REVIEW

Mechanisms underlying the control of responses to predator odours in aquatic prey

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ABSTRACT

In aquatic systems, chemical cues are a major source of information through which animals are able to assess the current state of their environment to gain information about local predation risk. Prey use chemicals released by predators (including cues from a predator's diet) and other prey (such as alarm cues and disturbance cues) to mediate a range of behavioural, morphological and life-history antipredator defences. Despite the wealth of knowledge on the ecology of antipredator defences, we know surprisingly little about the physiological mechanisms that control the expression of these defensive traits. Here, we summarise the current literature on the mechanisms known to specifically mediate responses to predator odours, including dietary cues. Interestingly, these studies suggest that independent pathways may control predator-specific responses, highlighting the need for greater focus on predator-derived cues when looking at the mechanistic control of responses. Thus, we urge researchers to tease apart the effects of predator-specific cues (i.e. chemicals representing a predator's identity) from those of diet-mediated cues (i.e. chemicals released from a predator's diet), which are known to mediate different ecological endpoints. Finally, we suggest some key areas of research that would greatly benefit from a more mechanistic approach.

KEY WORDS: Antipredator adaptation, Physiology, Endocrine response, Neuroplasticity, Kairomones

Introduction

Almost all animals are exposed to predators that come and go over time scales ranging from minutes to months, or predators that are present for only certain life-history stages. The risk of being eaten is therefore anything but static, and understanding how prey deal with the variation in predation risk is a long-standing theme of ecological research (Lima and Bednekoff, 1999; Lima and Dill, 1990; Sih et al., 2000). Although not always the case, investing in defensive traits can reduce overall fitness in variable environments if the costs associated with developing and maintaining defensive traits exceed their benefits during periods of low risk (Auld et al., 2010; DeWitt, 1998; Lima, 1998). Consequently, many prey species have evolved plastic defences that can be turned on or off in response to environmental fluctuations (Relyea, 2002; Tollrian and Harvell, 1999; Weiss et al., 2012a). Some of these responses stem from an innate recognition of the cue (this is the case for chemical alarm cues, diet cues and some predator-specific odours; Box 1), whereas others require experience and learning for the response to be

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triggered (as for most predator-specific odours; Box 1). Some defensive traits, such as behavioural responses, are quickly expressed and highly plastic, allowing prey to respond to short- and long-term changes in predation risk. Others, such as morphology and life-history traits, generally take more time and energy to express and are not easily reversed. The benefits of such traits are thus dependent on the speed at which they can be expressed relative to both the onset and duration of risk (Steiner and Pfeiffer, 2007). Their expression is therefore limited by their underlying control mechanisms.

In aquatic systems, chemical cues are one of the primary means by which prey detect predators and assess predation risk (Bronmark and Hansson, 2012; Ferrari et al., 2010). Chemical cues can provide temporal and spatial information about predation risk, allowing prey to regulate the expression of inducible defences. These cues fall into two categories: those derived from predators (predator odours and dietary cues; Box 1) and those from conspecifics or prey guild members (chemical alarm cues and disturbance cues; Box 1). While this overview is limited to cues derived from predators, and will not cover specifically those derived from injured prey (but see Døving and Lastein, 2009, for a recent review in fishes), the distinction between the two is not always clear cut, as smells from predators may contain chemicals derived from ingested conspecific prey – in other words, digested alarm cues (often referred to as diet cues) (Chivers and Mirza, 2001; Ferrari et al., 2010). Predator-specific cues allow prey to recognise and respond using defences that are adaptive against that predator, while diet cues may provide a general indication of risk (Ferrari et al., 2010). However, with a few exceptions, we know very little about the underlying mechanisms that control phenotypically plastic responses to predator odours and dietary cues. Part of the issue is that the chemical identity or composition of these cues, be it predator odour, alarm cues or diet cues, is still unknown. In this Review, we briefly summarise how predator odours and diet cues change prey phenotypes and highlight what is currently known about the mechanisms that control responses to the different odours released by predators. We suggest that a mechanistic approach can bridge gaps between physiological and ecological research.

Effects of predator odours and diet cues on prey ecology

Prey display a range of responses to predator odours and diet cues, which include alterations in behaviour, morphology, physiology and life histories (Bronmark and Miner, 1992; Chivers and Mirza, 2001; Dawidowicz and Loose, 1992; Fonner and Woodley, 2015; Gazzola et al., 2015; Hazlett, 1999). The expression of these various responses is sensitive to the time scales over which they might be beneficial in relation to the costs of induction and maintenance (Ferrari et al., 2009; Relyea, 2002; Steiner and Van Buskirk, 2009). Acute exposure to predator odours results in prey displaying a range of short-term antipredator behaviours, such as reduced activity (Gazzola et al., 2015), reduced feeding rate (Foam et al., 2005),

> **Box 1. Terminology: a need for better integration across fields**
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> In reviewing the literature for this paper, it became apparent that some terms are used interchangeably to describe a range of different cues. Yet, these cues induce distinct behavioural and physiological responses in prey; thus, there is a need for clear definitions of specific cues. Below, we define the main cue types available for risk perception.
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> **Predator kairomone**
> Kairomones are infochemicals released by an organism, which, when detected by another organism, provide an adaptive benefit for the receiver but not the sender. Predators release at least two types of kairomones: predator odour and diet cues. Because of this ambiguity, we advise against the use of the term 'kairomone' unless it is clearly defined in the study.
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> **Predator odour**
> The cues released by a predator that are unique to the species and do not include cues released as a result of prey digestion. This is a crucial component of the definition, because we know that diet cues (see below) can mediate a wide array of antipredator responses, from behaviour to life history. Hence, the term 'predator odour' should only be used when the diet component is carefully removed or controlled for.
>
> **Diet cue**
> Any odours that are released as a by-product of the digestive process. Diet cues can have a range of effects on prey antipredator responses depending on their composition. In this manuscript, we specifically refer to diet cues as those that provide direct information about predation risk and that elicit antipredator responses when encountered, e.g. those released following the consumption of prey conspecifics. Conclusions based on different types of predators may be confounded by differences in diets between predator types: is it the nature of the predator driving the response, or is it a difference in predator diets?
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> **Chemical alarm cue (originally 'Schreckstoff')**
> Chemicals released by prey as a by-product of mechanical damage to their body, eliciting overt antipredator responses in other prey individuals (e.g. conspecifics, closely related species and heterospecific prey guild members).
>
> **Disturbance cues**
> Chemicals that are actively released by disturbed prey and not as a by-product of mechanical damage. These cues elicit antipredator responses when detected by conspecifics but, on their own and without additional cues from conspecifics or predators, do not appear to mediate as wide a range of antipredator responses as do chemical alarm cues.

