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Author(s)	Okamiya, Hisanori; Kishida, Osamu
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1 **Proximate stimuli: An overlooked driving force for risk-induced trait**
2 **responses affecting interactions in aquatic ecosystems**

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4 Hisanori Okamiya¹ and Osamu Kishida²

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6 ¹Department of Biological Sciences, Graduate School of Sciences, Tokyo Metropolitan
7 University, Minami-ohsawa, Hachioji, Tokyo, Japan

8 ²Tomakomai Experimental Forest, Field Science Center for Northern Biosphere,
9 Hokkaido University, Takaoka, Tomakomai, Japan

10

11 **Correspondence**

12 Hisanori Okamiya, Department of Biological Sciences, Graduate School of Sciences,
13 Tokyo Metropolitan University, Minami-Osawa 1-1, Hachioji, Tokyo 192-0397, Japan

14 Email: h.okamiya@gmail.com

15 ORCID ID: 0000-0001-7708-2962

16

17 **Abstract**

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

32

33 **KEYWORDS**

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

36

37 **Introduction**

38

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

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

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

65 event.

66 Although usage of remote stimuli does not exclude availability of proximate stimuli as
67 alternative cues, very few studies have focused on proximate stimuli (Kishida &
68 Nishimura, 2004; Lagrue, Besson, & Lecerf, 2015; Sakamoto, Chang, & Hanazato, 2007).
69 For many predators, the majority of their attempts to capture prey are unsuccessful
70 (Abrams, 1989; Abrams, 2007; Vermeij, 1982) and such “unsuccessful predation”
71 opportunities allows the attacked prey to perceive proximate stimuli from the attacking
72 predators. In addition, proximate stimuli may be more reliable cues than remote stimuli,
73 since the actual attack experienced by prey means that a prey individual is the actual target
74 of the predators in the environment. Therefore, proximate stimuli from predators have the
75 potential to trigger risk-induced trait responses in prey. Because defensive phenotypes are
76 costly to produce and maintain, prey should adopt an optimal stimulus as an induction
77 cue of the costly defense, based on its reliability (Harvell, 1990; Lively, 1986; Tollrian &
78 Harvell, 1999). The reliability of cues is related not only to the sensory ability of prey,
79 but also the escape ability of prey and the attack strategy of the predator. Thus, proximate
80 stimuli as well as remote stimuli may serve as reliable cues for induction of defensive
81 phenotypes in prey. Indeed, evidence from a few previous studies (Kishida & Nishimura,
82 2004; Sakamoto et al., 2007) suggests that proximate stimuli can be necessary for
83 induction of defensive morphs of prey. Hence, previous studies in which effects of only
84 remote stimuli were examined may have failed to accurately estimate or underestimated
85 the risk-induced trait responses and their effects on other species and community
86 properties (i.e., trait-mediated indirect effects: TMIEs).

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

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

95

96 **Stimuli that evoke risk-induced trait responses**

97

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

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

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

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

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

137 For example, the effects of the remote tactile cues were often tested using artificial water
138 flows (Laforsch & Tollrian, 2004; Stauffer & Semlitsch, 1993). Laforsch and Tollrian
139 (2004) showed that water turbulence generated by an electric motor induces extreme
140 defensive helmet formation by the water flea (*Daphnia cucullata*) even in the absence of
141 predator kairomones.

