Physiology
Predation risk causes oxidative damage in prey

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While there is increasing interest in non-consumptive effects of predators on prey, physiological effects are understudied. While physiological stress responses play a crucial role in preparing escape responses, the increased metabolic rates and shunting of energy away from other body functions, including antioxidant defence, may generate costs in terms of increased oxidative stress. Here, we test whether predation risk increases oxidative damage in Enallagma cyathigerum damselfly larvae. Under predation risk, larvae showed higher lipid peroxidation, which was associated with lower levels of superoxide dismutase, a major antioxidant enzyme in insects, and higher superoxide anion concentrations, a potent reactive oxygen species. The mechanisms underlying oxidative damage are likely to be due to the shunting of energy away from antioxidant defence and to an increased metabolic rate, suggesting that the observed increased oxidative damage under predation risk may be widespread. Given the potentially severe fitness consequences of oxidative damage, this largely overlooked non-consumptive effect of predators may be contributing significantly to prey population dynamics.

1. Introduction
Non-consumptive predation is receiving increased attention driven by the insight that its effects may be as important for prey demographics as consumptive predation [1]. The focus of non-consumptive predation studies is largely on morphology, behaviour and life history [2]. By contrast, we know little about physiological responses to predation risk, especially in invertebrates [3]. Under predation risk, prey organisms are predicted to evolve a set of adaptive physiological responses that facilitate avoidance of predation. These involve an increase of metabolic rates and the allocation of resources to support emergency functions [3]. Yet, despite being beneficial to avoid predation, these physiological responses may alter individual nutritional budgets which may lead to prolonged inhibition of essential non-emergency body functions and accumulation of destructive effects at the molecular and cellular level [3].

Oxidative stress occurs when reactive oxygen species (ROS) are not fully neutralized by antioxidant defences (including repair), thereby generating oxidative damage [4]. Recent studies recognize that oxidative stress can be a mediator of trade-offs between life-history traits [4,5]. Oxidative damage may have profound fitness consequences, as it can reduce reproductive output and accelerate ageing [4]. Although predation risk has been shown to reduce antioxidant defence relative to oxidants [6,7], probably due to energetic constraints and increased metabolic rates associated with the fight-or-flight response [6], no studies have directly examined the effects of predation risk on oxidative damage. This is, however, crucial as changes in antioxidant defence may not necessarily translate into oxidative damage [4].

Here, we investigated whether predation risk results in increased oxidative damage in prey organisms. More specifically, we quantified the level of...
2. Material and methods

(a) Collecting and housing

Ten copulating females of the damselfly *Enallagma cyathigerum* were collected in ‘Het Stappersven’ (Kalmthout, Belgium), a fishless pond with large *Anax* dragonfly larvae as top predators. Females were transferred to the laboratory for egg laying. Larvae were reared individually in 200 ml cups under standard conditions of temperature (20°C), photoperiod (14 L : 10 D) and food (ad libitum *Artemia* nauplii 5 days a week). When larvae moulted into the final instar, they were used for the experimental trials and fed 7 days a week.

(b) Experimental setup

Twenty-five larvae were exposed to each of two predation risk treatments (absent versus present) for 7 days. During the exposure period, larvae were placed individually in glass vials (100 ml) filled with 50 ml synthetic pond water (composition in [10]). Glass vials were placed in groups of four in larger containers (750 ml) and daily randomly redistributed among containers of the same treatment. Predation risk was manipulated using a combination of visual and chemical predator cues, reflecting the cocktail of predator cues that damselfly larvae encounter in nature. *Enallagma* larvae are responsive to both types [11–13]. To provide visual predator cues, a large field-collected *Anax* dragonfly larva, an important predator of *Enallagma* larvae [14], was placed in the predation risk containers. Additionally, larvae could see the larvae in the other vials in the container (damselfly larvae are cannibalistic [15]). To preclude visual predator cues in the condition without predation risk, the sides of the vials of this condition were made non-transparent using dark tape. For the chemical predator cues, we daily homogenized one *E. cyathigerum* larva in 20 ml of water from an aquarium filled with 300 ml aged tap water in which a large *Anax* dragonfly larva had eaten an *E. cyathigerum* larva. We added 1 ml of this predator medium to each vial of the predation risk treatment. To the vials of the treatment without predation risk, we added 1 ml of water.

(c) Response variables

We weighed each larva to the nearest 0.01 mg at the start and end of the 7-day exposure period and quantified growth rate as \(\frac{\ln(\text{final mass}) - \ln(\text{initial mass})}{7 \text{ days}}\). Afterwards, to measure antioxidant defence, ROS and oxidative damage, individual larvae were homogenized and the resulting supernatant was used in the assays (for detailed protocols see the electronic supplementary material, S1).