increased hiding (Briones-Fourzán et al., 2008) and altered habitat use (Dawidowicz and Loose, 1992). The magnitude and duration of these behavioural defences reflect a trade-off against other fitness-enhancing activities, such as feeding, mating or guarding territories (Lima and Dill, 1990). Consequently, many prey display threat-sensitive responses to predator odours; that is, the intensity of the response correlates positively with the perceived level of risk based on the information contained in the chemical cues, such as the identity (Hawkins et al., 2007; Relyea, 2003a; Smith et al., 2008), size (Kusch et al., 2004) or – in some cases – density of predators (Ferrari et al., 2006; Relyea, 2003a). Furthermore, the concentration of predator odours indicates the spatial and/or temporal proximity of predators, allowing prey to further fine-tune their responses (Ferrari et al., 2006; Kats and Dill, 1998; van Buskirk and Arioli, 2002).

Upon prolonged exposure to predator odour, some species initiate a range of responses beyond behavioural changes. For instance, larval crabs exposed to predatory fish odours display increased visual sensitivity, thus enhancing their ability to detect predators (Charpentier and Cohen, 2015). Possibly the most striking responses to predator odours and diet cues are changes in morphology. Exposure to these odours can induce protective armaments, such as defensive spines, increased shell thickness or increased body depth, all of which reduce the chance of being attacked, caught or eaten by predators (Domenici et al., 2008; Engel et al., 2014; Laforsch et al., 2004; Middlemis Maher et al., 2013). Beyond morphological changes, exposure to predator odour can also alter the timing of major life-history transitions, such as egg hatching, ontogenetic switches and investment in reproduction (Ferrari et al., 2010; Lass and Spaak, 2003). Such responses can be specific to different predators and differ between populations (Templeton and Shriner, 2004; Wilson et al., 2005; Lakowitz et al., 2008; Orr et al., 2009). Because of the cost/benefit trade-off of developing, maintaining and using these traits, their onset may be limited to certain life-history periods and can be permanent or plastic in response to variation in predation levels (DeWitt, 1998). Additionally, individuals may demonstrate trait compensation, reducing other defences (such as behavioural responses to predators) following the development of morphological traits (Bourdeau et al., 2015). Below, we describe two systems that highlight the effects of predator odours on the biology of their prey.

Case study: frog larvae
Frog larvae, particularly wood frog tadpoles *Lithobates sylvaticus*, have become one of the main models for understanding the behavioural, cognitive, morphological and life-history responses to predator odours and diet cues. Like many prey, predator-naive larval amphibians do not innately respond to predator odours, and thus need to learn to recognise the odours of their predators in order to respond to them (Chivers et al., 2015; Gonzalo et al., 2009). As a result, behavioural and physiological responses to predators are often, but not always (see DeSantis et al., 2013; Orr et al., 2009), mediated by an individual's experience with that predator. Yet, predator-naive tadpoles can instantly recognise novel predators if these predators are releasing diet cues containing consumed conspecifics (van Buskirk and Arioli, 2002). In fact, some studies have shown that both predator odours and diet cues may be required to elicit full antipredator responses (Schoeppner and Relyea, 2005, 2009).

Chronic exposure to predators (3–18 days) results in tadpoles developing morphologies that are adapted to different predator groups, namely ambush invertebrate predators, such as larval dragonflies and beetles, or active pursuit predators, such as fish (Relyea, 2003b) (Fig. 1). Exposure to invertebrate predators results in tadpoles delaying hatching from eggs (Ireland et al., 2007), reducing growth rates and developing deeper, shorter tails for increased escape speeds (Middlemis Maher et al., 2013; Relyea, 2001b; Wilson and Lefort, 1993). Tadpoles exposed to fish predators develop similar morphological changes, but also develop larger tail muscles (Relyea, 2001a; Teplitsky et al., 2005). A consequence of these adaptive changes in morphology is a longer developmental time and delayed metamorphosis (Relyea, 2001b; Relyea and Auld, 2004). Some of these morphological responses may be reversible; in the absence of odours from predators, some tadpoles lose their adapted morphological phenotypes (Relyea, 2003b). Highlighting the complexity of cue-mediated effects, Van Buskirk and Arioli (2002) reported that behavioural responses of tadpoles were sensitive to the amount of conspecifics consumed by predators, whereas the expression of morphological traits was determined by the number of predators detected, regardless of the amount of prey eaten by these predators.