142 Likewise, effects of proximate stimuli have been rarely studied, despite their potential
143 to also serve as reliable cues of predation risk to prey. To our knowledge, only a few
144 studies have explicitly examined the effects of proximate stimuli (i.e., predator attack) on
145 prey morphology (frog tadpole [Kishida & Nishimura, 2004, 2005]; water flea [Sakamoto
146 et al. 2007]) and prey behavior (arthropod detritivores [Lagrue et al., 2015]). Tadpoles of
147 *Rana pirica* frogs enlarge their body by thickening their epithelium tissue (i.e., bulgy
148 morph) under predation risk from larval salamanders (*Hynobius retardatus*). Because
149 these predatory salamanders are a gape limited predator, the bulgy morph is highly
150 effective defensive morph (Takatsu & Kishida, 2013). Previous studies have showed that
151 direct contact by predatory salamanders is required for tadpoles to express the bulgy
152 morph: *R. pirica* frog tadpoles do not express the bulgy morph when exposed to caged-
153 salamanders but do express the bulgy morph when exposed to non-segregated
154 salamanders (Kishida & Nishimura, 2004; Kishida & Nishimura, 2005). Sakamoto et al.
155 (2007) have reported that the changes in the defensive antennule morphotypes of the
156 water flea (*Bosmina longirostris*) are induced by exposure of predation by copepods and
157 not by exposure of the predator kairomones alone, suggesting that the antennule type is
158 induced by proximate physical stimuli from the copepod. Lagrue et al. (2015) estimated
159 the effects of predator attack on defensive behavior of prey detritivores by comparing
160 refuge use between the prey exposed to the predatory dragonfly larvae that could attack

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

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

185 chemical cues alone.

186

187 **What experimental designs can examine the effects of proximate stimuli?**

188

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

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

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

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

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

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

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

281 the difference in prey trait value between the control and remote risk treatments (B, Figure
282 2b), we can conclude that the proximate stimuli play critical role in prey's response to
283 predation risk.

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

301 Here, we propose a method to test the TMIEs driven by proximate stimuli from
302 predator to prey. The method is based on the traditional method of TMIE empirical studies
303 (e.g., Schmitz et al., 1997). For example, first a food web consisting of predators, prey,
304 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

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

340

341 **Perspectives: Contact driven ecology**

342

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

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

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

359

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364

365 **REFERENCES**

- 366 Abrams, P. A. (1989). The evolution of rates of successful and unsuccessful
367 predation. *Evolutionary Ecology*, 3, 157–171.
- 368 Abrams, P. A. (2007). Defining and measuring the impact of dynamic traits on
369 interspecific interactions. *Ecology*, 88, 2555–2562.
- 370 Ball, S. L., & Baker, R. L. (1996). Predator-induced life history changes: antipredator
371 behavior costs or facultative life history shifts? *Ecology*, 77, 1116–1124.
- 372 Brönmark, C., & Hansson, L. A. (2000). Chemical communication in aquatic systems: an
373 introduction. *Oikos*, 88, 103–109.
- 374 Chivers, D. P., & Smith, R. J. F. (1998). Chemical alarm signalling in aquatic predator-
375 prey systems: a review and prospectus. *Ecoscience*, 5, 338–352.
- 376 Cowl, T. A., & Covich, A. P. (1994). Responses of a freshwater shrimp to chemical and

377 tactile stimuli from a large decapod predator. *Journal of the North American*
378 *Benthological Society*, 13, 291–298.

379 Culp, J. M., Glozier, N. E., & Scrimgeour, G. J. (1991). Reduction of predation risk under
380 the cover of darkness: avoidance responses of mayfly larvae to a benthic
381 fish. *Oecologia*, 86, 163–169.

382 Dell, A. I., Bender, J. A., Branson, K., Couzin, I. D., de Polavieja, G. G., Noldus, L. P., ...
383 & Brose, U. (2014). Automated image-based tracking and its application in
384 ecology. *Trends in Ecology & Evolution*, 29, 417–428.

385 Dodson, S. I., Crowl, T. A., Peckarsky, B. L., Kats, L. B., Covich, A. P., & Culp, J. M.
386 (1994). Non-visual communication in freshwater benthos: an overview. *Journal of*
387 *the North American Benthological Society*, 13, 268–282.

388 Edmunds, M. 1974. *Defence in Animals: A Survey of Anti-predator Defenses*. London,
389 England: Longman

390 Ferland-Raymond, B., March, R. E., Metcalfe, C. D., & Murray, D. L. (2010). Prey
391 detection of aquatic predators: assessing the identity of chemical cues eliciting prey
392 behavioral plasticity. *Biochemical Systematics and Ecology*, 38, 169–177.

