THE ECOLOGY OF STRESS
Predator-induced stress and the ecology of fear

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Summary

1. Predator-induced stress has been used to exemplify the concept of stress for close to a century because almost everyone can imagine the terror of fleeing for one's life from a lion or a tiger. Yet, because it has been assumed to be acute and transitory, predator-induced stress has not been much studied by either comparative physiologists or population ecologists, until relatively recently.

2. The focus in biomedical research has always been on chronic stress in humans, which most comparative physiologists would agree results from '*sustained psychological* stress – linked to mere thoughts' rather than '*acute physical* crises' (like surviving a predator attack) or '*chronic physical* challenges' (such as a shortage of food). Population ecologists have traditionally focused solely on the *acute* physical crisis of surviving a direct predator attack rather than whether the risk of such an attack may have a *sustained* effect on other demographic processes (e.g. the birth rate).

3. Demographic experiments have now demonstrated that exposure to predators or predator cues can have *sustained* effects that extend to affecting birth and survival in free-living animals, and a subset of these have documented associated physiological stress effects. These and similar results have prompted some authors to speak of an 'ecology of fear', but others object that 'the *cognitive* and emotional aspects of avoiding predation remain unknown'.

4. Recent biomedical studies on animals in the laboratory have demonstrated that exposure to predators or predator cues can induce '*sustained psychological* stress' that is directly comparable to chronic stress in humans, and this has now in fact become one of the most common stressors used in studies of the animal model of post-traumatic stress disorder (PTSD).

5. We review these recent findings and suggest ways the laboratory techniques developed to measure the 'neural circuitry of fear' could be adapted for use on free-living animals in the field, in order to: (i) test whether predator risk induces '*sustained psychological* stress' in wild animals, comparable to chronic stress in humans and (ii) directly investigate 'the *cognitive* and emotional aspects of avoiding predation' and hence the 'ecology of fear'.

Key-words: indirect predator effects, non-consumptive effects, non-lethal predator effects, post-traumatic stress disorder (PTSD), predation risk, risk effects

Introduction

Predator-induced stress has been used to exemplify the concept of stress for close to 100 years. Walter B. Cannon, one of the pioneers of the study of stress, used predator-induced stress in wildlife in 1915 to exemplify the 'fight or flight' response: 'the physiological provisions for fierce struggle are found not only in the bodies of lower animals, that must hunt and kill in order to live, but also in human beings… The increase in blood sugar, the secretion of adrenin and the altered circulation in pain and emotional excitement have been interpreted in the foregoing discussion as biological adaptations to conditions in wildlife which are likely to involve pain and emotional excitement, i.e., the necessities of fighting or flight… The cornering of an animal when in the headlong flight of fear may suddenly turn the fear to fury and the flight to a fighting in which all the strength of desperation is displayed' (Cannon

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1915, p. 211, 275, 286). Nearly a century later, Robert Sapolsky (2004) used a lion attacking a zebra to exemplify the concept of stress in his popular book *Why Zebras Don't Get Ulcers*.

Despite being used to exemplify the concept of stress for almost a century, there has been very little research directly addressing predator-induced stress, up until the past few years. There are two principal reasons for this. The first is that, until relatively recently, comparative physiologists have assumed that predator-induced stress is necessarily acute and transitory – and so has nothing to tell us about chronic stress, which is the focus in biomedical research. This is why Sapolsky entitled his book *Why Zebras Don't Get Ulcers*, because he argued that, unlike humans, wild animals cannot suffer from chronic stress; the thesis being that since the 'function' of the stress axis is to maintain homeostasis, chronic stress must be maladaptive and pathological, and hence cannot exist in nature (Sapolsky 2004; Rodrigues, LeDoux & Sapolsky 2009; see also Wingfield & Ramenofsky 2011). According to Sapolsky (2004, pp. 4–7, his emphasis) 'for animals like zebras, the most upsetting things in life are *acute physical* crises… An organism can also be plagued by *chronic physical* challenges' but 'viewed from the perspective of the evolution of the animal kingdom, sustained psychological stress is a recent invention, mostly limited to humans and other social primates… We can experience wildly strong emotions (provoking our bodies into an accompanying uproar) linked to mere thoughts… unlike less cognitively sophisticated species'.

The century-old idea that an *acute* stress response may be expected in 'lower animals' when exposed to a predator or predator cues (Cannon 1915, p. 211) is not something that any comparative physiologist would evidently disagree with, and numerous studies on diverse taxa have demonstrated just this. Hawlena & Schmitz (2010b) recently published a review that includes an excellent overview of field studies that have documented such acute responses. The questions we address are as follows: (i) what is the evidence that exposure to predators or predator cues induces '*sustained psychological* stress – linked to mere thoughts' in 'less cognitively sophisticated species', that is, *is* predator-induced stress comparable to chronic stress in humans and (ii) how can we best test this in the field?