Case study: *Daphnia*
Possibly the most well-studied prey system for responses to predator odour are water fleas (*Daphnia* spp.), for which the responses to fish
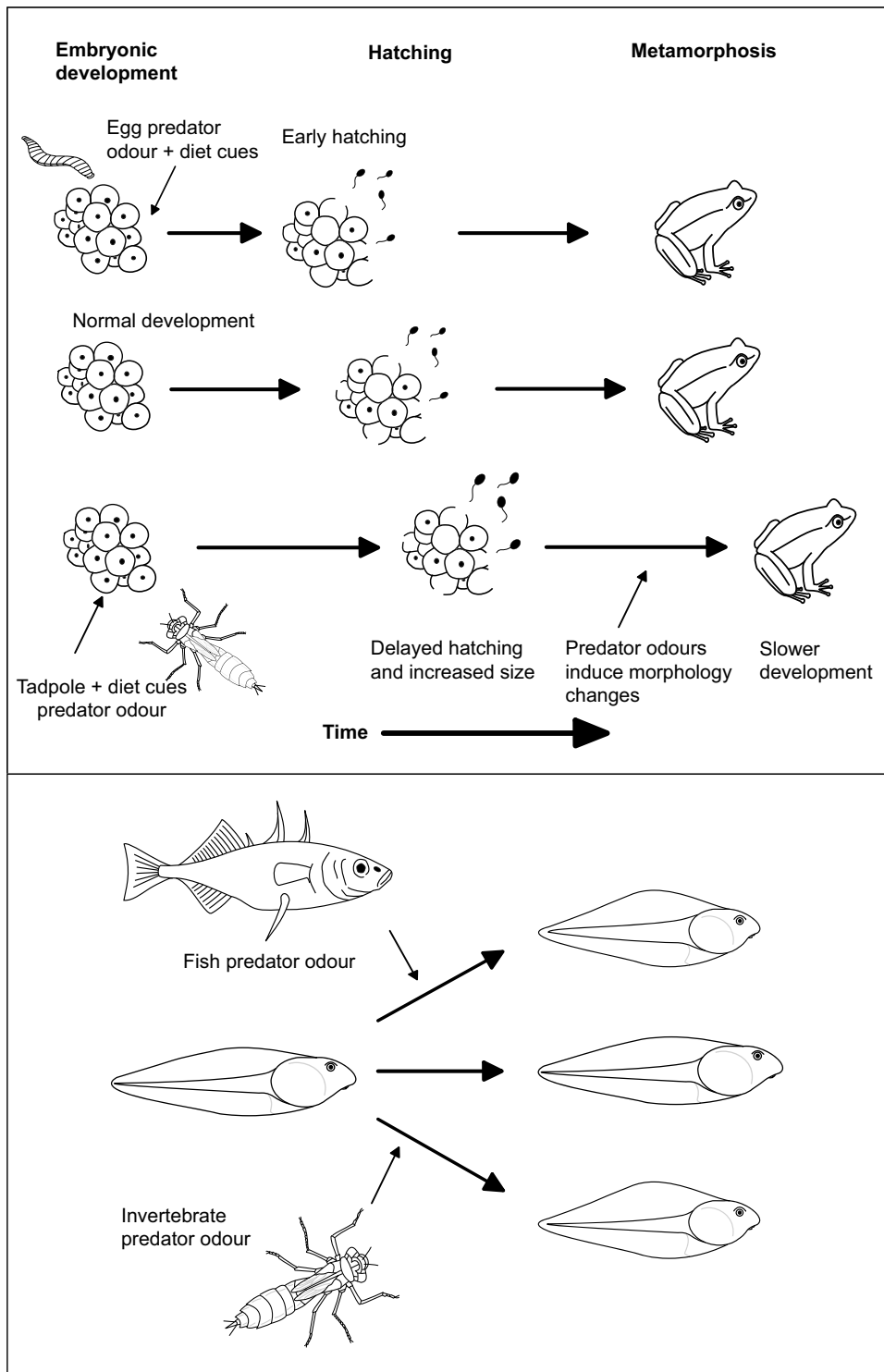
Fig. 1. The effects of exposure to predator odours and diet cues on the timing of life-history transitions and morphology of juvenile anurans. The top panel shows that juveniles exposed to predator odours as embryos alter the timing of hatching and their size at hatching; egg predators induce early hatching while tadpole predators induce delayed hatching (Chivers et al., 2001; Ireland et al., 2007). Furthermore, exposure to odours from predators fed conspecifics increases developmental time as a result of investment in morphological defences and reduced gut length (Relyea and Auld, 2004). The bottom panel shows the changes in morphology that result from exposure to predators as tadpoles. Invertebrate predators (e.g. *Anax* spp.) induce smaller bodies with relatively larger and deeper tails that increase escape speeds (Middlemis Maher et al., 2013; Relyea, 2001a), while fish predators induce deeper tail muscles as well as smaller bodies and larger tails (Relyea, 2001a; Teplitsky et al., 2005).

and invertebrate predators have been investigated. Exposure to the odours of predators fed *Daphnia* (the presence of diet cues derived from digested *Daphnia* seems to be crucial for recognition, e.g. Stabell et al., 2003) results in predator-specific polyphenism for behavioural, life-history and morphological responses. For instance, in order to avoid predation, *Daphnia* undergo a diel vertical migration in response to fish and diel horizontal migrations in response to phantom midge larvae (*Chaoborus* spp.) (Dawidowicz and Loose, 1992; Loose et al., 1993; Tollrian and Harvell, 1999; Wojtal-Frankiewicz et al., 2010). In addition, *Daphnia* exposed to

predatory fish cues undergo a life-history shift, reallocating energy from somatic growth to reproduction. These changes mean that *Daphnia* reproduce at a smaller size and produce greater numbers of smaller eggs, thus reducing the effects of predators that select for larger prey (Lass and Spaak, 2003; Tollrian and Harvell, 1999). In contrast, *Daphnia* exposed to phantom midge larvae odours reallocate resources from reproduction to somatic growth and induce morphological defences including neck teeth, helmets and strengthened carapaces in order to escape these gape-limited predators (Laforsch et al., 2004; Tollrian and Harvell, 1999).

