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Proximate stimuli: An overlooked driving force for risk-induced trait responses affecting interactions in aquatic ecosystems

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Abstract

Inducible responses in prey to predation risk can influence species interaction strength, with significant ecological consequences. Much of the past research on interactions in aquatic ecosystems has focused on remote stimuli (e.g., diffusible chemicals emitted from predators and injured conspecifics, which easily propagate through environmental water), as cues triggering trait responses in prey, and has overlooked the importance of proximate stimuli (e.g., physical disturbance and less-diffusible chemicals), which occur in attack or direct contact to prey by predators. Proximate stimuli from predators as well as remote stimuli may induce significant responses in prey functional traits such as behavior, morphology, and life history and, therefore, act as an important mechanism of top-down effects in aquatic ecosystems. In this opinion paper, we argue that studying the effects of proximate stimuli is essential to better understanding of individual adaptation to predation risk in nature and ecological consequences of predator-prey interactions. Here, we propose research directions to examine the role of proximate stimuli for phenotypic plasticity and interaction systems.

KEYWORDS

Aquatic ecosystems, Phenotypic plasticity, Predation attack, Predation-risk effects, Trait-mediated indirect effects (TMIEs)

Introduction

Plastic expression of defensive phenotypes in prey in response to predation risk has long been acknowledged as an important mechanism that strongly affects ecological

interactions (Miner, Sultan, Morgan, Padilla, & Relyea, 2005; Peacor, Barton, Kimbro, Sih, & Sheriff, 2020; Preisser, Bolnick, & Benard, 2005; Werner & Peacor, 2003) and thus can influence population and community dynamics (Kishida, Trussell, Mougi, & Nishimura, 2010; Matassa, Donelan, Luttbeg, & Trussell, 2016; Schmitz, Beckerman, & O'Brien, 1997; Trussell, Ewanchuk, & Bertness, 2003). To plastically express defensive phenotypes, prey individuals need to detect environmental cues indicating predation risk. Various factors such as odors, sounds, and physical contact by predators can be available for prey as potential cues indicating emergence of predation risk (Kishida et al., 2010; Relyea, 2001; Stauffer & Semlitsch, 1993). Regarding the occurrence of direct contact between prey and predator, we can categorize these cues into the following two types: (1) remote stimuli that are detected by prey without direct contact by predators (e.g., mediated by highly diffusible chemicals emitted from predator and injured conspecifics) and (2) proximate stimuli that are detected by prey during an actual attack when they are directly contact by the predator (e.g., mediated by direct physical contact and less-diffusible chemicals).

Traditionally, studies on predator-prey interactions in aquatic ecosystems have focused on remote stimuli as cues triggering trait responses in prey, especially water-soluble chemicals emitted from predators (often called "kairomones"), which propagate through water (e.g., Brönmark & Hansson, 2000; Kats & Dill, 1998; Tollrian & Harvell, 1999). In general, water-soluble chemicals are assumed as a reasonable cue for induction of prey responses due to their highly transmittable nature (Tollrian & Harvell, 1999). Moderate diffusivity of environmental water is considered to allow the chemicals to reach in a distance, at detectable concentrations for prey (Tollrian & Harvell, 1999). By using remote stimuli, prey can detect impending predation threat before an actual predation

event.

Although usage of remote stimuli does not exclude availability of proximate stimuli as alternative cues, very few studies have focused on proximate stimuli (Kishida & Nishimura, 2004; Lagrue, Besson, & Lecerf, 2015; Sakamoto, Chang, & Hanazato, 2007). For many predators, the majority of their attempts to capture prey are unsuccessful (Abrams, 1989; Abrams, 2007; Vermeij, 1982) and such "unsuccessful predation" opportunities allows the attacked prey to perceive proximate stimuli from the attacking predators. In addition, proximate stimuli may be more reliable cues than remote stimuli, since the actual attack experienced by prey means that a prey individual is the actual target of the predators in the environment. Therefore, proximate stimuli from predators have the potential to trigger risk-induced trait responses in prey. Because defensive phenotypes are costly to produce and maintain, prey should adopt an optimal stimulus as an induction cue of the costly defense, based on its reliability (Harvell, 1990; Lively, 1986; Tollrian & Harvell, 1999). The reliability of cues is related not only to the sensory ability of prey, but also the escape ability of prey and the attack strategy of the predator. Thus, proximate stimuli as well as remote stimuli may serve as reliable cues for induction of defensive phenotypes in prey. Indeed, evidence from a few previous studies (Kishida & Nishimura, 2004; Sakamoto et al., 2007) suggests that proximate stimuli can be necessary for induction of defensive morphs of prey. Hence, previous studies in which effects of only remote stimuli were examined may have failed to accurately estimate or underestimated the risk-induced trait responses and their effects on other species and community properties (i.e., trait-mediated indirect effects: TMIEs).

