{-1}], baseline plasma corticosterone (base CORT; ESP = −3.2 ng ml^{-1}, ESF = −3.9 ng ml^{-1}), plasma free fatty acid levels (FFA, ESP = −0.3 mmol 1^{-1}, ESF = −0.3 mmol 1^{-1}) and haematocrit (PCV, ESP = 1.5 %, ESF = 1.3 %)}, predator pressure alone affected one variable (basophils; a type of white blood cell; ESP = −5.6 %, ESF = 0.8 %) and food alone affected two more (glucose, ESP = −8.2 mg dl^{-1}, ESF = −32.4 mg dl^{-1} and polychromasia, ESP = 0.4 %, ESF = −2.3 %). There were no significant predator pressure by food interaction effects but there were trends indicative of an interaction in three variables (base CORT, FFA and PCV). Considering the five variables showing a main effect of predator pressure (max CORT, base CORT, FFA, PCV and basophils) we tested whether these five measures in combination could differentiate the effect of predation risk from food supply. Using a two-factor MANOVA we found significant main effects of predation risk ($F_{5,28} = 4.0$, $P = 0.01$), and food supply ($F_{5,28} = 3.0$, $P = 0.02$) with no interaction ($F_{5,28} = 0.3$, $P = 0.98$). To illustrate how model selection can aid in more clearly identifying predation risk effects, we next removed base CORT and PCV from our analysis (base CORT showed the strongest trend toward an interaction and the predator effect was weakest on PCV) which led to a stronger *P*-value for predation risk ($F_{3,31} = 5.0$, $P = 0.009$), a much weaker and now non-significant effect of food supply ($F_{3,31} = 1.9$, $P = 0.08$), and no interaction ($F_{3,31} = 0.3$, $P = 0.98$). Utilizing a robust model selection technique [e.g. Akaike information criterion (Burnham and Anderson 2002)] would provide an even better means to determine the degree to which different combinations of variables best explain variation due to predation risk at any level of food supply. Once physiological measures diagnostic of predation risk have been identified, again, it would be important to establish that predation risk has affected demography and results from physiology could also be tested for direct correlations with demography.

Another approach to take in diagnosing predation risk effects on physiology using our two-factor manipulations is to focus on food quality rather than quantity. For example,

grasshopper nymphs exposed to risky but non-lethal predatory spiders have a distinct body C:N ratio, that is much higher compared to those in prey-only control treatments. The explanation is that predation risk leads to elevated metabolism (Hawlena and Schmitz 2010a, b; Hawlena et al. 2012) thereby altering the nutritional requirements of the prey who forgo protein (i.e. N) in favour of carbohydrates (C) which can fuel the heightened energy demands of the prey's elevated metabolism. Presumably, the resulting high C:N ratio would not be dependent on the quantity of food available (i.e. the absolute food supply), but this could be verified in two-factor manipulations. It has yet to be shown that a high C:N ratio affects demography, but previous work in this system has demonstrated demographic effects of exposure to risky spiders (Schmitz et al. 1997) suggesting that C:N ratio is likely to be a good predictor of predation risk effects on demography in this and related systems.

Measuring fear activity in the brain using a downstream physiological marker

Fear is something that can be measured in the brain, as we have elucidated previously (Clinchy et al. 2011a, 2013). As we outlined in these previous papers, another productive avenue of research to pursue in identifying physiological measures diagnostic of predation risk is to conduct predation risk manipulations on wildlife and examine the physiological profile of the individual to determine which physiological variables are best correlated with fear effects in the brain. The prospect of this providing useful means for diagnosing predation risk has never been better since recent research has demonstrated that not only can fear be measured in the brain, it is possible to specifically measure the neural circuitry associated with the 'fear of predators'. Gross and Canteras (2012) reviewed this accumulating literature that demonstrates that the fear of predators, fear of an aggressive conspecific and fear of pain are each processed by independent neural circuits in the amygdala and hypothalamus. Marzluff et al. (2012) and Cross et al. (2013) report measuring fear effects in the brains of wild-caught crows using neuroimaging, demonstrating that neurobiological studies of wildlife need not entail destructive sampling. The great advantage of being able to identify fear effects in the brain is that this ought to allow us to eliminate ambiguities as to whether physiological changes are the result of predation risk effects. For example, if physiological or demographic differences are evident between populations under naturally high and low levels of predation risk, but the brain shows the absence of fear effects, then clearly those changes cannot be due to predation risk. Moreover, identifying fear effects in the brain could assist in partitioning predation risk effects on physiology into those related to fear vs. predator-induced reductions in food intake (Fig. 2d).