Understanding how predator odours and diet cues can mediate such a wide array of responses is a challenge that few researchers have tackled. In particular, a clear mechanistic understanding of prey responses to predator odours and diet cues is lacking. Below, we discuss the mechanisms of detection and neurosignalling, neuroplasticity and cognition, endocrine responses and genetic responses involved in prey responses to the different cues released by predators.

Mechanisms underlying prey responses to predator odours and diet cues

The physiological processes that control responses to general olfactory cues have been well studied across multiple systems and taxa (Bell, 2009; Dennis et al., 2014; Døving and Lastein, 2009; Firestein, 2001; Hegab and Wei, 2014). However, for responses to cues related to predation, much of the work is limited to a few terrestrial organisms, such as the responses of rats to predatory cat urine (Hegab and Wei, 2014). The response cascade initiated by exposure to predator odours and diet cues generally involves the detection of cues, which initiates neuronal and endocrine signalling, changes in gene expression and synaptic sensitisation, and, ultimately, changes in phenotypes associated with antipredator defences (Fig. 2). Below, we summarise what is currently known about the mechanisms that control the responses of aquatic prey to predator odours and diet cues.

Odour detection and neurosignalling pathways

There is a distinct paucity of information regarding the sensory and neurological pathways through which prey detect and process predator odours and diet cues, particularly when compared with studies on sex pheromone and feeding cue reception. This is partly a reflection of the fact that the chemical composition of predator odours and alarm cues is currently unknown. The main sensory pathways for the detection of chemicals present in the aqueous medium are olfaction and gustation, although chemicals that are indicative of risk appear to be mostly processed though olfactory pathways (Atema, 1977; Døving and Lastein, 2009; Shabani et al., 2006).

In vertebrates, the basic organisation of the olfactory system is conserved (Derby and Sorensen, 2008; Firestein, 2001; Moreno et al., 2008). In fish, there are three types of olfactory receptor neurones (ORNs) – ciliated, microvillar and crypt cells – which are clustered into rosettes in the olfactory epithelium. These different ORNs project to glomeruli located in specific regions within the olfactory bulb, resulting in glomeruli with similar chemosensitivity being located near each other (Derby and Sorensen, 2008; Døving and Lastein, 2009). Chemical information is then passed from the olfactory bulb via mitral cells to the forebrain, where higher-order processing of the olfactory information occurs. The role of the olfactory pathway in processing predator odours is further supported by the fact that prolonged exposure to predator odours alters the activity of mitral cells and their sensitivity to predator odours in *Rana dalmatina* tadpoles (Gazzola et al., 2015). The different types of ORNs are sensitive to different odour classes, meaning food odours, pheromones and alarm cues are processed along predominantly separate pathways (Derby and Sorensen, 2008; Døving and Lastein, 2009; Hamdani and Døving, 2003). The alarm cue pathway consists of ciliated cells which project into the dorsomedial region of the olfactory bulb and activate mitral cells that have axons within the medial region of the olfactory tract. Diet cues are thought to be digested derivatives of chemical alarm cues (Ferrari et al., 2007a) and thus may be processed via alarm cue-sensitive ORNs, while predator odours are likely to involve food odour-sensitive ORNs (Derby and Sorensen, 2008). However, the high interconnectivity between glomeruli should allow integration of complex information from predator odours and diet cues prior to processing in higher regions of the brain (Derby and Sorensen, 2008).

Unlike those of vertebrates, chemosensory systems are diverse across aquatic invertebrates. In crustaceans, the antennular chemosensory pathway, consisting of the aesthetasc sensilla and
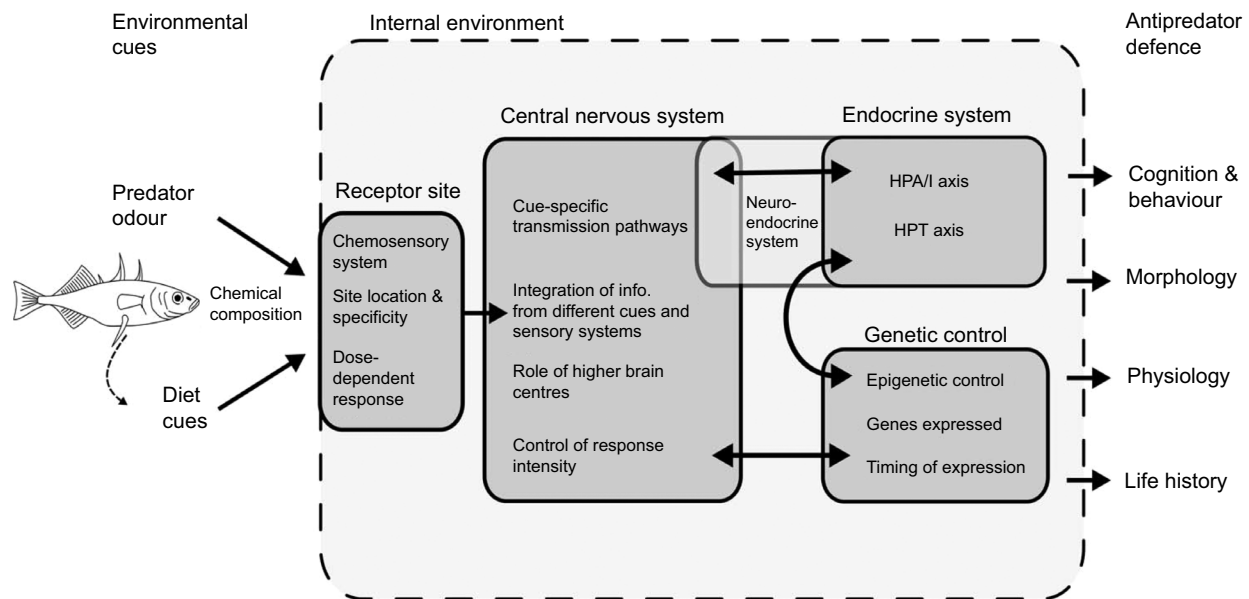
Fig. 2. Conceptual overview of the physiological pathways and systems involved in controlling prey responses to predator odours and diet cues. Within each box are general topics that should prove fruitful in furthering our understanding of the proximate mechanisms controlling antipredator responses in aquatic prey. These topics are based on the findings discussed in the main text and also on papers looking at mechanistic responses to different predation cues, developmental pathways and neurobiology (Denver, 2009; Derby and Sorensen, 2008; Firestein, 2001; Hamdani and Døving, 2003; Hegab and Wei, 2014; Li, 2014). HPA/I axis, hypothalamic-pituitary–adrenal/interrenal axis; HPT axis, hypothalamic-pituitary–thyroid axis.