Here, we argue that studying the effects of proximate stimuli on the trait responses in prey is essential to better understand trait variation in prey and the consequences of

predator-prey interactions, and that such an approach will likely lead to new insights for population and community ecology. In this paper, we first summarize the types of stimuli that evoke risk-induced trait responses by defining remote stimuli and proximate stimuli in existing studies on predator-prey systems. We then propose an experimental approach to test the effects of proximate stimuli. Finally, we describe the prospects of studies on species interaction driven by proximate stimuli.

Stimuli that evoke risk-induced trait responses

Here, we categorize stimuli into remote and proximate stimuli based on whether direct contact with a predator mediates prey's perception of predation risk. We define remote stimuli as stimuli that the prey recognizes as predation risk without direct contact with a predator, and proximate stimuli as stimuli that require direct contact (many of which are attacks from predators) with a predator for their recognition.

Remote stimuli include diffusible chemicals released by predators or conspecifics as olfactory cues (note that it is commonly termed as chemical cues in many papers) (Hettyey et al., 2015), the appearance of predator as visual cues (Edmunds, 1974), sounds released by predators as auditory cues (e.g., Wilson, Schack, Madsen, Surlykke, & Wahlberg, 2011) and the disturbance of water by predators (i.e., water flow and vibration) as tactile cues (Dodson et al., 1994). Proximate stimuli include direct physical contact as a tactile cue, and less diffusible chemicals that can be only perceived through physical contact as olfactory cues.

Previous studies documented how each type of stimuli induces prey's responses in various traits such as behavior, life history and morphology (Chivers & Smith, 1998; Kats

& Dill, 1998; Stauffer & Semlitsch, 1993). Most of them focused on remote stimuli. In particular, the remote olfactory cues have received the most attention as a trigger of prey responses in aquatic systems (Ferland-Raymond, March, Metcalfe, & Murray, 2010; Hettyey et al., 2015; Winkler & Van Buskirk, 2012). The prevailing view that diffusible chemicals are the most useful cues because they are easily and widely transmitted at perceptional concentration for prey animals may be partly responsible for the research bias. Commonly, effects of remote olfactory cues are examined by comparing prey traits in the presence and absence of olfactory cues from predators (e.g., between a treatment with caged predator and a treatment without caged predator [e.g., Hews, 1988; Hoverman & Relyea, 2009; Matassa, 2010] and between a treatment containing water with predator kairomones and a treatment containing water without predator kairomones [e.g., Imai, Naraki, Tochinai, & Miura, 2009; Stauffer & Semlitsch, 1993; Weiss et al., 2018; Yasumoto et al., 2005]). Through these comparative experiments, many studies have demonstrated that aquatic animals from various taxa exhibit defensive phenotypes in behavior, morphology, and life history in response to the remote olfactory cues (Chivers & Smith, 1998; Kats & Dill, 1998).

Despite limited publications, trait responses induced by the other types of remote stimuli (i.e., visual and tactile cues) have also been documented. The effects of remote visual cues are often tested presenting a predator through a transparent partition (e.g., Plexiglas). For example, Hettyey, Rölli, Thürlimann, Zürcher, and Van Buskirk (2012) showed that predators viewed through Plexiglas suppress prey tadpole activity. Disturbance can be used as remote tactile cues indicating emergence of predation risk. When predators approach prey individuals, disturbance of the environmental elements (e.g., water turbulence) can occur. Such a disturbance may response of prey individuals.

For example, the effects of the remote tactile cues were often tested using artificial water flows (Laforsch & Tollrian, 2004; Stauffer & Semlitsch, 1993). Laforsch and Tollrian (2004) showed that water turbulence generated by an electric motor induces extreme defensive helmet formation by the water flea (*Daphnia cucullata*) even in the absence of predator kairomones.