393 Harvell, C. D. (1990). The ecology and evolution of inducible defenses. *The Quarterly*
394 *Review of Biology*, 65, 323–340.

395 Havel, J. E., & Dodson, S. I. (1985). Environmental cues for cyclomorphosis in *Daphnia*
396 *retrocurva* Forbes. *Freshwater Biology*, 15, 469–478.

397 Hettyey A, Rölli F, Thürlimann N, Zürcher A-C, & Van Buskirk J (2012). Visual cues
398 contribute to predator detection in anuran larvae. *Biological Journal of the Linnean*
399 *Society*, 106, 820–827.

400 Hettyey, A., Tóth, Z., Thonhauser, K. E., Frommen, J. G., Penn, D. J., & Van Buskirk, J.

401 (2015). The relative importance of prey-borne and predator-borne chemical cues for
402 inducible antipredator responses in tadpoles. *Oecologia*, *179*, 699–710.

403 Hews, D. K. (1988). Alarm response in larval western toads, *Bufo boreas*: release of larval
404 chemicals by a natural predator and its effect on predator capture efficiency. *Animal*
405 *Behaviour*, *36*, 125–133.

406 Hoverman, J. T., & Relyea, R. A. (2009). Survival trade-offs associated with inducible
407 defences in snails: The roles of multiple predators and developmental
408 plasticity. *Functional Ecology*, *23*, 1179–1188.

409 Imai, M., Naraki, Y., Tochinai, S., & Miura, T. (2009). Elaborate regulations of the
410 predator-induced polyphenism in the water flea *Daphnia pulex*: kairomone-sensitive
411 periods and life-history tradeoffs. *Journal of Experimental Zoology Part A:*
412 *Ecological Genetics and Physiology*, *311*, 788–795.

413 Kats, L. B., & Dill, L. M. (1998). The scent of death: chemosensory assessment of
414 predation risk by prey animals. *Ecoscience*, *5*, 361–394.

415 Kishida, O., & Nishimura, K. (2004). Bulgy tadpoles: inducible defense
416 morph. *Oecologia*, *140*, 414–421.

417 Kishida, O., & Nishimura, K. (2005). Multiple inducible defenses against multiple
418 predators in anuran tadpoles (*Rana pirica*). *Evolutionary Ecology Research*, *7*, 619–
419 631.

420 Kishida, O., Trussell, G. C., Mougi, A., & Nishimura, K. (2010). Evolutionary ecology of
421 inducible morphological plasticity in predator-prey interaction: toward the practical
422 links with population ecology. *Population Ecology*, *52*, 37–46.

423 Laforsch, C., & Tollrian, R. (2004). Extreme helmet formation in *Daphnia cucullata*
424 induced by small-scale turbulence. *Journal of Plankton Research*, *26*, 81–87.

425 Lagrue, C., Azémar, F., Besson, A., Lamothe, S., & Lecerf, A. (2011). Novel ligature
426 methods for studying sublethal effects of sit-and-wait predators: test using
427 *Cordulegaster boltonii* (Donovan, 1807) larvae (Anisoptera: Cordulegasteridae).
428 *Odonatologica*, *40*, 95–103.

429 Lagrue, C., Besson, A. A., & Lecerf, A. (2015). Interspecific differences in antipredator
430 strategies determine the strength of non-consumptive predator effects on stream
431 detritivores. *Oikos*, *124*, 1589–1596.

432 Lively, C. M. (1986). Predator-induced shell dimorphism in the acorn barnacle
433 *Chthamalus anisopoma*. *Evolution*, *40*, 232–242.

434 Matassa, C. M. (2010). Purple sea urchins *Strongylocentrotus purpuratus* reduce grazing
435 rates in response to risk cues from the spiny lobster *Panulirus interruptus*. *Marine*
436 *Ecology Progress Series*, *400*, 283–288.