The second principal reason why predator-induced stress has been so little studied is that ecologists interested in the effects of predators on prey population size have not, until relatively recently, paid much heed to predator-induced stress. Predators obviously affect the number of prey in a population when they directly kill one of them and population ecologists have thus tended to focus solely on direct killing in considering the effects of predators on prey demography (Lima 1998; Preisser, Bolnick & Benard 2005). A direct predator attack constitutes an *acute physical crisis* that the prey either survives or does not. Other demographic processes, such as reproduction (giving birth and rearing young) or death from malnutrition or disease,

occur on longer time scales, and ecologists have traditionally assumed that these processes are affected by *chronic physical challenges*, such as a shortage of food or a parasitic infection (Krebs 2002; Creel & Christianson 2008). Until such time as predators could be shown to affect these slower demographic processes, there was no reason to consider whether chronic predator-induced stress was pertinent to population ecology.

Because demographic experiments (e.g. Peckarsky *et al.* 1993; Krebs *et al.* 1995; Schmitz, Beckerman & O'Brien 1997) increasingly began to suggest that predators may affect prey demography in more ways than by just directly killing prey, population ecologists began in the 1990s to investigate whether 'sustained psychological stress' in response to predators could indeed affect the demography of 'less cognitively sophisticated species' (Boonstra *et al.* 1998). We briefly describe below the types of demographic experiments that have now shown that predators can affect prey demography – even in the absence of direct killing. These studies demonstrate that exposure to predators or predator cues can have *sustained* effects that are sufficiently long-lasting to affect the prey's reproduction and long-term (rather than just acute) survival. Demographic effects may be demonstrated without addressing the mechanism, and we also review the modest number of field studies that have (i) documented physiological stress effects in prey in response to predation risk; in the context of (ii) also demonstrating a predator risk effect on demography.

'Chronic *physical* challenges', such as a shortage of food (i.e. 'famine'), can induce a physiological stress response (Sapolsky 2004, p. 4). Physiological changes may be observed in association with variation in food intake (Lima 1998; Beckerman, Wieski & Baird 2007), food demand (e.g. Hawlena & Schmitz 2010a) and conversion efficiency (e.g. McPeek 2004), all of which may be affected by the presence of predators. A decrease in food intake may be expected in most animals when exposed to a predator or predator cues because literally hundreds of behavioural studies on diverse species have demonstrated that foraging is impaired in the presence of predators (Lima 1998; Brown & Kotler 2004; Caro 2005). Decreased food intake, whether due to a shortage of food or the impairment of foraging, may be expected to produce the same physiological changes, such as a decrease in body condition (e.g. body mass/body length[3]; Romero & Wikelski 2001) or an alteration in glucocorticoid levels (e.g. Boonstra *et al.* 1998; Clinchy *et al.* 2004; Forristal *et al.* 2012).

Many authors have begun referring to the 'ecology of fear' (Brown, Laundré & Gurung 1999), 'degree of "fear"' (Stankowich & Blumstein 2005), or 'cost of fear' (Martin 2011), when describing predator-induced physiological, behavioural and demographic changes in prey, such as we have been discussing. As already noted, Cannon (1915, p. 275) spoke of animals 'in the headlong flight of fear'. Creel, Winnie & Christianson (2009, p. 12391) recently argued that predator risk effects ought not to be referred

to as 'fear' effects because 'the cognitive and emotional aspects of avoiding predation remain unknown … in virtually all studies of "the ecology of fear".' Creel, Winnie & Christianson (2009, p. 12391) emphasized that predator 'risk effects can logically arise through mechanisms that do not involve the stress response'. Sapolsky (2004), like many other comparative physiologists (e.g. McEwen & Wingfield 2003), distinguishes between physiological stress resulting from 'chronic *physical* challenges', such as a shortage of food, and 'sustained *psychological* stress – linked to mere thoughts' (Sapolsky 2004, pp. 4–5). Hence, just as predator risk effects can logically arise through mechanisms that do not involve the stress response, predator risk can affect the stress response through mechanisms, for example, the impairment of foraging, that do not necessarily involve 'sustained *psychological* stress – linked to mere thoughts', that is, 'the *cognitive* and emotional aspects of avoiding predation'.

We agree with the assessment of Creel, Winnie & Christianson (2009) that 'the cognitive and emotional aspects of avoiding predation remain unknown … in virtually all studies of "the ecology of fear",' but we also see this as a testable hypothesis. Fear *is* something that can be measured in the brain (Rosen & Schulkin 1998, 2004), as we will discuss. At roughly the same time that ecologists began to consider whether predator-induced stress may affect prey demography, biomedical researchers coincidentally began using predator-induced stress in animal studies in the laboratory designed to address chronic stress effects in humans and, in particular, post-traumatic stress disorder (PTSD; e.g. Adamec & Shallow 1993). These studies suggest that exposure to predators or predator cues has quantifiable effects on the 'neural circuitry of fear' (Rosen & Schulkin 1998, 2004) and can induce 'sustained psychological stress' in 'less cognitively sophisticated species'. However, 'experiments in captivity … leave room to wonder how the results generalize to natural conditions' (Creel & Christianson 2008, p. 199). Although 'sustained psychological stress' can be induced in 'less cognitively sophisticated species' in the laboratory, such studies are designed to investigate pathologies in humans and it could thus be argued that the effects seen are necessarily maladaptive and pathological, and hence cannot exist in nature (Sapolsky 2004; Rodrigues, LeDoux & Sapolsky 2009; Wingfield & Ramenofsky 2011).