Likewise, effects of proximate stimuli have been rarely studied, despite their potential to also serve as reliable cues of predation risk to prey. To our knowledge, only a few studies have explicitly examined the effects of proximate stimuli (i.e., predator attack) on prey morphology (frog tadpole [Kishida & Nishimura, 2004, 2005]; water flea [Sakamoto et al. 2007]) and prey behavior (arthropod detritivores [Lagrue et al., 2015]). Tadpoles of *Rana pirica* frogs enlarge their body by thickening their epithelium tissue (i.e., bulgy morph) under predation risk from larval salamanders (*Hynobius retardatus*). Because these predatory salamanders are a gape limited predator, the bulgy morph is highly effective defensive morph (Takatsu & Kishida, 2013). Previous studies have showed that direct contact by predatory salamanders is required for tadpoles to express the bulgy morph: *R. pirica* frog tadpoles do not express the bulgy morph when exposed to caged-salamanders but do express the bulgy morph when exposed to non-segregated salamanders (Kishida & Nishimura, 2004; Kishida & Nishimura, 2005). Sakamoto et al. (2007) have reported that the changes in the defensive antennule morphotypes of the water flea (*Bosmina longirostris*) are induced by exposure of predation by copepods and not by exposure of the predator kairomones alone, suggesting that the antennule type is induced by proximate physical stimuli from the copepod. Lagrue et al. (2015) estimated the effects of predator attack on defensive behavior of prey detritivores by comparing refuge use between the prey exposed to the predatory dragonfly larvae that could attack

but not consume the prey (i.e., predator with non-lethal attack) and those that neither attack nor consume the prey (i.e., predator without attack), using ingenious ligature methods (Lagrue, Azémar, Besson, Lamothe, & Lecerf, 2011). The prey exposed to the predator with non-lethal attack tended to use refuge greater than those exposed to the predator without attack. Furthermore, Lagrue et al. (2015) found that the behavioral response of prey to the proximate stimuli influenced decomposition rate of leaf litter (i.e., TMIEs, see below), although some conflicting result patterns were observed between field and laboratory experiments. These studies are very pioneering achievements and have strongly suggested that experimental studies that do not consider proximate stimuli as potential cues may underestimate the risk-induced trait responses.

Although we illustrated various types of stimuli separately, it should be noted that each type of stimuli may not independently operate as a trigger. If simultaneous occurrence of different types of stimuli increases the reliability of predation risk, the use of multiple types of stimuli jointly can be optimal for prey. For example, defensive morphs in the water flea, which are induced by predator kairomones, are less extreme than those observed in the field (Havel & Dodson, 1985), suggesting that water fleas use multiple cues to express their defensive morphs more strongly in the field (Havel & Dodson, 1985). Tollrian and Laforsch (2006) demonstrated that both chemical and tactile cues in combination induce significantly larger responses of the water flea (*D. cucullata*) compared to each cue alone, such that the defensive helmets were as large as those found in natural lakes. Similarly, the behavioral responses of prey may be larger when multiple cues are combined. Stauffer and Semlitsch (1993) reported that the combination of chemical and other cues (i.e., visual and tactile cues) causes the strongest behavioral responses of prey tadpoles, although its degree was only slightly stronger than that from

chemical cues alone.

What experimental designs can examine the effects of proximate stimuli?

Here, we propose experimental designs to study the effects of proximate stimuli from predators on prey traits. Revealing risk-induced trait responses evoked by proximate stimuli as well as remote stimuli should lead to a more accurate estimation of predation-risk effects. Quantifying the effects of proximate stimuli is difficult compared to those of remote stimuli. In general, when researchers investigate independent effects of a factor in a manipulative experiment, they manipulate only the focal factor without changes in other prospective factors in order to eliminate potential confounding effects. Commonly, researchers have investigated the independent effects of remote stimuli on prey traits by comparing trait values between the following two conditions: (1) prey that are exposed to predation risk without direct contact with a predator by, for example, separating predators from focal prey using compartments or administering water treated with predator chemical cues and (2) prey that are not exposed to predation risk (i.e., control) (Crowl & Covich, 1994; Hettyey et al., 2012; Tollrian & Laforsch, 2006). Such a robust experimental design to evaluate the independent effect of proximate stimuli is difficult because separating them from remote stimuli is challenging. When researchers seek to detect the effects of proximate stimuli on prey response, prey should be exposed to a predator that can physically attack the prey. In this case, remote stimuli also exist because a predator continues to produce remote stimuli through its visual appearance or diffusible chemicals.

