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Eat or be eaten? Modifications of *Aplysia californica* feeding behaviour in response to natural aversive stimuli

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Prey often modify their behaviours to diminish predation risk, but the persistence of such behavioural changes are not well understood. We investigated the effects of predation risk in the aquatic mollusc *Aplysia californica* to determine whether various natural aversive stimuli alter the expression of feeding behaviour (i.e. bites) over short-term (15 min), intermediate-term (2 h) and long-term (24 h) periods. *Aplysia* were presented with a variety of natural aversive stimuli that are indicative of predation risk, including exposure to the spiny lobster *Panulirus interruptus* (a natural predator), the blue crab *Callinectes sapidus* (a novel predator), and the defensive secretions ink and opaline that are normally released by *Aplysia* when attacked. Feeding was significantly suppressed immediately after *Aplysia* were exposed to ink but not to opaline. Additionally, several stimuli, including exposure to lobsters, ink, opaline and attacks from crabs, suppressed feeding, but only for a short time (i.e. 15 min) after the end of the stimulus. No single-exposure treatment elicited intermediate (2 h) or long-term (24 h) changes in feeding, and multiple exposures to ink did not induce long-term feeding suppression. Thus, changes in feeding behaviour in response to predation risk cues appear short-lived in *Aplysia*, unlike feeding suppression after electric shocks that suppress biting behaviour over longer periods. Since *Aplysia* possess chemical defences to defend against consumers, it is perhaps unnecessary or too costly to maintain feeding suppression after risk subsides.

By altering prey behaviours, habitat selection or foraging patterns, predators can have significant effects on entire food webs. Several recent studies highlight the ecological importance of sublethal effects (i.e. nonlethal or nonconsumptive effects) in structuring communities and influencing their function, stability and biodiversity (Orrock et al., 2008; Peckarsky et al., 2008; Preisser, Bolnick, & Benard, 2005; Weissburg, Smee, & Ferner, 2014; Werner & Peacor, 2003). Such effects, manifesting as adaptive changes in behaviour, morphology, physiology or life history in response to predation risk (Clinchy et al., 2010; Schoeppner & Relyea, 2005, 2009; Sheriff, Krebs, & Boonstra, 2009), are effective antipredatory strategies and increase survival (Flynn & Smee, 2010; Robinson, Lunt, Marshall, & Smee, 2014; Smee & Weissburg, 2006).

Although beneficial, these defences are energetically costly (Adolphs, 2013; Kavaliers & Choleris, 2001; Lima, 1998a, 1998b; Lima & Bednekoff, 1999), and organisms often limit their expression to scenarios likely to pose imminent risk of injury or death (Clinchy et al., 2010). To optimally trade off the benefits and costs of antipredation behaviours, prey require reliable information about the presence and intentions of potential consumers. To date, most studies on sublethal effects focus upon changes in prey behaviour occurring during or immediately following exposure to predators or other cues indicative or predation risk, including injured conspecifics (Kats & Dill, 1998), alarm cues (Kicklighter, Germann, Kamio, & Derby, 2007) and predator encounters (Frost, Brandon, & Mongeluzi, 1998). Despite such efforts, it is unclear how long behavioural sublethal effects persist after the exposure to risky stimuli ceases (Adamec & Shallow, 1993; Figueiredo, Bodie, Tauchi, Dolgas, & Herman, 2003).

Predation risk is often variable in time and space, and organisms may vary reactions to it depending upon the frequency and intensity of exposure to cues indicative of predation risk (Lima & Bednekoff, 1999). The risk allocation hypothesis (Lima & Bednekoff, 1999) suggests that intense and infrequent risk detection will cause the greatest displays of predator avoidance strategies. Yet, the predictability of risk may also affect the likelihood and intensity of reactions by prey (Ferrari, Brown, Bortolotti, & Chivers, 2010; Ferrari, Rive,

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MacNaughton, Brown, & Chivers, 2008). For example, in situations where risk is unpredictable, heightened sensitivity to any cues indicative of risk would be prudent (Ferrari et al., 2008), while in conditions where risk is minimal, responding to it would incur unnecessary costs and foraging should be favoured over risk detection and response (Ferrari et al., 2008, 2010).

Learning and memory are mechanisms by which prey could vary response frequency and intensity. For example, wood frogs that experience heightened levels of risk retain longer memories of and subsequent reactions to predators than do those in uncertain or low-risk environments (Ferrari et al., 2010). Yet, few studies have assessed the latency of changes in response to predation risk, which have important consequences for costs to prey and the propagation of nonlethal predator effects across trophic levels (Weissburg et al., 2014; Werner & Peacor, 2003).

Costs and benefits of behavioural responses to risk can be enhanced through learning and memory (e.g. Ferrari et al., 2010). Different forms of learning and memory can influence future behaviour through the storage of information, such as the memory of a risky event. However, such forms of learning and memory are distinct and differ by the type, intensity and time frame of delivery of a stimulus as well as environmental conditions and physiological status of the prey (e.g. Lima & Dill, 1990; Mita et al., 2014; Orr, Hittel, Lukowiak, Han, & Lukowiak, 2009; Weissburg et al., 2014). Learning may be associative or nonassociative. In associative forms of learning, animals learn the association either between two stimuli (classical conditioning) or between a behaviour and a stimulus (operant conditioning), whereas in nonassociative forms of learning, behavioural changes occur in response to a single stimulus (habituation) or to two stimuli not temporally related (sensitization; Mozzachiodi & Byrne, 2010).