437 Matassa, C. M., Donelan, S. C., Luttbeg, B., & Trussell, G. C. (2016). Resource levels and
438 prey state influence antipredator behavior and the strength of nonconsumptive
439 predator effects. *Oikos*, *125*, 1478–1488.

440 Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K., & Relyea, R. A. (2005).
441 Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution*, *20*,
442 685–692.

443 Nelson, E. H., Matthews, C. E., & Rosenheim, J. A. (2004). Predators reduce prey
444 population growth by inducing changes in prey behavior. *Ecology*, *85*, 1853–1858.

445 Peacor, S. D., Barton, B. T., Kimbro, D. L., Sih, A., & Sheriff, M. J. (2020). A framework
446 and standardized terminology to facilitate the study of predation-risk effects. *Ecology*,
447 *101*, e03152.

448 Peckarsky, B. L., Cowan, C. A., Penton, M. A., & Anderson, C. (1993). Sublethal

449 consequences of stream-dwelling predatory stoneflies on mayfly growth and
450 fecundity. *Ecology*, 74, 1836–1846.

451 Preisser, E. L., Bolnick, D. I., & Benard, M. F. (2005). Scared to death? The effects of
452 intimidation and consumption in predator-prey interactions. *Ecology*, 86, 501–509.

453 Relyea, R. A. (2001). Morphological and behavioral plasticity of larval anurans in
454 response to different predators. *Ecology*, 82, 523–540.

455 Rudolf, V. H. (2008). The impact of cannibalism in the prey on predator-prey systems.
456 *Ecology*, 89, 3116–3127.

457 Sakamoto, M., Chang, K. H., & Hanazato, T. (2007). Plastic phenotypes of antennule
458 shape in *Bosmina longirostris* controlled by physical stimuli from
459 predators. *Limnology and Oceanography*, 52, 2072–2078.

460 Schmitz, O. J. (1998). Direct and indirect effects of predation and predation risk in old-
461 field interaction webs. *The American Naturalist*, 151, 327–342.

462 Schmitz, O. J., Beckerman, A. P., & O'Brien, K. M. (1997). Behaviorally mediated
463 trophic cascades: effects of predation risk on food web interactions. *Ecology*, 78,
464 1388–1399.

465 Sparrevik, E., & Leonardsson, K. (1995). Effects of large *Saduria entomon* (Isopoda) on
466 spatial distribution of their small *S. entomon* and *Monoporeia affinis* (Amphipoda)
467 prey. *Oecologia*, 101, 177–184.

468 Stauffer, H. P., & Semlitsch, R. D. (1993). Effects of visual, chemical and tactile cues of
469 fish on the behavioural responses of tadpoles. *Animal Behaviour*, 46, 355–364.

470 Takatsu, K., & Kishida, O. (2013). An offensive predator phenotype selects for an
471 amplified defensive phenotype in its prey. *Evolutionary Ecology*, 27, 1–11.

472 Takatsu, K., & Kishida, O. (2020). Enhanced recruitment of larger predators in the

473 presence of large prey. *Journal of Animal Ecology* (in press).

474 Tollrian, R., & Harvell, C. D. (Eds.). (1999). *The Ecology and Evolution of Inducible*
475 *Defenses*. New Jersey, USA: Princeton University Press.

476 Tollrian, R., & Laforsch, C. (2006). Linking predator kairomones and turbulence:
477 synergistic effects and ultimate reasons for phenotypic plasticity in *Daphnia*
478 *cucullata*. *Archiv für Hydrobiologie*, *167*, 135–146.

479 Trussell, G. C., Ewanchuk, P. J., & Bertness, M. D. (2003). Trait-mediated effects in
480 rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology*, *84*,
481 629–640.