olfactory lobe, functions as the olfactory pathway (Derby and Sorensen, 2008) and processes information from predator odours in spiny lobsters, *Panulirus argus* (Shabani et al., 2006). In *Daphnia longicephala*, predator odours are detected by sensory systems located on the first antennae (Weiss et al., 2015a), which connect to the deutocerebrum in the central nervous system, where olfactory cues are processed (Weiss et al., 2012c). Similarly, predator odours are detected by receptors located on the rhinophores of the nudibranch *Tritonia diomedea* (Wyeth, 2006), which form part of the system analogous to olfaction (Di Cosmo and Winlow, 2014). In the snail *Lymnaea stagnalis*, predator odours are detected by the osphradium, an externally located chemosensory organ, which is innervated by the osphradial nerve, a small nerve branching off the internal pallial nerve (Bullock and Horridge, 1965; Il-Han et al., 2010; Karnik et al., 2012). This pathway allows for information about predators to be transferred to the central nervous system and affects long-term memory (LTM) and behaviour.

Neuroplasticity and cognition

Exposure to predator odours has been shown to alter various cognitive traits relating to behaviour. For example, exposure to predator odour can enhance learning and the formation of LTM or can result in individuals becoming neophobic (Brown et al., 2013; Mitchell et al., 2016a; Orr et al., 2007). However, while exposure to predation risk can enhance cognitive traits relating to predator recognition, it may impair other cognitive functions such as spatial learning (Brown and Braithwaite, 2005), suggesting that there is a trade-off which might result from changes to the structure and functioning of the brain. A few studies described below have examined how exposure to predator odours and diet cues affect neurological structures, but generally did not demonstrate how these changes relate to cognitive function.

Woodley et al. (2015) demonstrated that anuran larvae show significant changes in brain structure following exposure to predators and their diet cues. Leopard frog tadpoles, *Lithobates pipiens*, exposed to predator odours and diet cues, developed brains that were narrower and shorter in several dimensions compared with those of non-predator controls. However, these changes did not persist beyond metamorphosis (Woodley et al., 2015). Similarly, nine-spined stickleback, *Pungitius pungitius*, developed larger bulbus olfactorius (chemosensory centre) regions in the brain when exposed to the odours of predatory perch (Gonda et al., 2012). Unfortunately, neither study explored whether these changes directly modified cognition or behaviour. Gazzola et al. (2015), working on *R. dalmatina* tadpoles, found that baseline activity of mitral neurons and their sensitivity to predator odours increased following prenatal exposure to predator odours. In addition to these changes in neuronal activity, tadpoles changed their baseline activity and overall activity levels following exposure to predator odours. However, the differences in neuronal activity persisted for longer than the behavioural changes, suggesting that more work is needed to understand the relationship between neuronal activity and behaviour.

A direct link between neurological function and changes in cognitive traits resulting from exposure to predator odours has been demonstrated in *L. stagnalis* snails. In juvenile snails, exposure to crayfish odour enhances their ability to form LTM following operant conditioning of aerial respiration. Exactly how LTM acts as an antipredator defence is unclear (Forest et al., 2016; Lukowiak et al., 2014; Orr et al., 2010). RPeD1 is a key neuron that controls rhythmic neuronal activity within the central pattern generator (region controlling aerial respiration), and a decrease in its

excitability is required for the formation of LTM. RPeD1 is more excitable in juveniles than in adults, and adults are able to regulate RPeD1 excitability and the central pattern generator via synaptic connections with the peripheral pneumostoma area; these connections do not have the same suppressive input in juveniles. Exposure to predator odours changes the excitability of RPeD1, allowing the formation of LTM more easily than in the absence of predator odours (Karnik et al., 2012; Orr et al., 2010).

Endocrine responses

Hormones play a central role in mediating predation risk effects on organisms, and they influence the behaviour and physiology of prey (Denver, 2009). In both vertebrates and invertebrates, hormones that control growth and metamorphosis mediate many responses to predator odours and diet cues (Dennis et al., 2014; Denver, 2009). For instance, ecdysteroids (insect moulting and sex hormones) and juvenile hormones are highly conserved in arthropods, and together control the timing of moulting and sexual development (Dennis et al., 2014).