We argue that by applying the myriad techniques for measuring the 'neural circuitry of fear' developed in the laboratory, to studies on free-living animals in the field, it ought to be possible to (i) test whether exposure to predators or predator cues induces '*sustained psychological* stress' in 'less cognitively sophisticated species' in the wild and (ii) directly investigate 'the *cognitive* and emotional aspects of avoiding predation' and hence the 'ecology of fear'. We provide a brief overview of the various methods developed in laboratory animal studies that have used exposure to predators or predator cues to explore chronic stress effects in humans, and specifically PTSD, and we suggest ways in which these approaches could be adapted for use on free-living animals in the field. Although we discuss other taxa, we give emphasis to studies on birds and mammals because the existing techniques for quantifying psychological and cognitive effects in laboratory animals are most readily translatable to these species.

Predator effects on prey demography in the absence of direct killing

From about the early 1990s onwards, there have been an ever-increasing number of elegant experiments conducted showing that predators can affect prey demography – even when direct killing is actively prevented (Lima 1998; Preisser, Bolnick & Benard 2005). Direct killing by predators has often been actively eliminated in such studies by gluing shut (e.g. Peckarsky *et al*. 1993; Schmitz, Beckerman & O'Brien 1997) or partially amputating (e.g. Nelson, Matthews & Rosenheim 2004) the mouthparts of predators (e.g. stoneflies, spiders and damsel bugs). These 'risk' predators (Schmitz, Beckerman & O'Brien 1997), which can 'intimidate' but not directly kill any prey (Preisser, Bolnick & Benard 2005), are then placed together with prey (e.g. mayfly larvae, grasshopper nymphs, pea aphids) constrained in artificial enclosures. The effect on the prey's birth, survival or population growth rate, due to 'intimidation' alone, is then quantified by comparing prey in enclosures containing 'risk' predators, vs. prey housed by themselves. Other studies have used caged predators or predator odour (Kats & Dill 1998) to 'intimidate' prey. Preisser, Bolnick & Benard's (2005) conducted a meta-analysis concerning such experiments and concluded that the general pattern is that 'intimidation' by predators affects prey demography as much, or more, than direct killing does.

The fact that predators can affect prey demography in the absence of direct killing demonstrates that exposure to predators or predator cues can have *sustained* effects that are sufficiently long-lasting to affect slower demographic processes, such as reproduction, consistent with what might be expected in response to '*sustained* psychological stress'. Nonetheless, mechanisms other than 'sustained psychological stress' must necessarily be involved in many cases. Preisser, Bolnick & Benard's (2005) meta-analysis included examples in which the prey were algae ($n = 17$), invertebrates ($n = 111$), fish ($n = 14$) and amphibians ($n = 24$), but none in which the prey were reptiles, birds or mammals. That algae show such effects demonstrates that predator 'risk effects can logically arise through mechanisms that do not involve the stress response' (Creel, Winnie & Christianson 2009), let alone 'sustained *psychological* stress'.

As described in the Introduction, 'sustained *psychological* stress – linked to mere thoughts' represents a more easily comprehended and readily testable mechanism when considering predator risk effects on the demography of higher vertebrates, and experiments and correlative studies

on birds and mammals conducted since, or not included in, Preisser, Bolnick & Benard's (2005) meta-analysis, demonstrate that predators can affect prey demography – even in the absence of direct killing, in these taxa as well. Zanette *et al.* (2011) recently reported a 40% reduction in the number of offspring produced per year in a population of free-living song sparrows, in an experiment directly modelled on those described earlier, in which direct predation was actively eliminated (using electric fences and netting) and 'intimidation' (perceived predation risk) was manipulated using predator call playbacks. 'Intimidation' due solely to hearing the sound of predators caused females to lay fewer eggs, a greater proportion of which failed to hatch because incubation was disrupted, and a greater proportion of their nestlings starved to death because 'intimidation' impaired their foraging as evidenced by their bringing less food to the nest. Eggers *et al.* (2006) similarly showed that predator call playbacks caused Siberian jays to lay fewer eggs in the first clutch of the season, and Travers *et al.* (2010) experimentally demonstrated that the experience of nest predation caused female song sparrows to lay fewer eggs in subsequent clutches.

In mammals, Krebs *et al.* (1995) reported that the experimental combination of both predator removal and food supplementation caused an increase in the density of snowshoe hares (11-fold) much greater than that expected from the additive effect of both treatments (fivefold), indicating that 'intimidation' by predators prevented the hares from taking full advantage of the supplemental food by impairing their foraging. Karels *et al.* (2000) similarly experimentally demonstrated that the weaning success of arctic ground squirrels was greater where predators were removed and hence both direct predation and 'intimidation' by predators had been eliminated. Following the reintroduction of wolves to Yellowstone National Park in the United States, there was a decline in the pregnancy rate of the elk in the Park, which a large body of work suggests was due to 'intimidation' of the elk by the wolves (Creel *et al.* 2007; Creel & Christianson 2008; Creel, Christianson & Winnie 2012).