As mentioned above, it is difficult to evaluate the independent effect of proximate

stimuli in experiments using predator individuals as an inducing agent. In the experimental treatment where preys are exposed to predators that can physically attack prey, the independent effects of proximate stimuli, the independent effects of remote stimuli, and their interactive effects can operate concurrently. Therefore, experimental treatments that can show whether proximate stimuli are involved in prey's responses include: (1) a proximate risk treatment in which prey individuals are exposed to predators that can attack prey, (2) a remote risk treatment in which prey individuals are exposed to a predator that are not allowed to attack prey or exposed to the remote stimuli alone (e.g., diffusible chemicals emitted from predators), and (3) a no predator treatment in which prey are not exposed to any factor representing predation risk (Figure 1a). Significant differences in prey trait values between the proximate and remote risk treatments would suggest that prey individuals respond to proximate stimuli from predators.

Although we can create a proximate risk treatment by placing predators and prey together in a compartment, it should be noted that in such a treatment, actual predation can occur if researchers use lethal predators. An actual predation event may be problematic because it can cause confounding effects on prey traits through phenotypic selection. When defensive performance of prey varies among prey individuals within experimental group, actual predation will likely select against prey individuals that express less defensive traits. In this case, even if researchers detect a difference in average trait values of prey between the remote and proximate risk treatments, it is impossible to conclude that prey individuals plastically change their functional traits in response to the presence of the lethal predators because selective predation would bias the average trait value of prey in the proximate risk treatment. Likewise, when assessing the predation-risk effects from lethal predators to receiver prey species via prey trait changes, the effects

233 detected when an actual predation event occurs include not only effects of risk-induced
234 trait responses in prey, but also those of selective trait changes in prey. In general, the
235 difference in the average value of the measurements between the proximate risk treatment
236 that contains a lethal predator and the no-predator treatment should not be considered as
237 the consequence of predator-induced plastic responses in prey. Therefore, it is better to
238 design an experimental treatment using a non-lethal predator that can attack but cannot
239 consume prey individuals in order to preclude selective predation and detect risk-induced
240 trait responses and the resulting consequence for TMIEs.

241 One approach to solve this issue is to prevent actual consumption by predators by
242 disabling their capturing organs (e.g., mouth, mandible, claw). For example, Schmitz
243 (1998) examined the non-consumptive effects of predators by gluing the mouthparts of a
244 predator spider, which allowed grasshoppers to attack their grasshopper prey but not
245 consume them. Other methods for disabling predator's capture organs have been devised,
246 such as cutting (Nelson, Matthews, & Rosenheim, 2004; Peckarsky, Cowan, Penton, &
247 Anderson, 1993; Sparrevik, & Leonardsson, 1995; Van Buskirk, 1989), ligating (Lagrue
248 et al. 2015; Rudolf, 2008), and suturing the mouthparts (Ball & Baker, 1996). Although
249 these studies were not intended to detect the importance of proximate stimuli except in
250 Lagrue et al. (2015), similar treatment of predator individuals may be useful to design the
251 proximate risk treatment (Figure 1b). By using predators that cannot consume prey
252 individuals, researchers can evaluate prey's plastic response caused by proximate stimuli
253 from predators based on the differences in prey trait value between the proximate risk and
254 remote risk treatments. However, such surgical manipulation is likely to be applicable
255 only for Arthropods because the capturing organ of aquatic Arthropods is structured by
256 chitinous exoskeleton. Since surgical operation such as gluing and suturing the capturing

organ may cause damage in predators other than Arthropods and also lead to unnatural behavior of the predators, we need to adopt an alternative method for predator species other than Arthropods. For example, using thin elastic net as a separator is a prospective method (Figure 1c). This type of net is useful because it enables the predator individuals to attack (directly contact with) prey, but not consume prey.

Using an artificial predator (e.g., plastic model) may be an alternative method. However, production of realistic predation risk using an artificial model predator is difficult and may can cause unnatural or limited response among prey individuals. In nature, prey may perceive specific cues that artificial model predators cannot produce when they are in contact with predators (e.g., negative pressure when being swallowed by predators and less-diffusible chemicals). Therefore, when we use an artificial model predator as an inducing agent, careful assessment of prey responses is required.