Conclusion

Physiology has the potential to provide us with a set of tools that can be used to diagnose the extent to which prey populations are suffering from predation risk effects. Although we are far from having this diagnostic toolkit assembled, in our view, building it is a worthwhile effort. Identifying the extent to which predation risk affects physiology is of value in and of itself, and useful to a wide range of researchers in many disciplines. In addition to being useful, we have argued that it is indispensable for developing our understanding of predator–prey interactions per se. Determining the extent to which predation risk affects animals across taxa and ecosystems is critical for assessing both the importance of predation risk as a biological force, in addition to the total impact that predators have on prey.

Acknowledgments We thank Michael Sheriff and Jennifer Thaler for organizing this special issue of Oecologia on the ecophysiological effects of predation risk, and for organizing and inviting L. Y. Z. and M. C. to present talks at an organized oral session at the Ecological Society of America meeting in 2013 which was the impetus for this paper. Michael Sheriff and three anonymous reviewers provided helpful comments on a previous version of the manuscript.

References

Adamo SA, Baker JL (2011) Conserved features of chronic stress across phyla: the effects of long-term stress on behavior and the concentration of the neurohormone octopamine in the cricket, *Gryllus texensis*. Horm Behav 60:478–483

Alton LA, White CR, Wilson RS, Franklin CE (2012) The energetic cost of exposure to UV radiation for tadpoles is greater when they live with predators. Funct Ecol 26:94–103

Archard GA, Earley RL, Hanninen AF, Braithwaite VA (2012) Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. Funct Ecol 26:637–645

Barry MJ, Syal S (2013) Metabolic responses of tadpoles to chemical predation cues. Hydrobiologia 700:267–276

Bell AM, Henderson L, Huntingford FA (2010) Behavioral and respiratory responses to stressors in multiple populations of three-spined sticklebacks that differ in predation pressure. J Comp Physiol B 180:211–220

Beschta RL, Ripple WJ (2013) Are wolves saving Yellowstone's aspen? A landscape-level test of a behaviorally mediated trophic cascade: comment. Ecology 94:1420–1425

Blaustein L (1997) Non-consumptive effects of larval Salamandra on crustacean prey: can eggs detect predators? Oecologia 110:212–217

Bolnick D, Preisser E (2005) Resource competition modifies the strength of trait-mediated predator-prey interactions: a meta-analysis. Ecology 86:2771–2779

Boonstra R, Hik D, Singleton G, Tinnikov A (1998) The impact of predator-induced stress on the snowshoe hare cycle. Ecol Monogr 68:371–394

Breuner CW, Delehanty B, Boonstra R (2013) Evaluating stress in natural populations of vertebrates: total CORT is not good enough. Funct Ecol 27:24–36

Brown J, Kotler B (2004) Hazardous duty pay and the foraging cost of predation. Ecol Lett 7:999–1014

Brown J, Laundre J, Gurung M (1999) The ecology of fear: optimal foraging, game theory, and trophic interactions. J Mammal 80:385–399

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York

Christianson D, Creel S (2010) A nutritionally mediated risk effect of wolves on elk. Ecology 91:1184–1191

Clinchy M, Zanette L, Boonstra R, Wingfield JC, Smith JNM (2004) Balancing food and predator pressure induces chronic stress in songbirds. Proc R Soc B 271:2473–2479

Clinchy M, Schulkin J, Zanette LY, Sheriff MJ, McGowan PO, Boonstra R (2011a) The neurological ecology of fear: insights neuroscientists and ecologists have to offer one another. Front Behav Neurosci 5:21

Clinchy M, Zanette L, Charlier TD, Newman AEM, Schmidt KL, Boonstra R, Soma KK (2011b) Multiple measures elucidate glucocorticoid responses to environmental variation in predation threat. Oecologia 166:607–614

Clinchy M, Sheriff MJ, Zanette LY (2013) Predator-induced stress and the ecology of fear. Funct Ecol 27:56–65

Coslovsky M, Groothuis T, de Vries B, Richner H (2012) Maternal steroids in egg yolk as a pathway to translate predation risk to offspring: experiments with great tits. Gen Comp Endocrinol 176:211–214

Costello DM, Michel MJ (2013) Predator-induced defenses in tadpoles confound body stoichiometry predictions of the general stress paradigm. Ecology 94:2229–2236

Creel S, Christianson D (2008) Relationships between direct predation and risk effects. Trends Ecol Evol 23:194–201