Both predator odours and diet cues induce stress in prey animals; hence, work has generally focused on hormones that mediate stress responses. For vertebrates, these are hormones such as glucocorticoids, which are primarily associated with the sympathetic nervous system and the hypothalamic-pituitary–adrenal (HPA) axis [or the hypothalamic-pituitary–interrenal (HPI) axis for fishes] (Barton, 2002; Denver, 2009; Rehnberg and Schreck, 1987; Ricciardella et al., 2010). The few studies examining vertebrate prey responses to predator odours and diet cues have focused on the HPA axis and associated glucocorticoids (Denver, 2009; Fonner and Woodley, 2015; Middlemis Maher et al., 2013; Sunardi et al., 2007). Exposure to predator odours normally results in the release of glucocorticoids and an increased metabolic rate that readies prey for the fight-or-flight response. However, this is not always the case, as predator odours do not always activate the HPA axis (Fonner and Woodley, 2015). Time-sensitive activation of the HPA axis controls antipredator responses in *L. sylvaticus* tadpoles. Upon initial exposure to predator odours, tadpoles suppress HPA axis activity, which reduces corticosterone levels. The lower corticosterone levels reduce tadpole activity and, hence, the chances of detection by predators (Fraker et al., 2009; Middlemis Maher et al., 2013). Yet, extended periods of exposure to predator odours lead to an increase in corticosterone levels and activation of the HPA axis, which interacts with the development of antipredator morphological phenotypes in response to predators (Denver, 2009; Middlemis Maher et al., 2013). Furthermore, tadpoles exposed to corticosterone in the absence of predator odours developed the tail morphology of predator-exposed tadpoles, while tadpoles exposed to predator odours but treated with a corticosteroid biosynthesis inhibitor failed to develop the antipredator morphology (Middlemis Maher et al., 2013). Although not directly tested in the context of responses to predator odours or diet cues, the hypothalamic-pituitary–thyroid axis has been shown to work alongside the HPA axis to regulate metamorphosis, and thus may influence the timing of prey life-history transitions in response to predator odours (Denver, 2009).

For invertebrates such as pond snails, crayfish and the sea hare, serotonin appears to play a key role in the stress response and anxiety-like behaviour. For *L. stagnalis*, predator odours detected in the osphradium are transferred to the central pattern generator via serotonergic pathways. When serotonin reception is blocked, individuals fail to respond to predator odours (Il-Han et al., 2010). Similarly, increased serotonin in crayfish (*Procambarus*

clarkii) and sea hares (*Aplysia californica*) has been found to lead to increased responses and sensitivity to acute stressors such as an electrical shock (Fossat et al., 2015; Marinesco and Carew, 2002).

Genetic responses

Identifying the genes that control phenotypic plasticity – along with the control mechanisms (e.g. changes in gene expression versus genetic polymorphism) – is crucial to understanding the genetic basis of plasticity (Auld et al., 2010; DeWitt, 1998). Despite the difficulties associated with identifying the genes involved in plasticity (Bell, 2009; Edmunds et al., 2016), some progress has been made in identifying specific genes that control the expression of antipredator traits. Again, much work on identifying genes that control predator odour-induced plasticity has involved *Daphnia pulex*, in which the eco-responsive genome has been sequenced (Colbourne et al., 2011). In the related species *Daphnia magna*, exposure to fish predator odour results in the upregulation of *cyclophilin* (Dappu-92663), a gene involved in protein folding, while exposure to an invertebrate predator leads to downregulation of the same gene (Schwarzenberger et al., 2009). While this differential expression may reflect different life-history responses to different predators, it is unclear how *cyclophilin* mediates the expression of these different traits.

Hokkaido salamander larvae (*Hynobius retardatus*) express five times the number of genes when inducing a defensive (antipredator) morphology following exposure to odours and diet cues from a predator (*Aeshna nigroflava*) than when inducing a predatory (foraging) morphology following exposure to food cues from the prey, *Rana pirica*; the difference in gene expression probably reflects the heavier investment required to express the defensive compared with the predatory morphotype (Matsunami et al., 2015). In both cases, genes related to responses to reactive oxygen species were upregulated to account for the increased metabolic demand of morphogenesis. Clearly, numerous genes are involved in controlling antipredator responses and there is a long way to go before we really understand the genetic basis of antipredator defences.

Understanding the mechanisms of predator-specific prey responses: insights from *Daphnia*

Daphnia have long been a model system for understanding the indirect ecological effects of predators on prey, and are known to display distinct morphological, behavioural and life-history responses to invertebrate versus fish predators. To complement this, there is now a growing body of work examining the mechanisms controlling these predator-specific responses (Fig. 3). Following detection, predator-specific signals are transferred via independent neurosignalling pathways. Responses of *D. pulex* to fish predators are mediated via the suppression of the inhibitory GABAergic pathways, resulting in the release of ecdysteroids (Dennis et al., 2014; Weiss et al., 2012b, 2015b). Ecdysteroids promote the reallocation of energy from somatic growth to reproduction, which results in the life-history changes described above. Responses to invertebrate predators are controlled by cholinergic pathways (Barry, 2002; Miyakawa et al., 2013; Weiss et al., 2012b). Blocking these pathways using physostigmine (an acetylcholine inhibitor) prevents the expression of defensive traits, but appropriate stimulation of these pathways alone does not induce the relevant defences, suggesting there are additional intermediate steps involved in the expression pathway (Weiss et al., 2012b). One potential candidate is ionotropic glutamate receptors (which are involved in neurotransmission), as exposure to *Chaoborus* odours

results in the upregulation of genes coding for the production of these receptors (Miyakawa et al., 2015).