Sensitization may help elucidate the persistence of behavioural sublethal effects through time. Sensitization occurs when animals amplify defensive responses to a mild stimulus following exposure to an aversive event (Byrne & Hawkins, 2015; Fioravante, Antzoulatos, & Byrne, 2008). This form of nonassociative learning may have significant effects on the latency of initial reactions to predation risk as well as on the frequency and intensity of future risk responses. In the context of sensitization, the duration of memory storage depends on the number of aversive events and their intensity or duration. As noxious events intensify and/or the number of events increases, short-term (minutes) defensive responses become more robust and consolidate into more persistent (hours to days) changes (Frost, Castellucci, Hawkins, & Kandel, 1985; Mason et al., 2014; Watkins et al., 2010). In addition, nondefensive behaviours, such as feeding, are suppressed as defensive behaviours are sensitized (Acheampong et al., 2012).

The strength of short-term behavioural changes is also contingent upon the source and reliability of information indicative of predation risk. Organisms may use a variety of cues including chemical, visual and mechanical to evaluate risk (Weissburg et al., 2014), although olfaction seems to be the predominant means of risk detection in both terrestrial and aquatic systems (Derby & Sorensen, 2008). Aquatic animals, in particular, express high sensitivity to a variety of chemosensory cues, including chemical exudates released by predators (Hay, 2009; Kats & Dill, 1998; Large, Smee, & Trussell, 2011; Orr, El-Bekai, Lui, Watson, & Lukowiak, 2007; Scherer & Smee, 2016; Schoeppner & Relyea, 2009) and alarm signals secreted by damaged or frightened conspecifics (Chivers, Mirza, & Johnston, 2002; Kicklighter, Kamio, Nguyen, Germann, & Derby, 2011; Kicklighter et al., 2007; Mathuru et al., 2012; Stensmyr & Maderspacher, 2012). Prey animals also enhance their defensive behaviours following various mechanosensory stimuli, such as predator contact (Frost et al., 1998) or sublethal attack (Watkins et al., 2010).

Although much is understood regarding how specific cues trigger immediate or short-term behavioural changes in prey across multiple taxa, the current understanding of the persistence of these behavioural changes in response to different types and delivery time frames of various predatory stimuli remains unclear. Therefore, several important questions remain unaddressed. First, how rapidly do behavioural changes revert once a threat has subsided? Understanding the potential medium and long-term effects of risk cues on an individual can resolve to what extent sublethal effects extend over time and the conditions influencing these behavioural patterns. Additionally, analysing the temporal domains by which behaviours are modified would yield insight to the adaptive nature of these responses and the strategies prey use to minimize risk. Second, does the persistence of these responses depend upon the source of stimulus detected? If this indeed occurs, it would allow for a deeper understanding of the causes of sublethal effects observed in natural systems once predation risk is partially or wholly diminished.

In the current study, we utilized a neurobiological model organism, the sea hare *Aplysia californica* (henceforth *Aplysia*), to analyse the expression of behavioural changes in response to different cues that are indicative of predation risk. *Aplysia* are attacked by the spiny lobster *Panulirus interruptus* (Kicklighter, Shabani, Johnson, & Derby, 2005; Mason et al., 2014; Watkins et al., 2010). When threatened, *Aplysia* release the defensive secretions ink and opaline, both of which act as antipredatory defences (Kicklighter & Derby, 2006; Kicklighter et al., 2005) as well as alarm compounds to alert nearby conspecifics (Kicklighter et al., 2007, 2011). Ink is primarily composed of water-soluble compounds and disperses widely when released, contrasting with the sticky and viscous opaline (Kicklighter et al., 2005; Love-Chezem, Aggio, & Derby, 2013). In ink, immediate defensive responses are triggered by uracil, a nitrogenous base, and by the nucleosides uridine and cytidine (Kicklighter et al., 2007).

Behavioural changes persist in *Aplysia* as a result of the amount of stimuli delivered. A single 10 s trial of electric shocks (Acheampong et al., 2012) or lobster attack (Mason et al., 2014; Watkins et al., 2010) produces short-term to intermediate-term sensitization (up to 2 h) of a defensive withdrawal reflex, whereas multiple trials of electric shocks (Acheampong et al., 2012) or lobster attacks (Mason et al., 2014) produce long-term alterations persisting for at least 24 h. At the cellular level, each form of memory (short, intermediate and long term) is distinct in *Aplysia* and accompanied by a unique set of biochemical mechanisms within neurons in the circuits underlying these behaviours (Kandel, 2001). However, how predator exudates or prey alarm cues induce behavioural changes across different temporal scales has not been investigated.

Our previous work has revealed that, when *Aplysia* is presented with noxious electric shocks that mimic a sublethal predator attack (Watkins et al., 2010), a clear behavioural profile emerges in which biting is suppressed whereas defensive withdrawal responses are enhanced (Acheampong et al., 2012). These two behavioural changes were consistently observed following different training protocols and across multiple temporal domains, and appeared mechanistically linked (i.e. they both either occurred or did not occur; Acheampong et al., 2012; Shields-Johnson et al., 2013).

The ultimate goal of this research was to analyse the mechanisms underlying the persistence of sublethal effects by linking previous research on *Aplysia* feeding suppression with ecologically realistic scenarios (i.e. exposure to predation risk cues). In this study, feeding was selected because it is one of the behaviours that are modified by aversive paradigms in *Aplysia* (Kandel, 2001; Walters, Carew, & Kandel, 1981) and because, in general, feeding suppression is common among invertebrates after exposure to