Creel S, Christianson D, Liley S, Winnie JA Jr (2007) Predation risk affects reproductive physiology and demography of elk. Science 315:960

Creel S, Winnie JA Jr, Christianson D (2009) Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. Proc Natl Acad Sci USA 106:12388–12393

Creel S, Christianson DA, Winnie JA Jr (2011) A survey of the effects of wolf predation risk on pregnancy rates and calf recruitment in elk. Ecol Appl 21:2847–2853

Creel S, Winnie JA Jr, Christianson D (2013) Underestimating the frequency, strength and cost of antipredator responses with data from GPS collars: an example with wolves and elk. Ecol Evol 3:5189–5200

Cross DJ, Marzluff JM, Palmquist I, Minoshima S, Shimizu T, Miyaoka R (2013) Distinct neural circuits underlie assessment of a diversity of natural dangers by American crows. Proc R Soc B 280:20131046

Dahl E, Orizaola G, Winberg S, Laurila A (2012) Geographic variation in corticosterone response to chronic predator stress in tadpoles. J Evol Biol 25:1066–1076

Dickens MJ, Romero LM (2013) A consensus endocrine profile for chronically stressed wild animals does not exist. Gen Comp Endocrinol 191:177–189

Eggers S, Griesser M, Nystrand M, Ekman J (2006) Predation risk induces changes in nest-site selection and clutch size in the siberian jay. Proc R Soc B 273:701–706

El-Sabaawi RW, Zandonà E, Kohler TJ, Marshall MC, Moslemi JM, Travis J, Lopez-Sepulcre A, Ferriere R, Pringle CM, Thomas SA, Reznick DN, Flecker AS (2012) Widespread intraspecific organismal stoichiometry among populations of the Trinidadian guppy. Funct Ecol 26:666–676

Fontaine JJ, Arriero E, Schwabl H, Martin TE (2011) Nest predation and circulating corticosterone levels within and among species. Condor 113:825–833

Giesing ER, Suski CD, Warner RE, Bell AM (2011) Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. Proc R Soc B 278:1753–1759

Glazier DS, Butler EM, Lombardi SA, Deptola TJ, Reese AJ, Satterthwaite EV (2011) Ecological effects on metabolic scaling: amphipod responses to fish predators in freshwater springs. Ecol Monogr 81:599–618

Gosler A, Greenwood J, Perrins C (1995) Predation risk and the cost of being fat. Nature 377:621–623

Gravel M-A, Suski CD, Cooke SJ (2011) Behavioral and physiological consequences of nest predation pressure for larval fish. Behav Ecol 22:510–519

Groner ML, Buck JC, Gervasi S, Blaustein AR, Reinert LK, Rollins-Smith LA, Bier ME, Hempel J, Relyea RA (2013) Larval exposure to predator cues alters immune function and response to a fungal pathogen in post-metamorphic wood frogs. Ecol Appl 23:1443–1454

Gross CT, Canteras NS (2012) The many paths to fear. Nat Rev Neurosci 13:651–658

Haislip NA, Hoverman JT, Miller DL, Gray MJ (2012) Natural stressors and disease risk: does the threat of predation increase amphibian susceptibility to ranavirus? Can J Zool 90:893–902

Hamlin KL, Garrott RA, White PJ, Cunningham JA (2009) Contrasting wolf-ungulate interactions in the greater Yellowstone ecosystem. In: Garrott RA, White PJ, Watson FGR (eds) The ecology of large mammals in central Yellowstone: sixteen years of integrated field studies. Elsevier, San Diego, pp 541–577

Harshman LG, Zera AJ (2007) The cost of reproduction: the devil in the details. Trends Ecol Evol 22:80–86

Hawlena D, Schmitz OJ (2010a) Herbivore physiological response to fear of predation alters ecosystem nutrient dynamics. Proc Natl Acad Sci USA 107:15503–15507

Hawlena D, Schmitz OJ (2010b) Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. Am Nat 176:537–556

Hawlena D, Strickland MS, Bradford MA, Schmitz OJ (2012) Fear of predation slows plant-litter decomposition. Science 336:1434–1438

Houston A, Mcnamara J, Hutchinson J (1993) General results concerning the trade-off between gaining energy and avoiding predation. Philos Trans R Soc B 341:375–397

Ibáñez-Álamo JD, Chastel O, Soler M (2011) Hormonal response of nestlings to predator calls. Gen Comp Endocrinol 171:232–236