The cholinergic system controls the release of dopamine and juvenile hormones, which themselves control the expression of inducible defences in response to invertebrate predators (Dennis et al., 2014; Miyakawa et al., 2013; Weiss et al., 2015b). Dopamine is stored and released from polyploid cells surrounding the regions where inducible defence structures develop. Exposure to *Chaoborus* odours results in the upregulation of the genes encoding the dopamine-synthesising enzyme dopamine decarboxylase and the juvenile hormone pathway-related genes *JHAMT* and *Met* (Miyakawa et al., 2010; Weiss et al., 2015b). The release of dopamine causes increased sclerotisation that strengthens the carapace (Laforsch et al., 2004). Additionally, dopamine also acts as a proliferation agent either alone or in conjunction with juvenile hormones to stimulate the development of neck teeth (Dennis et al., 2014; Miyakawa et al., 2013; Weiss et al., 2015b). Mirroring these morphological changes is a significant up-regulation of genes coding for cuticle-associated proteins required to produce neck teeth and the strengthening of the carapace, along with an up-regulation of the genes involved in chromatin restructuring and cell cyclin – genes likely to be involved in cell proliferation at the site where neck teeth form (Rozenberg et al., 2015). Finally, the interactive effect of dopamine and juvenile hormones increases overall body size and delays reproduction (Weiss et al., 2015b). Interestingly, increasing concentrations of *Chaoborus* odour result in a dose-dependent expression of genes involved in the juvenoid hormone pathway, which in turn mediates the threat-sensitive expression of morphological defences and, thus, predator-induced plasticity in prey (Dennis et al., 2014).

Which cues are responsible?

There is a need to clearly define which cues are being investigated when it comes to understanding the mechanisms that control antipredator responses. Many of the studies reviewed here exposed prey to predator odours and diet cues simultaneously, and yet antipredator responses of prey to predator odours can differ when diet cues are present or absent (Mitchell et al., 2015, 2016b; Schoeppner and Relyea, 2005, 2009; Stabell et al., 2003). While there are a growing number of studies showing innate predator recognition (Dalesman et al., 2006; DeSantis et al., 2013; Langerhans and DeWitt, 2002; Vilhunen and Hirvonen, 2003), many studies have shown that, when diet cues are controlled for, predator-naive prey do not display antipredator responses to predator odours. Rather, prey must learn to recognise predator odours in order for an antipredator response, be it behavioural or physiological, to be observable (Chivers and Mirza, 2001; Ferrari et al., 2010; Kelley and Magurran, 2003; Stabell et al., 2003). This would suggest that chemical alarm cues and their diet cue derivatives might, in many cases, be the primary cues responsible for inducing defences in prey.

Investigating the pathways through which these predator odours and diet cues are processed and how they interact to control the expression of antipredator traits is further complicated by the fact that we currently have little knowledge about the chemical composition of these different cues. While the analytical tools to isolate and identify these chemicals are available, the cues rapidly degrade and are generally found at low concentrations. Thus, isolating these chemicals from their chemical milieu is problematic (Pohnert, 2012). Furthermore, the cues used by different prey to assess risk are likely to be species specific, dependent on the
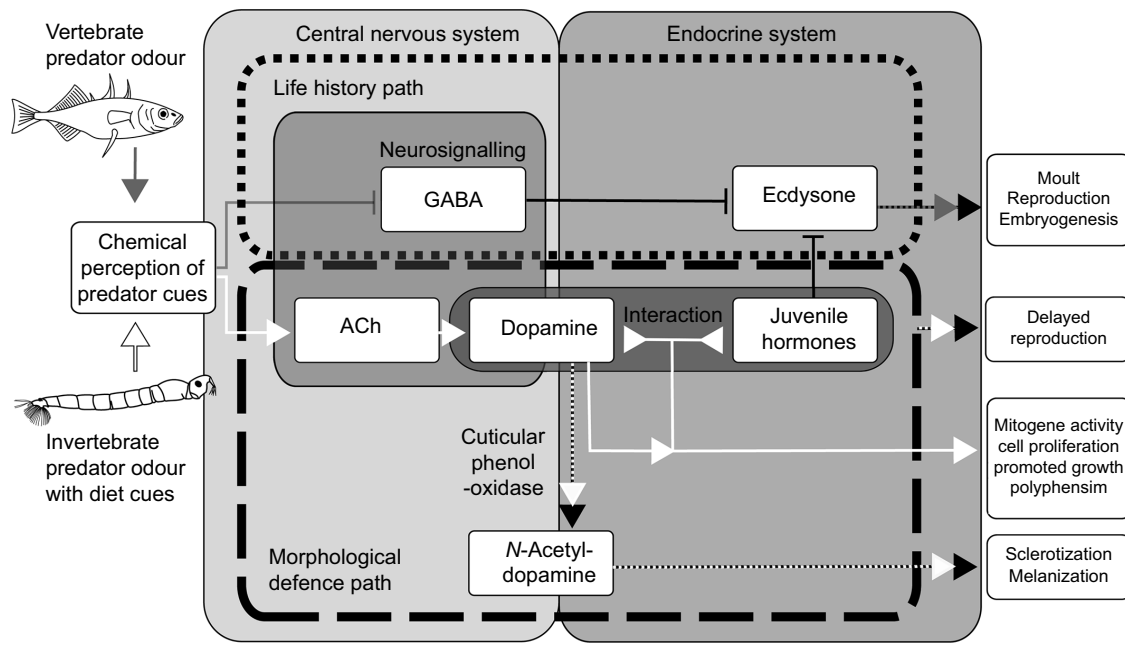
Fig. 3. Conceptual model of physiological mechanisms that control the expression of defensive traits in *Daphnia*. Based on the model of Weiss et al. (2015b). The network includes chemical perception of predator odours and diet cues, changes in neural signalling within the central nervous system and neurochemical changes. Two predator-specific pathways are illustrated: grey arrows indicate the pathway activated in response to predatory fish odours that result in changes to life history, while white arrows indicate the pathway activated in response to predatory invertebrate odours and diet cues that result in the development of morphological defences. Black arrows indicate pathways that are normally active in the absence of predator odours or diet cues.

species' historical interactions with predators, and can differ from one prey species to the next, or even between populations (Ferrari et al., 2007b; Orr et al., 2009). For instance, conspecific alarm cues, i.e. the cues released from injured conspecifics, elicit an innate antipredator response in all aquatic species tested to date, from corals to amphibians. Some phylogenetic relationship exists, with closely related congeneric species responding to each other's alarm cues, albeit with a lower intensity of response (Dalesman et al., 2007; Mirza and Chivers, 2001; Mitchell et al., 2012). Yet, distantly related species do not respond to each other's alarm cues, indicating that the chemical mixture that elicits the optimal response is unique for each species.