How can a comparison of the three experimental treatments (proximate stimuli, remote stimuli, and control treatments) reveal the importance of proximate stimuli for risk-induced trait responses? Figure 2 shows a schematic diagram of the expected results of an experiment. This figure shows the trait values (e.g., defensive morph, activity, and food consumption) of the prey individuals observed in the experimental treatments. The phenotypic changes of prey individuals in response to the cues in each stimulus treatment can be detected from the difference in trait values between the control and each stimulus treatment. We can conclude that the proximate stimuli are not important factors as cues if there is no significant variation between (A) the difference in trait values between the control and proximate risk treatments and (B) the difference in trait values between the control and remote risk treatments (Figure 2a). On the other hand, if the difference in prey's trait value between the control and proximate risk treatments (A) is greater than

the difference in prey trait value between the control and remote risk treatments (B, Figure 2b), we can conclude that the proximate stimuli play critical role in prey's response to predation risk.

It has been known that the relative importance of different types of remote stimuli can vary depending on environmental context. For example, in lotic environments, chemicals are washed away quickly, so visual and tactile cues are relatively more important for prey to detect predation risk (Culp, Glozier, & Scrimgeour, 1991). On the other hand, in turbid water, visual cues are unavailable and the relative importance of chemical cues is high (Brönmark & Hansson, 2000). Accordingly, it is expected that proximate stimuli also vary in their importance depending on the environmental conditions. For example, in lotic environments, where availability of remote stimuli, especially chemicals, is likely low, proximate stimuli should play important role in trait response of prey under predation risk. Comparisons in prey trait values among several experimental treatments with different water exchange rates could reveal the hypothetical dependence of the importance of proximate stimuli on water flow rate. If prey respond more strongly in the treatments where the degree of exchanges in environmental water exchange was larger, the importance of proximate stimuli can be expected to high in lotic environments. Such experimental results will deepen our insight of variability of the ecological role of proximate stimuli in nature and evolution of utilization of proximate stimuli in prey species.

Here, we propose a method to test the TMIEs driven by proximate stimuli from predator to prey. The method is based on the traditional method of TMIE empirical studies (e.g., Schmitz et al., 1997). For example, first a food web consisting of predators, prey, and basal resources (e.g., algae, plants material and detritus consumed by preys) is

305 prepared in enclosures. Then, a proximate risk treatment where predators can attack but
306 cannot consume prey, a remote risk treatment where predators cannot attack, and control
307 treatments without predators (see Figure 1) are provided for a period of time. At the end
308 of the experiment, the abundance of basal resources in each type of treatments is measured.
309 Comparison of the differences in abundance of bottom resources between control and
310 remote risk treatments with those between control and proximate risk treatments will
311 enable researchers to examine the importance of proximate stimuli in TMIEs. Lagrue et
312 al. (2015) used a similar experimental procedure to determine whether predator attack
313 influences litter decomposition of prey (i.e., TMIEs). In the laboratory experiment, they
314 showed more leaf litters remained if the prey detritivores were exposed to predatory
315 dragonfly with non-lethal attacks compared to the prey were exposed to predatory
316 dragonfly without any attacks. The lower decomposition rate of leaf litter in the presence
317 of dragonfly with non-lethal attacks represented the TMIE evoked by proximate stimuli
318 of predators, since prey detritivores exhibited greater refuge use in the presence of the
319 dragonfly with non-lethal attacks than in the presence of the dragonfly without any attacks
320 (see above).

321 Proximate stimuli may play a role in another overlooked process of TMIEs. Recently,
322 Yamaguchi, Takatsu, and Kishida (2016) demonstrated that direct contact of large *R.*
323 *pirica* frog tadpoles with small conspecifics increase the likelihood that the small tadpoles
324 would be consumed by the predatory *H. retardatus* salamander larvae. This likely
325 occurred because disturbance created by the large tadpoles affects the activity of small
326 frog tadpoles, which increases the encounter rate between small tadpoles and predatory
327 salamanders. Although this demonstrates the importance of direct contact in the context
328 of a size-dependent intraspecific interaction, similar processes likely operate in different

contexts (Takatsu & Kishida, 2020). For example, prey should exhibit escape behavior after they are attacked by a predator. This escape behavior stimulated by one by predator's attack may increase the likelihood of predation from other predators through a potential increase in encounter rates. In addition, sudden escape behavior may cause further contact with other nearby individuals. The chain of contact-escape-contact may result in a temporary increase in predator-prey interactions in system. Thus, the proximate stimuli may be common causes of various indirect effects in nature. Although it is generally difficult to investigate sequential process of multiple events that occur concurrently, recent developments of automated individual tracking system (Dell et al., 2014) allow researchers to investigate such complicated indirect effects mediated by the chain of proximate stimuli.