The fact that only a modest number of field experiments on birds and mammals have yet demonstrated predator risk effects on demography is most readily explained by the logistical challenges involved in studying free-living vertebrates (Lima 1998; Creel & Christianson 2008), and this also provides the most ready explanation for why no such field experiment has evidently yet been conducted on a reptile. We expect that, just as among other taxa (Preisser, Bolnick & Benard 2005), predator risk effects on demography will be found to be norm among reptiles, birds and mammals, as further rigorously designed field experiments are conducted.

Field studies of predator-induced stress effects on demography

It is now clear that exposure to predators or predator cues can have *sustained* effects that extend to affecting birth and survival in free-living animals (Preisser, Bolnick & Benard 2005; Zanette *et al.* 2011). In this section, we describe those field studies that have (i) documented physiological stress effects, in the context of (ii) also demonstrating predator risk effects on demography. To our knowledge, only two experiments (Sheriff, Krebs & Boonstra 2009; Travers *et al.* 2010) have measured effects on stress physiology in relation to predator-induced changes in the birth rate – neither of which can be considered definitive; and we are not aware of any field experiment on free-living animals that has manipulated predator risk and demonstrated an effect on both (i) stress physiology and (ii) mortality not due to direct predation (see also Hawlena & Schmitz 2010b). Although the studies described in this section suggest that predator-induced stress can affect the demography of free-living animals in ways consistent with what might be expected in response to 'sustained *psychological* stress', the physiological stress effects reported could also result from 'chronic *physical* challenges', such as a decrease in food intake caused by the impairment of foraging. As noted in the Introduction, it remains unknown whether effects on demography can result solely from 'sustained *psychological* stress – linked to mere thoughts', that is, 'the *cognitive* and emotional aspects of avoiding predation'.

Sheriff, Krebs & Boonstra (2009) presented a live predator (a trained dog) to pregnant snowshoe hares housed in 4×4 m outdoor pens, and reported that predator-exposed females had elevated faecal glucocorticoid metabolite concentrations, and were significantly less likely to give birth to live young. Though the experiment was conducted on captive hares and so 'leaves room to wonder how the results generalize to natural conditions', correlative studies on the same population of hares (Boonstra *et al.* 1998; Sheriff, Krebs & Boonstra 2009, 2011) have reported that variation in predator abundance is associated with both, variation in the birth rate, and variation in a suite of physiological measures suggestive of predator-induced stress [faecal glucocorticoid metabolite concentration; plasma: glucocorticoid, corticosteroid-binding globulin (CBG), glucose and free fatty acid level; white blood cell count; haematocrit].

Travers *et al.* (2010) reported effects on stress physiology in a field experiment that demonstrated predator-induced effects on the birth rate (number of eggs laid) in free-living female song sparrows. Females that experienced frequent experimental nest predation (the removal of all their eggs) laid fewer eggs in their next nest, and thus had a lower birth rate, compared to females that did not experience nest predation. To evaluate the effects on the physiology of the birds the authors measured multiple physiological variables (15 in total; including glucocorticoid and CBG levels and oxidative stress) and used multivariate statistical procedures [e.g. discriminant function analysis (DFA)]. In addition to a lower birth rate, females that experienced frequent experimental nest predation showed significantly more evidence of 'physiological dysregulation' (Seeman *et al.* 2001, 2004; Travers *et al.* 2010).

Though the effects on stress physiology were consistent with the birds perceiving the experience of nest predation as a cue concerning predator risk (Lima 2009), the same effects could also have resulted from the 'stress' of reproduction. Consequently, Travers *et al.*'s results do not provide definitive evidence of a link between predator-induced stress and the birth rate.

The potential effect of predator-induced stress on mortality not due to direct predation was recently evaluated in an experiment looking at the effect of 'risk' predators (spiders with their mouthparts glued shut) on prey (grasshopper nymphs) constrained in artificial enclosures (Hawlena & Schmitz 2010a). Hawlena & Schmitz (2010a) reported that the metabolic rate (measured by the rate of carbon dioxide emission) of grasshopper nymphs exposed to 'risk' spiders was 40% higher than that of control grasshoppers, and this difference in metabolic rate was associated with a heightened energy demand, and corresponding shift in diet. Schmitz, Beckerman & O'Brien (1997) earlier established that the presence of 'risk' spiders increases grasshopper mortality. Hawlena & Schmitz's (2010a) findings suggest that predator-induced stress may result from an increase in the *demand* for food. Whether such a predator-induced increase in the *demand* for food occurs in free-living animals has not, to our knowledge, been tested, but seems highly probable. Obesity, and hence an increase in the *demand* for food, is a common feature of 'sustained *psychological* stress' in humans (McEwen & Wingfield 2003), and McEwen & Wingfield (2010) recently pondered whether this could also occur in wild animals.