Janssens L, Stoks R (2013) Predation risk causes oxidative damage in prey. Biol Lett 9:20130350

Kats L, Dill L (1998) The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience 5:361–394

Kauffman MJ, Brodie JF, Jules ES (2013) Are wolves saving Yellowstone's aspen? A landscape-level test of a behaviorally mediated trophic cascade: reply. Ecology 94:1425–1431

Liesenjohann M, Liesenjohann T, Palme R, Eccard JA (2013) Differential behavioural and endocrine responses of common voles (*Microtus arvalis*) to nest predators and resource competitors. BMC Ecol 13:33

Lima S (1986) Predation risk and unpredictable feeding conditions—determinants of body-mass in birds. Ecology 67:377–385

Lima S (1998) Nonlethal effects in the ecology of predator-prey interactions—what are the ecological effects of anti-predator decision-making? Bioscience 48:25–34

Lima SL (2009) Predators and the breeding bird: behavioral and reproductive flexibility under the risk of predation. Biol Rev 84:485–513

Lima S, Dill L (1990) Behavioral decisions made under the risk of predation—a review and prospectus. Can J Zool 68:619–640

MacLeod R, MacLeod CD, Learmonth JA, Jepson PD, Reid RJ, Deaville R, Pierce GJ (2007) Mass-dependent predation risk and lethal dolphin porpoise interactions. Proc R Soc B 274:2587–2593

MacLeod CD, MacLeod R, Learmonth JA, Cresswell W, Pierce GJ (2014) Predicting population level risk effects of predation from the responses of individuals. Ecology (in press)

Maher JM, Werner EE, Denver RJ (2013) Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. Proc R Soc B 280:20123075

Marzluff JM, Miyaoka R, Minoshima S, Cross DJ (2012) Brain imaging reveals neuronal circuitry underlying the crow's perception of human faces. Proc Natl Acad Sci USA 109:15912–15917

Mateo JM (2010) Alarm calls elicit predator-specific physiological responses. Biol Lett 6:623–625

Middleton AD, Kauffman MJ, McWhirter DE, Jimenez MD, Cook RC, Cook JG, Albeke SE, Sawyer H, White PJ (2013) Linking anti-predator behaviour to prey demography reveals limited risk effects of an actively hunting large carnivore. Ecol Lett 16:1023–1030

Monclús R, Tiulim J, Blumstein DT (2011) Older mothers follow conservative strategies under predator pressure: the adaptive role of maternal glucocorticoids in yellow-bellied marmots. Horm Behav 60:660–665

Morosinotto C, Ruuskanen S, Thomson RL, Siitari H, Korpimäki E, Laaksonen T (2013) Predation risk affects the levels of maternal immune factors in avian eggs. J Avian Biol 44:427–436

Nelson EH, Matthews CE, Rosenheim JA (2004) Predators reduce prey population growth by inducing changes in prey behavior. Ecology 85:1853–1858

Pakkala JJ, Ryan Norris D, Newman AEM (2013) An experimental test of the capture-restraint protocol for estimating the acute stress response. Physiol Biochem Zool 86:279–284

Paterson RA, Pritchard DW, Dick JTA, Alexander ME, Hatcher MJ, Dunn AM (2013) Predator cue studies reveal strong trait-mediated effects in communities despite variation in experimental designs. Anim Behav 86:1301–1313

Pauwels K, Stoks R, De Meester L (2010) Enhanced anti-predator defence in the presence of food stress in the water flea daphnia magna. Funct Ecol 24:322–329

Peacor SD, Pangle KL, Schiesari L, Werner EE (2012) Scaling-up anti-predator phenotypic responses of prey: impacts over multiple generations in a complex aquatic community. Proc R Soc B 279:122–128

Peckarsky BL, Cowan CA, Penton MA, Anderson C (1993) Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. Ecology 74:1836–1846

Peckarsky BL, Abrams PA, Bolnick DI, Dill LM, Grabowski JH, Luttbeg B, Orrock JL, Peacor SD, Preisser EL, Schmitz OJ, Trussell GC (2008) Revisiting the classics: considering nonconsumptive effects in textbook examples of predator-prey interactions. Ecology 89:2416–2425

Perez-Tris J, Diaz J, Telleria J (2004) Loss of body mass under predation risk: cost of antipredatory behaviour or adaptive fit-for-escape? Anim Behav 67:511–521

Plautz SC, Guest T, Funkhouser MA, Salice CJ (2013) Transgenerational cross-tolerance to stress: parental exposure to predators increases offspring contaminant tolerance. Ecotoxicology 22:854–861