Perspectives: Contact driven ecology

It has long been believed that remote chemical cues are the most important sensory modality in aquatic ecosystem (Hettyey et al., 2015). However, since preys may come into direct contact with predators frequently in nature (Abrams, 1989; Abrams, 2007; Vermeij, 1982), the proximate stimuli itself can be a useful cue. Indeed, prey's defensive traits that are enhanced and induced by proximate stimuli from predators have been reported recently (Kishida & Nishimura, 2004; Sakamoto et al., 2007, Lagrue et al. 2015). This shows that proximate stimuli may play an unexpected, important role in aquatic interaction systems by eliciting changes in prey phenotypes. Revealing the magnitude of the responses of prey to and other cascading indirect effects of proximate stimuli using our proposed approach will lead to a more accurate estimate of the effects of predators on

prey trait responses and the indirect effects in the natural settings. Moreover, these results will prove the importance of considering proximate stimuli.

The impacts of proximate stimuli on species interaction are largely unknown. We believe that reinterpreting species interactions through the lens of contact driven ecology opens up a new frontier in population and community ecology as well as evolutionary ecology. We hope that our opinion paper provides more fertile ground for this endeavor.

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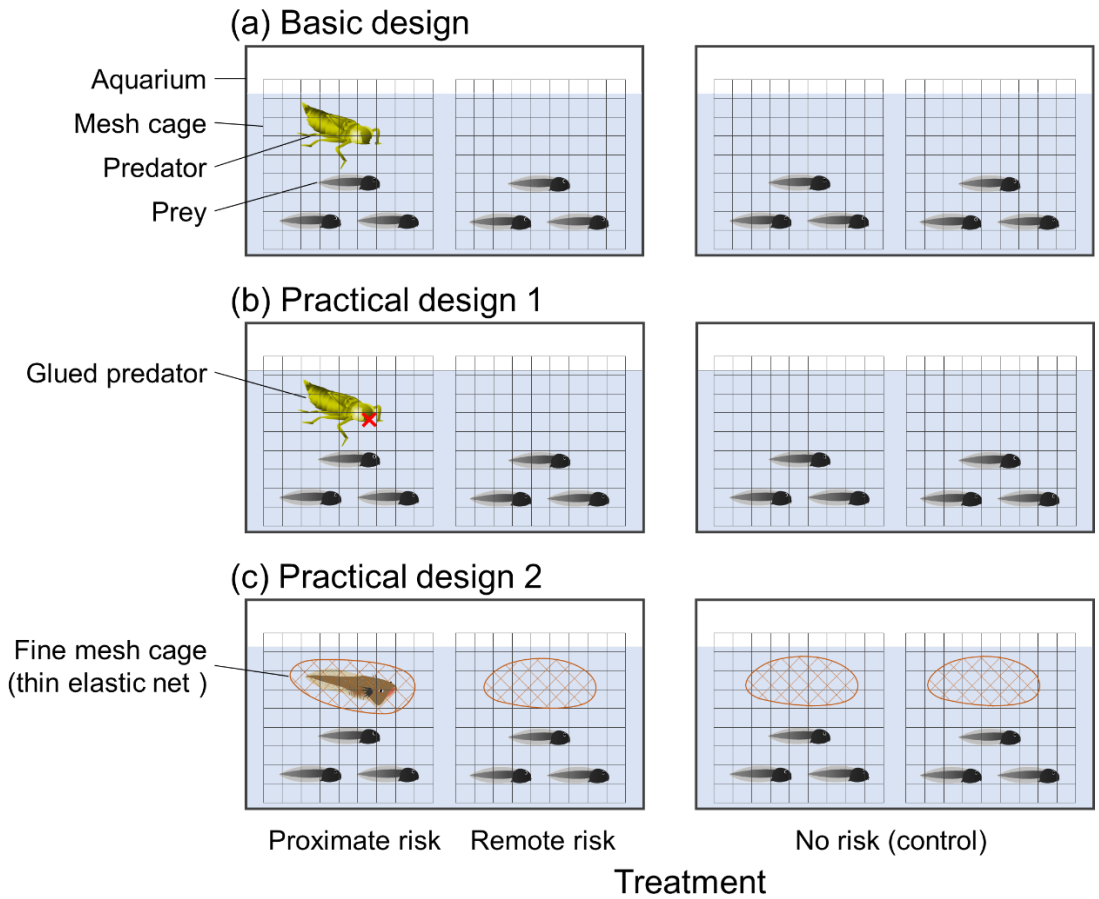
503 **Figure Legends**