Several correlative studies have evaluated the association between predator risk and demography in free-living animals and whether this may be mediated by predator-induced stress. As noted earlier, predator risk appears responsible for declines in the birth rate (pregnancy rate) of elk in Yellowstone National Park following the reintroduction of wolves to the Park in 1995 (Creel *et al.* 2007; Creel & Christianson 2008; Creel, Christianson & Winnie 2012). Behavioural and dietary data are consistent with this reduction in the birth rate being due to predator-induced impairment of foraging (Creel, Winnie & Christianson 2009; Christianson & Creel 2010). Creel, Winnie & Christianson (2009) measured elk faecal glucocorticoid metabolite concentrations and found no relationship to either predator risk (the ratio of elk to wolves) or the birth rate (the ratio of calves to cows). If glucocorticoid levels vary with food intake (e.g. Boonstra *et al.* 1998; Clinchy *et al.* 2004; Forristal *et al.* 2012) then the well-documented evidence of predator-induced impairment of foraging in the elk arguably ought to have been evident in the faecal glucocorticoid metabolite concentrations. Fontaine *et al.* (2011) similarly found no effect on plasma glucocorticoid levels in grey-headed juncos in response to a predator-removal experiment, even though they observed behavioural changes consistent with predator-induced impairment of foraging.

Monclús, Tiulim & Blumstein (2011) recently reported an association between predator risk, the birth rate (litter size) and faecal glucocorticoid metabolite concentrations in older (>3 years old), but not younger (<3 years old), yellow-bellied marmot mothers. Comparing among marmot colonies that differed in predator abundance, there was a significant relationship between predator abundance and maternal faecal glucocorticoid metabolite concentration. Litter size was associated with maternal faecal glucocorticoid metabolite concentration, but only in older (>3 years old) mothers. Presumably because of the age-specific relationship between predator-induced stress and litter size, there was evidently no overall association between predator risk and the birth rate (litter size).

Rather than an age-specific association like that reported by Monclús, Tiulim & Blumstein (2011), Scheuerlein, Van't Hof & Gwinner (2001) earlier reported a sex-specific association between predator risk, the birth rate, and both body condition and plasma glucocorticoid levels, in tropical stonechats. Stonechats subject to greater predator risk due to the presence of predatory fiscal shrikes on their territories had a lower birth rate, because they were less likely to initiate a second brood and took longer to do so if they did, compared to stonechats without shrikes on their territories. Parental males with shrikes on their territories had lower body condition index scores (body mass/tarsus length) and elevated plasma glucocorticoid levels. Parental females, in contrast, did not show differences in either body condition or plasma glucocorticoid levels in association with the presence or absence of shrikes on their territories. Neither was there an association between predator risk and restraint-induced plasma glucocorticoid levels, in either sex.

Clinchy *et al.* (2004) documented an association between predator risk, demography, and the stress physiology of parental male song sparrows, in the same study populations where Zanette *et al.* (2011) subsequently experimentally demonstrated that the perception of predation risk alone can affect the demography of free-living wildlife. Clinchy *et al.* (2004) compared sites differing in predator abundance that demonstrated differences in several demographic measures which Zanette *et al.*'s (2011) results experimentally confirm are affected by the perception of predation risk (number of eggs laid, proportion that failed to hatch, proportion of nestlings expiring; Zanette, Clinchy & Smith 2006a,b). Clinchy *et al.* (2004) reported that these differences in predator risk and demography were associated with differences between parental males in a suite of physiological stress measures (baseline plasma glucocorticoid; restraint-induced plasma glucocorticoid; free fatty acid; white blood cell count; haematocrit). Clinchy *et al.* (2011b) later re-sampled these populations and collected physiological measures of both parental males and parental females. Just as Scheuerlein, Van't Hof & Gwinner (2001) found in stonechats, in the song sparrows, parental males showed differences in baseline plasma total glucocorticoid levels, but parental females did not. Clinchy *et al.*

(2011b) also measured plasma CBG levels and found an inverse pattern – CBG levels differed in females but not males. The net result of these inverse differences in total glucocorticoid and CBG levels being that both parental males and parental females demonstrated elevated free glucocorticoid levels (glucocorticoids not bound to CBG) in response to greater predator risk.

The preceding studies included measures of both, the potential effect of predator risk on demography, and whether predator risk evidently affected stress physiology. As noted in the Introduction, Hawlena & Schmitz (2010b) recently reviewed the large number of studies that have not included measures of the potential effects on demography, which have nonetheless reported an association between predator risk and stress physiology. In the remainder of this section, we briefly review the small number of such studies, not included, or published since, Hawlena & Schmitz's (2010b) review.

Experimental field studies on a fish, a mammal and a bird have all recently demonstrated that playbacks of predator calls or sounds, or alarm calls to predators, can affect the stress physiology of prey. Remage-Healey, Nowacek & Bass (2006) demonstrated that playbacks of dolphin (predator) vocalizations induced elevated glucocorticoid levels in Gulf toadfish. Mateo (2010) reported that playbacks of the alarm calls that Belding's ground squirrels use to warn each other of predator risk, increased glucocorticoid levels in the hearer. Finally, Ibáñez-Álamo, Chastel & Soler (2011) found that playbacks of magpie (predator) calls affected glucocorticoid levels in nestling blackbirds.