482 Van Buskirk, J. (1989). Density-dependent cannibalism in larval dragonflies. *Ecology*,
483 *70*, 1442–1449.

484 Vermeij, G. J. (1982). Unsuccessful predation and evolution. *The American*
485 *Naturalist*, *120*, 701–720.

486 Weiss, L. C., Albada, B., Becker, S. M., Meckelmann, S. W., Klein, J., Meyer, M., ... &
487 Metzler-Nolte, N. (2018). Identification of *Chaoborus* kairomone chemicals that
488 induce defences in *Daphnia*. *Nature chemical biology*, *14*, 1133–1139.

489 Werner, E. E., & Peacor, S. D. (2003). A review of trait-mediated indirect interactions in
490 ecological communities. *Ecology*, *84*, 1083–1100.

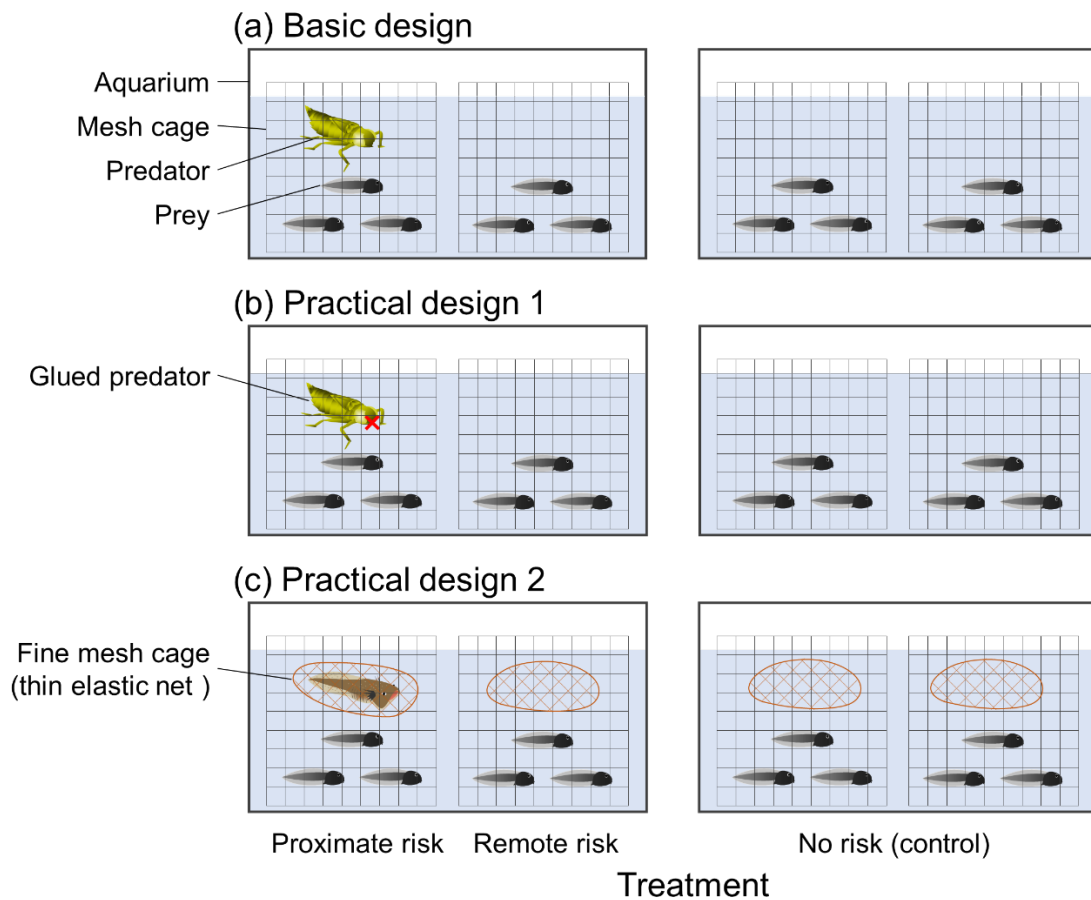
491 Wilson, M., Schack, H. B., Madsen, P. T., Surlykke, A., & Wahlberg, M. (2011).
492 Directional escape behavior in allis shad (*Alosa alosa*) exposed to ultrasonic clicks
493 mimicking an approaching toothed whale. *Journal of Experimental Biology*, *214*,
494 22–29.