504

505 **FIGURE 1** Experimental design for detecting the effects of proximate stimuli on
506 prey's trait values: basic design (a) and practical design precluding actual predation of
507 invertebrate predators (b) and vertebrate predators (c). Description of the illustrations are
508 below: aquarium (external rectangular), mesh cage (inside rectangular with coarse mesh),
509 a predator (dragonfly larva or salamander larva), prey (frog tadpoles). In figure 1b
510 (practical design for invertebrate predator), the glued predator has its capturing organs
511 disabled, which allowed the predator to attack prey but prevent the predator from actual
512 predation. In figure 1c (practical design for vertebrate predators), fine mesh with
513 indeterminate form inside the mesh cage represents a thin elastic net as a separator which
514 allow the predator to attack prey but prevent the predator from actual predation. In the

Proximate risk treatment, the prey receive both proximate and remote stimuli from the predator. In the Remote risk treatment, the prey can receive remote stimuli from the predator in the adjacent cage through the environmental water, but cannot receive direct contact by the predator. The No risk treatment as a control provides baseline data for the description of the predator-naive prey phenotype. The effects of the proximate stimuli can be detected based on the variation in the trait values among the treatments (see Figure 2).

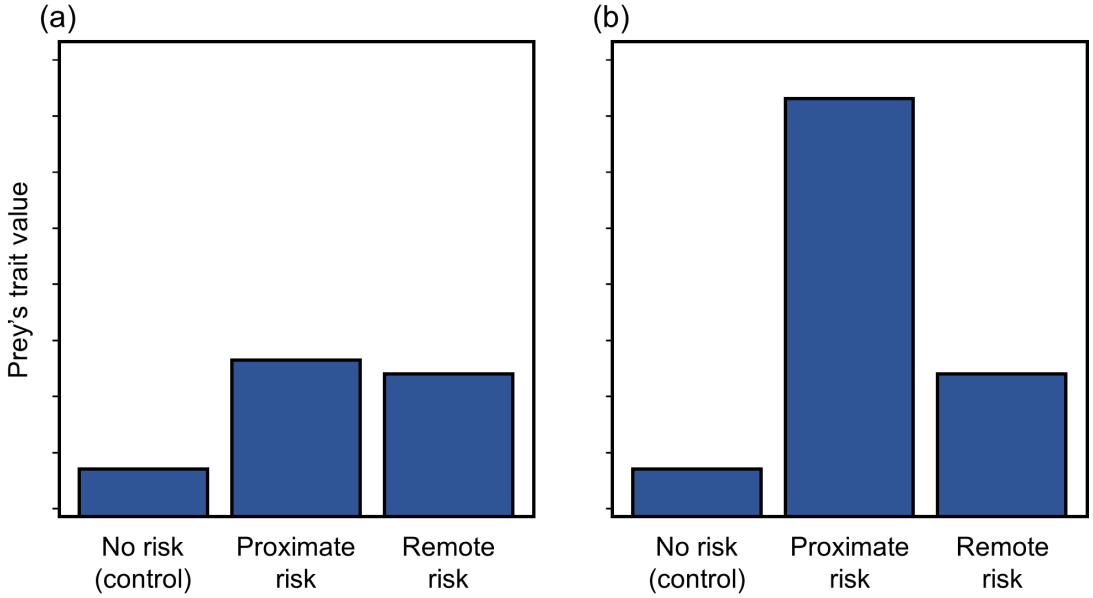


FIGURE 2 Schematic diagrams of possible results of the experiment. The vertical line indicates the prey's trait value that is assumed to be changed by the presence of the predator. If the trait value between the proximate risk and remote risk treatments is similar (i.e., scenario (a)), proximate stimuli is not relatively important as a trigger of prey's trait response, compared to remote stimuli. On the contrary, if difference in the trait value between the proximate risk treatment and No risk (control) treatment is larger than that between the remote risk treatment and No risk (control) treatment (i.e., scenario (b)), the result shows that proximate stimuli is a significantly important cues for prey's trait responses.