Four recent correlative studies on two mammals, a bird and a lizard, have reported results regarding a variety of physiological measures that are all consistent with predator-induced stress. Hodges, Boonstra & Krebs (2006) reported that overwinter change in body mass varied with predator risk (predation rate) in snowshoe hares. Mateo (2007) found differences in faecal glucocorticoid metabolite concentrations among Belding's ground squirrels inhabiting sites differing in apparent predator risk. Thomson et al. (2010) showed that body mass, blood stress protein (heat-shock protein) and immunoglobulin levels in pied flycatchers, varied with the distance between their nest and that of the nearest sparrowhawk (predator) nest. Finally, Olsson et al. (2010) reported evidence of chromosomal damage (shortened telomere length) in sand lizards that had survived a direct predator attack, as evidenced by their having a re-grown tail (tail 'dropping' being an anti-predator defence).

Predator-induced fear in laboratory animal studies of chronic stress

A plethora of studies have been conducted that have identified and elucidated not only which parts of the brain are activated by fearful stimuli, but also the endocrinology and molecular biology of the 'neural circuitry of fear' (Rosen & Schulkin 1998, 2004). The amygdala is the neuroanatomical region of the brain that appears central to the phenomenon of fear, although other areas are involved (Rosen & Schulkin 1998, 2004). Humans with lesions of the amygdala have impaired fear-related behaviour (e.g. LaBar et al. 1995), and neuroimaging studies have shown greater activation of the amygdala in response to fear-provoking stimuli (Bryant et al. 2008; Kim et al. 2011). Laboratory studies on primates, rodents and rabbits have similarly shown that lesions of the amygdala disrupt the expression of fear-related behaviour (Rosen & Schulkin 1998, 2004; e.g. Campeau et al. 2008; Amano et al. 2011).

Laboratory work on primates, rodents, sheep and frogs has demonstrated that glucocorticoids stimulate the expression of corticotrophin releasing hormone (CRH) in the amygdala (Rosen & Schulkin 2004; Schulkin, Morgan & Rosen 2005; Yao, Schulkin & Denver 2008), and in the species tested to date (primates, rodents and sheep), this pathway appears to play a critical role in eliciting fear-related behavioural responses. Predator-induced fear, caused by exposure to live predators (e.g. cat, ferret, dog) or predator odour (e.g. cat, fox) has been shown to affect this pathway in both rats and sheep (Dielenberg, Hunt & McGregor 2001; Cook 2002; Takahashi et al. 2005; Roseboom et al. 2007). Because glucocorticoids are thus associated with fear, measuring glucocorticoid levels can assist in evaluating the *psychological* effects of predator-induced stress; however, 'glucocorticoids are not the molecules of fear' but instead play a fundamental role in energy balance (Rosen & Schulkin 2004, p. 177), and so may be expected to vary in response to not just fear, but food shortage, and a host of other stressors, as well (McEwen & Wingfield 2003, 2010).

Post-traumatic stress disorder (PTSD) represents arguably the most salient example of how fear and stress can have chronic effects that may last a lifetime. Because experimental studies of PTSD can obviously not be conducted on humans, it is necessary to use an 'animal model' to elucidate the aetiology of PTSD and explore the associated neurological changes (Cohen et al. 2010). As noted in the Introduction, at roughly the same time that population ecologists began conducting experiments using 'risk' predators to test whether exposure to predators or predator cues can have *sustained* effects on prey demography (e.g. Peckarsky et al. 1993), biomedical researchers studying the animal model of PTSD quite independently began utilizing exposure to a predator as a stressor (e.g. showing a rat a cat; Adamec & Shallow 1993) to understand the *sustained* neurological effects of life-threatening events. Today, exposure to a predator (or predator odour) is one of the most common stressors used in studies of the animal model of PTSD (Cohen et al. 2010; Mackenzie et al. 2010). Predator exposure was initially seized upon for purely practical reasons as this permits the researcher to utilize a *psychological* stressor, that is *life-threatening*, but does not involve pain; all consistent with the aetiology of PTSD in humans (Adamec & Shallow 1993; Roseboom et al. 2007; Campeau et al. 2008; Staples, McGregor & Hunt 2009; Cohen et al.

2010; Mackenzie *et al.* 2010). Of greatest importance with respect to understanding PTSD, the hallmark of which is the long-lasting or 'transformational' change in the patient in response to a trauma (Yehuda & Bierer 2009), predator exposure has been shown to have *sustained* effects on: anxiety-like behaviours, glucocorticoid levels, gene expression and the release of CRH in the amygdala, and many other phenomena associated with PTSD (Adamec & Shallow 1993; Armario, Escorihuela & Nadal 2008; Campeau *et al.* 2008; Staples, McGregor & Hunt 2009; Cohen *et al.* 2010; Clinchy *et al.* 2011a).