Preisser E, Bolnick D, Benard M (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86:501–509

Reeve BC, Crespi EJ, Whipps CM, Brunner JL (2013) Natural stressors and ranavirus susceptibility in larval wood frogs (*Rana sylvatica*). EcoHealth 10:190–200

Rivers JW, Martin LB, Liebl AL, Betts MG (2011) Parental alarm calls of the white-crowned sparrow fail to stimulate corticosterone production in nest-bound offspring. Ethology 117:374–384

Soares MC, Bshary R, Cardoso SC, Cote IM, Oliveira RF (2012) Face your fears: cleaning gobies inspect predators despite being stressed by them. PLOS ONE 7:e39781

Scheuerlein A, Van't Hof TJ, Gwinner E (2001) Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). Proc R Soc B 268:1575–1582

Schmitz O, Beckerman A, OBrien K (1997) Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology 78:1388–1399

Seeman TE, McEwen BS, Rowe JW, Singer BH (2001) Allostatic load as a marker of cumulative biological risk: macArthur studies of successful aging. Proc Natl Acad Sci USA 98:4770–4775

Seeman TE, Crimmins E, Huang M-H, Singer B, Bucur A, Gruenewald T, Berkman LF, Reuben DB (2004) Cumulative biological risk and socio-economic differences in mortality: MacArthur studies of successful aging. Soc Sci Med 58:1985–1997

Seiter SA (2011) Predator presence suppresses immune function in a larval amphibian. Evol Ecol Res 13:283–293

Sheriff MJ, Krebs CJ, Boonstra R (2009) The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. J Anim Ecol 78:1249–1258

Sheriff MJ, Krebs CJ, Boonstra R (2010) The ghosts of predators past: population cycles and the role of maternal programming under fluctuating predation risk. Ecology 91:2983–2994

Sheriff MJ, Krebs CJ, Boonstra R (2011) From process to pattern: how fluctuating predation risk impacts the stress axis of snowshoe hares during the 10-year cycle. Oecologia 166:593–605

Speakman JR, Garratt M (2014) Oxidative stress as a cost of reproduction: beyond the simplistic trade-off model. Bioessays 36:93–106

Strobbe F, McPeek MA, De Block M, Stoks R (2010) Survival selection imposed by predation on a physiological trait underlying escape speed. Funct Ecol 24:1306–1312

Thaker M, Vanak AT, Lima SL, Hews DK (2010) Stress and aversive learning in a wild vertebrate: the role of corticosterone in mediating escape from a novel stressor. Am Nat 175:50–60

Thaler JS, McArt SH, Kaplan I (2012) Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. Proc Natl Acad Sci USA 109:12075–12080

Thomson RL, Tomás G, Forsman JT, Broggi J, Mönkkönen M (2010) Predator proximity as a stressor in breeding flycatchers: mass loss, stress protein induction, and elevated provisioning. Ecology 91:1832–1840

Thomson RL, Tomás G, Forsman JT, Mönkkönen M (2012) Manipulating individual decisions and environmental conditions reveal individual quality in decision-making and non-lethal costs of predation risk. PLoS One 7:e52226

Travers M, Clinchy M, Zanette L, Boonstra R, Williams TD (2010) Indirect predator effects on clutch size and the cost of egg production. Ecol Lett 13:980–988

Vucetich JA, Smith DW, Stahler DR (2005) Influence of harvest, climate and wolf predation on Yellowstone elk, 1961–2004. Oikos 111:259–270

White PJ, Garrott RA, Hamlin KL, Cook RC, Cook JG, Cunningham JA (2011) Body condition and pregnancy in northern yellowstone elk: evidence for predation risk effects? Ecol Appl 21:3–8

Wikelski M, Cooke S (2006) Conservation physiology. Trends Ecol Evol 21:38–46

Williams T (2005) Mechanisms underlying the costs of egg production. Bioscience 55:39–48

Zanette L, Clinchy M, Smith J (2006) Combined food and predator effects on songbird nest survival and annual reproductive success: results from a bi-factorial experiment. Oecologia 147:632–640

Zanette LY, White AF, Allen MC, Clinchy M (2011) Perceived predation risk reduces the number of offspring songbirds produce per year. Science 334:1398–1401

Zanette LY, Hobson KA, Clinchy M, Travers M, Williams TD (2013) Food use is affected by the experience of nest predation: implications for indirect predator effects on clutch size. Oecologia 172:1031–1039