For the SOD activity, we used the protocol of De Block & Stoks [16]. One SOD unit is the amount of enzyme needed to cause 50 per cent inhibition of the rate of the colorimetric reaction. To measure CAT activity, we used the protocol of De Block & Stoks [16]. One CAT unit is the amount of enzyme needed to decompose 1 μmol \(H_2O_2\) min\(^{-1}\). Superoxide anion concentrations were quantified based on the protocol by Oracz et al. [17] and expressed in M. Sample preparation for quantification of the formation of MDA was based on the protocol of Miyamoto et al. [18], and injection in the high-performance liquid chromatography/ultraviolet-visible spectroscopic detector (HPLC/UV-Vis) system was based on the protocol of Karatas et al. [19]. MDA levels were expressed in nmol ml\(^{-1}\).

The statistical analyses and the raw data are presented in more detail in the electronic supplementary material, S2 and S3, respectively.

3. Results

Analyses at the level of the treatment groups showed that growth rate was lower in larvae exposed to predation risk (\(t_{46} = -2.35, p = 0.023\; \text{figure 1}\)) of the two antioxidant enzymes measured, SOD activity was lower under predation risk (\(t_{46} = -3.53, p < 0.001\; \text{figure 2a}\)), whereas CAT activity was unaffected (\(t_{46} = -0.11, p = 0.91\; \text{figure 2b}\)). Larvae exposed to predation risk had higher superoxide anion levels (\(t_{46} = 2.33, p = 0.024\; \text{figure 2c}\)) and higher MDA levels (\(t_{48} = 6.82, p < 0.001\; \text{figure 2d}\)).

Analyses testing for relationships at the individual level showed that larvae exposed to predation risk had higher superoxide anion concentrations for a given SOD level (ANCOVA, predation risk: \(F_{1,46} = 4.67, p < 0.001\); SOD: \(F_{1,47} = 3.09, p = 0.085\); predation risk × SOD: \(F_{1,46} = 0.0036, p = 0.96\), and higher MDA levels for a given superoxide anion concentration (ANCOVA, predation risk: \(F_{1,46} = 4.83, p = 0.033\); \(O_2^\cdot\): \(F_{1,46} = 1.19, p = 0.28\); predation risk × \(O_2^\cdot\): \(F_{1,46} = 0.56, p = 0.46\); electronic supplementary material, figure S1).

4. Discussion

Our study demonstrates for the first time that predation risk generates oxidative damage in prey and starts exploring the underlying mechanistic insights, thereby extending previous studies indicating that predation risk may reduce antioxidant defence [6,7]. The absence of covariation at the individual level between SOD, superoxide anion and MDA levels reflects the complexity of the antioxidant response and may indicate (i) the fine-scale individual adjustment of the antioxidant defence, (ii) the use of other antioxidant defence mechanisms in addition to SOD, and (iii) the transient nature of the antioxidant defence (see the electronic supplementary material, S2).
Under predation risk, more ROS were apparently produced as suggested by the higher superoxide anion concentrations. This was further suggested by the higher superoxide anion concentration for a given SOD level in larvae exposed to predation risk. It is probable that an increased ROS production was the by-product of increased metabolic rates associated with the fight-or-flight response. In line with this, we observed increased respiration under predation risk in this species [6].

The increased ROS production was not balanced by an upregulation of the antioxidant defence; instead, SOD activity was reduced. Previous research in damselflies has shown that antioxidant levels are costly to maintain [16,20]. Although larvae of the study species do not reduce food intake under predation risk [16], it is likely that they shunted more energy towards the fight-or-flight response, specifically mobilizing energy for escape burst swimming [21]. This may also explain the observed predator-induced reduction in growth rate as has previously been documented in the study species [6] and in many other taxa [2] and which explicitly has been shown to be driven by a reduced conversion of food into biomass in the presence of predation risk [11,22–24].

The higher MDA levels for a given concentration of superoxide anion in larvae exposed to predation risk suggest that (i) larvae relied on other mechanisms (in addition to SOD) to reduce MDA levels and (ii) that larvae under predation risk invested less in these other mechanisms, again probably because they were more energy-limited due to their investment in the fight-or-flight response. Candidate mechanisms are the use of scavenger molecules (such as glutathione) and enzymes which repair oxidative damage to lipids [8].

The fight-or-flight response is an adaptive set of physiological responses that evolved to avoid predation. Yet, the increased metabolic rate and shunting of energy away from other functions may also generate costly physiological effects [3] such as the increase in oxidative damage to lipids that we have documented here. Given the association between physiological responses and predation risk (this study, see also [3]), we hypothesize that oxidative damage may be a common side effect of the fight-or-flight response which may have widespread negative fitness consequences [4]. Such negative side effects could play an evolutionary role by acting as a selective force causing prey organisms to adjust the magnitude of the fight-or-flight response to the perceived levels of risk and by reducing the severity of the physiological stress responses over time [3]. A next challenge will therefore be to link the predator-induced oxidative damage to fitness consequences in prey, thereby unravelling a novel pathway for non-consumptive effects of predation on prey population dynamics.

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Figure 2. Mean activity of the antioxidant enzymes SOD (a) and CAT (b), concentrations of the superoxide anion (c) and oxidative damage to lipids (MDA levels; d) of Enallagma cyathigerum larvae as a function of exposure to predation risk. Given are least-squares means ± 1 s.e.
References