That predator exposure can have *sustained* – or even permanent – effects on morphology is well-established in invertebrate and aquatic species (Tollrian & Harvell 1999; Benard 2004). Such 'inducible defences' may include changes in body size or shape or the development of defensive armature (e.g. spines or thickened shells; Hawlena *et al.* 2011). Some researchers have suggested that PTSD may derive from evolutionarily adaptive neuronal changes facilitating rapid and enduring learning in response to a life-threatening experience (Silove 1998; Mineka & Zinbarg 2006; Cantor 2009); the *sustained* – or even permanent – effects on learning and memory, and neural circuitry, thus constituting in essence another form of inducible defence (in this case involving neurons, rather than body size, shape or defensive armature). The idea that long-term memory may constitute another form of inducible defence was recently proposed by Orr, Hittel & Lukowiak (2010), who showed that pond snails exposed to the odour of a predator (crayfish) develop the ability to retain a long-term memory of an operant learning task, whereas control snails not exposed to predator odour do not. In a related study, Orr & Lukowiak (2008) showed that these changes in memory retention were associated with predator odour induced changes in the electrophysiology of the relevant neurons. The neuronal changes induced by predator exposure in vertebrates seen in studies of the animal model of PTSD, certainly entail a difference in scale, given the more complex neural circuitry involved compared to pond snails, but may not involve a difference in kind; induced long-term memory formation arguably being the evolutionary 'function' in both cases. With respect to this evolutionary 'function', retaining a long-lasting memory of a life-threatening experience and the responses that thus permitted the individual to avoid death is clearly adaptive, if this permits the individual to avoid death in similar circumstances in the future (Clinchy *et al.* 2011a).

The large literature addressing the animal model of PTSD demonstrates that exposure to predators or predator cues can induce 'sustained *psychological* stress' in 'less cognitively sophisticated species' – that is, directly comparable to chronic stress in humans; in other words, that 'zebras *may* get ulcers'. As we have discussed, field experiments on free-living animals have now demonstrated that exposure to predators or predator cues can have *sustained* effects on demography that are accompanied by physiological stress effects, consistent with what might be expected in response to 'sustained psychological stress'. This similarly suggests that 'zebras *may* get ulcers'. In the next section, we propose ways of applying the techniques for quantifying 'sustained *psychological* stress' that have been developed in the laboratory to studies on free-living animals in the field. By integrating these approaches, it ought to be possible to directly test whether predator-induced stress in wild animals *is* comparable to chronic stress in humans and hence whether 'zebras *do* get ulcers'.

Measuring predator-induced fear and stress in the field

At present, measuring the 'neural circuitry of fear' in wild animals will in most cases involve destructive sampling. Non-destructive neuroimaging of predator-induced fear and stress in wild animals may be feasible in some circumstances, as recently demonstrated by Marzluff *et al.* (2012). Using Positron Emission Tomography, Marzluff *et al.* (2012) were able to document that the perception of threatening human faces by captive wild crows activated neuronal circuits that included amygdalar, thalamic and brainstem regions, known in humans and other vertebrates to be related to emotion, motivation and conditioned fear learning. Although the procedure involved the use of a radioactive marker, there were no adverse health effects and all subjects were released back into the wild. This provides the exciting prospect of being able to follow the individual's subsequent reproduction and survival in the field, and hence *not* having to sacrifice demographic data on known individual's to measure the neuronal effects of predator-induced fear. Like any method, however, there are limitations. Marzluff *et al.* (2012) held their crows in captivity for 4 weeks, partly to ensure there were no adverse health effects. There are also a limited number of suitable scanners, the procedure is currently very expensive, and the trauma of capturing and transporting an animal to wherever the scanner is might render the results uninterpretable (Clinchy *et al.* 2011a). We suggest that the best approach, at present, to test whether 'sustained *psychological* stress – linked to mere thoughts' can itself affect demography, is to collect multiple non-destructive measures of stress physiology in the field, coupled with the destructive sampling of a subset of individuals, to calibrate which variables measured in the field are in fact associated with measurable fear effects in the brain. Where neuroimaging is feasible, this can be used to complement other neuronal measurements and so reduce the amount of destructive sampling required.

As already noted, 'glucocorticoids are not the molecules of fear' (Rosen & Schulkin 2004), and measuring glucocorticoid levels does not permit one to distinguish between changes due to predator-induced effects on, for example, food intake or demand, and effects due to 'the *cognitive* and emotional aspects of avoiding predation'. Collecting just one measure of the action of glucocorticoids can lead to further ambiguities (e.g. Scheuerlein, Van't Hof &

Gwinner 2001; Creel, Winnie & Christianson 2009), because the effects that glucocorticoids have on the body are mediated by all of (i) the amount released into the blood, (ii) plasma CBG levels and (iii) the number of receptor sites in target tissues (e.g. Breuner *et al.* 2003). Even collecting multiple measures of the action of glucocorticoids (e.g. both glucocorticoid and CBG levels) can provide ambiguous results, which may be clarified by also evaluating multiple 'downstream' physiological responses (e.g. glucose; free fatty acids; white blood cell counts; haematocrit; Delehanty & Boonstra 2011). There are, in addition, a large number of potential measures of physiological stress not directly tied to glucocorticoid levels, which we have mentioned, such as oxidative stress (Travers *et al.* 2010; see also Costantini, Marasco & Møller 2011), metabolic rate (Hawlena & Schmitz 2010a), immunoglobulin levels (Thomson *et al.* 2010; Travers *et al.* 2010), heat-shock proteins (Thomson *et al.* 2010; see also Slos & Stoks 2008) and telomere length (Olsson *et al.* 2010); and still others may be found in Hawlena & Schmitz's (2010b) recent overview.