495 Winkler, J. D., & Van Buskirk, J. (2012). Influence of experimental venue on phenotype:
496 multiple traits reveal multiple answers. *Functional Ecology*, *26*, 513–521.

497 Yamaguchi, A., Takatsu, K., & Kishida, O. (2016). Contacts with large, active individuals
498 intensify the predation risk of small conspecifics. *Ecology*, *97*, 3206–3218.

499 Yasumoto, K., Nishigami, A., Yasumoto, M., Kasai, F., Okada, Y., Kusumi, T., & Ooi, T.
500 (2005). Aliphatic sulfates released from *Daphnia* induce morphological defense of
501 phytoplankton: isolation and synthesis of kairomones. *Tetrahedron Letters*, *46*,
502 4765–4767.

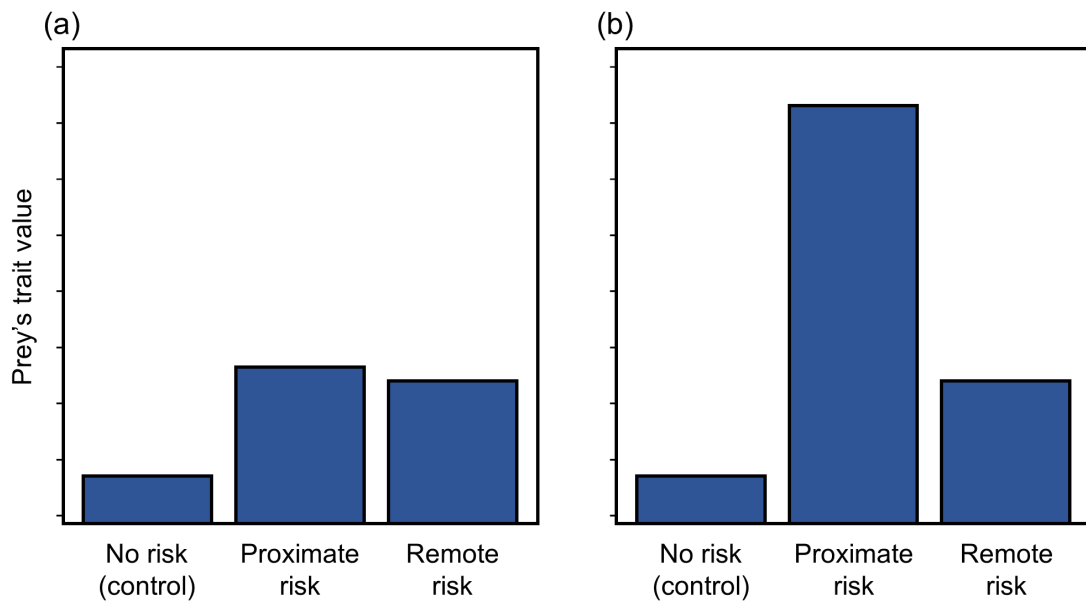
503 **Figure Legends**



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

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



521

522

FIGURE 2 Schematic diagrams of possible results of the experiment. The vertical line

523

indicates the prey's trait value that is assumed to be changed by the presence of the

524

predator. If the trait value between the proximate risk and remote risk treatments is similar

525

(i.e., scenario (a)), proximate stimuli is not relatively important as a trigger of prey's trait

526

response, compared to remote stimuli. On the contrary, if difference in the trait value

527

between the proximate risk treatment and No risk (control) treatment is larger than that

528

between the remote risk treatment and No risk (control) treatment (i.e., scenario (b)), the

529

result shows that proximate stimuli is a significantly important cues for prey's trait

530

responses.