Controlling for diet cues represents a useful first step to understanding how predator odours and diet cues control antipredator responses. To distinguish between the effects of predator odour and diet cues, predator odour collections require a diet-control step that ensures that chemicals that might elicit an innate response, such as digested alarm cues, are removed. This involves the predator being fed distantly related and sometimes sympatric heterospecific prey, whose alarm cues do not elicit an antipredator response from the test subject (Ferrari et al., 2010), for a few days prior to odour collection. This step is sometimes followed by a non-feeding period to allow for complete gut evacuation. However, it should also be noted that in natural environments, diet cues cannot be isolated from predator odours and, thus, experimenters must carefully control for predator diet when the experiment directly examines the effects of predator odour. While the combination of both predator odour and diet cues may allow for a maximal response from an ecological viewpoint, the distinction between the two may highlight different pathways and modes of action of predator odour and diet cues, the latter often eliciting an innate response, while the former needs to be learned prior to observing a response for those species lacking innate responses. Hence, caution should be taken when designing experiments, and

the inclusion of either or both cues should depend on the objectives of the study.

Conclusions and future directions
Over the last few decades, we have developed a detailed understanding of how chemical cues relating to predation induce antipredator responses, mediate both consumptive and non-consumptive effects of predation and ultimately shape communities. Yet, the field is only just starting to investigate the mechanisms underlying responses to olfactory cues from predators. As discussed, surprisingly little is known about the physiological pathways through which prey develop antipredator responses to predator odours and their associated diet cues, with the majority of work so far focusing on anurans and crustaceans. What is clear is that predator odours and diet cues have a significant effect on all physiological stages relating to the detection, processing and expression of antipredator defences. This raises interesting questions about how adaptation at different physiological locations might limit the plasticity of antipredator responses, how prey respond to fluctuations in risk and the evolutionary origins of plastic antipredator responses. Such questions might be addressed through comparative studies using one of the many systems where populations have developed different antipredator responses to their local predator community.

While this overview is limited to work focusing on predator odours and diet cues specifically, several studies have investigated the mechanisms responsible for antipredator responses induced by alarm cues from conspecifics or visual cues of predators. While the early sensory-detection sequences might differ, there is likely to be much overlap in the downstream mechanisms of antipredator responses. Teasing apart the relative contributions of different predator-derived odours and more general alarm cues to prey response mechanisms may be an important area of future research. Knowledge of how different cues contribute to the expression of

antipredator traits would also be of use when investigating innate versus learned responses and their ecological origins.

Another fruitful avenue of investigation will be to understand the mechanisms that control threat-sensitive responses in prey and how long-term exposure to risk might change feedback systems that alter sensitivity to predation cues. Taking a mechanistic approach might provide researchers with a way to quantitatively compare the effects of all predation cues across different sensory systems (visual versus chemical versus mechanical) and, more importantly, could demonstrate how prey integrate the different sensory inputs. A recent study demonstrated that, in zebrafish, axons originating from the olfactory bulb innervate the retina and increase luminance sensitivity, suggesting that the interaction between these two sensory systems might influence reproductive behaviour by increasing sensitivity to visual courtship coloration in potential mates (Dowling, 2013; Esposti et al., 2013). It is not hard to imagine that such interactions between sensory systems would be adaptive upon the detection by prey of visual and chemical cues from predators. Indeed, prey are known to integrate information from different sensory systems when assessing risk (Mikheev et al., 2006; Ward and Mehner, 2010) and to compensate for the loss of information from one sensory system with another when assessing risk (Leahy et al., 2011).

A mechanistic understanding of prey responses to predators should also enhance our capacity to predict how animals will respond to human-altered environments, such as those resulting from ocean acidification and pollutants. Knowledge of the physiological processes involved in antipredator defences will allow researchers to assess how different stressors or contaminants interfere with trait expression and determine how different species will be affected. For example, the predicted increase in ocean acidification has been shown to interfere with olfaction in coral reef fishes and prevents fish from detecting predator odours (Ferrari et al., 2011; Munday et al., 2009). This inhibition of olfaction is attributed to the alteration of the neurotransmitter function of $GABA_A$ receptors in the brain (Nilsson et al., 2012). Given the conserved nature of the olfactory system, plasticity occurring at the same locations across animals may result in many vertebrate species in marine systems suffering a similar fate. However, there is a surprisingly high variation in the effect of ocean acidification among closely related species (Ferrari et al., 2011), indicating that recognising the $GABA_A$ involvement is only the first step towards understanding the complex effect of such stressors on prey risk assessment. There is an abundance of information on the structure and function of the olfactory system (Derby and Sorensen, 2008; Dowling, 2013; Firestein, 2001; Hamdani and Døving, 2003; Li, 2014) and the neuroendocrine system of aquatic organisms (Dennis et al., 2014; Døving and Lastein, 2009; Dufty et al., 2002). In addition, much is known about the way predator odours are processed in terrestrial animals (Hegab and Wei, 2014; Li, 2014). Consequently, a thorough understanding of how predator odours and diet cues are detected and processed by aquatic species could be readily obtained in the near future.

In summary, while there has been some impressive progress made for a few species, we still have a long way to go before there is a comprehensive understanding of the mechanisms that control antipredator responses. Yet, this represents an exciting opportunity to increase our understanding of how antipredator traits develop. Through greater integration of ecological studies with the fields of physiology, neurobiology and genetics, we will not only be able to identify the mechanisms underlying the expression of antipredator traits but also be able to answer key

questions about their evolution and the trade-offs associated with phenotypic plasticity.

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