Various multivariate statistical procedures exist which can be used to determine the relationship between predator risk, effects on birth and survival, the array of physiological measures that one has ideally been able to collect, and measurable fear effects in the brain (Tabachnick & Fidell 2007; Grace *et al.* 2010). To evaluate whether predator risk is associated with changes in the overall physiological profile of the animals under study, independent of any assumptions about the relevant importance of any given variable or the expected direction of change in any of them, one can employ such procedures as a discriminant function analysis (DFA; e.g. Travers *et al.* 2010), or a principal component analysis (PCA). To next determine whether animals subject to greater predator risk are in poorer overall condition, based on expectations from the literature, procedures may be used such as the multivariate ranking of 'physiological dysregulation' developed by Seeman *et al.* (2001, 2004; e.g. Travers *et al.* 2010). This procedure, developed by biomedical researchers, involves calculating a score for each individual, which is the sum of the number of physiological variables for which the value for that individual falls within the quartile, which the researcher deems is indicative of poor condition (e.g. a cholesterol level in the highest quartile, in humans; Seeman *et al.* 2001). Finally, these evaluations based on the literature can then be calibrated against one's own data by using, for example, stepwise multiple regression, to determine which physiological measures are most closely associated with (i) birth or survival and (ii) measurable fear effects in the brain.

As discussed in the section on laboratory animal studies, the amygdala is the neuroanatomical region of the brain that appears central to the phenomenon of fear, and testing for the expression of the immediate-early gene *fos*B and its protein products FosB/ΔFosB in the medial amygdala, using immunohistochemistry, appears to presently provide the most widely applicable means of testing for predator-induced fear effects in the brains of wild animals (Dielenberg, Hunt & McGregor 2001; Roseboom *et al.* 2007; Campeau *et al.* 2008; Staples, McGregor & Hunt 2009; Mackenzie *et al.* 2010; Clinchy *et al.* 2011a). The nucleus taeniae of the amygdala in birds appears to be the analogue of the medial amygdala in mammals (Yamamoto *et al.* 2005), so testing this brain region in birds ought to provide evidence of predator-induced fear effects, as recently confirmed by Marzluff *et al.* (2012). Clinchy *et al.* (2011a) recently reviewed various other neurological measures developed in laboratory animal studies of predator-induced fear and stress that also ought to be adaptable to use in the field, given the less than ideal conditions that will typically apply when collecting samples from the brains of wild animals. Pilsner *et al.* (2010) recently reported results concerning DNA methylation in the brains of polar bears shot by aboriginal hunters in eastern Greenland, demonstrating that it is entirely feasible to collect perfectly useful neurological samples under circumstances that are about as far from stereotypical laboratory conditions as it is possible to imagine.

Finding measurable fear effects in the brain would appear to provide the best evidence that the mechanism underlying predator risk effects on demography includes 'sustained *psychological* stress – linked to mere thoughts', that is, 'the *cognitive* and emotional aspects of avoiding predation'. Assuming one finds fear effects in the brain then it will be possible to use the multivariate statistical procedures described above to determine, (i) which demographic variables appear to be most strongly affected by 'sustained *psychological* stress' and (ii) which physiological measures are most reflective of 'the *cognitive* and emotional aspects of avoiding predation', as opposed to being more reflective of 'chronic *physical* challenges', such as a decrease in food intake caused by the impairment of foraging (Sapolsky 2004; Creel, Winnie & Christianson 2009).

Conclusions

Research on predator-induced stress has developed along two independent parallel paths, among biomedical researchers addressing chronic stress in humans, and population ecologists studying predator risk effects in wild animals. We suggest that both groups have much to gain from integrating these two approaches. Although use of predator exposure as a stressor in the laboratory was initially adopted for practical reasons, more and more biomedical researchers have begun to consider its ecological validity (e.g. Roseboom *et al.* 2007; Staples, McGregor & Hunt 2009; Cohen *et al.* 2010) and what this can tell us about the 'evolution of PTSD' and other anxiety disorders (e.g. Silove 1998; Mineka & Zinbarg 2006; Cantor 2009). For ecologists, addressing neurobiological effects provides the prospect of resolving whether predator-induced stress is always the result of *physical* challenges caused by the risk of predation (e.g. the impairment of foraging), or

whether it is ever 'linked to mere thoughts' and so resembles chronic stress in humans. We expect that it will be found that exposure to predators or predator cues induces 'sustained *psychological* stress' in many wild animals and that it will consequently often be accurate to refer to the 'ecology of fear'.

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