

REVIEW

Ecological Monographs, 92(1), 2022, e01487
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Chemical disturbance cues in aquatic systems: a review and prospectus

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Citation: Crane, A. L., K. R. Bairos-Novak, J. A. Goldman, and G. E. Brown. 2022. Chemical disturbance cues in aquatic systems: a review and prospectus. *Ecological Monographs* 92(1):e01487. 10.1002/ecm.1487

Abstract. In the natural environment, animals can face potentially dangerous and often regular exposure to major environmental fluctuations such as flash flooding and drought, or the approach of a predator. For many aquatic species, exposure to these ecological disturbances triggers the release of “disturbance cues” – generally characterized as chemicals released when animals are startled but not injured. While the chemistry of such cues remains largely unexplored, they appear to provide early warning information to nearby individuals, potentially leading to behavioral decisions that increase overall fitness, particularly for social species that may coordinate group defense. In the literature, disturbance cues have received little attention relative to other chemical cues, such as damage-released alarm cues. However, recent advances in the study of disturbance cue communication have led an uptick in research on the subject. Here, we review the existing literature on responses to disturbance cues in aquatic systems. Although the majority of studies involve behavioral responses to a simulated predator approach, we describe various disturbance types across a broad range of taxa. We discuss the ecological implications of disturbance cues, including their role in risk assessment, signaling, learning, and species specificity. We also address several methodological challenges for this developing field of study, as well as the ethical and conservation implications of this research going forward. Future research on disturbance cues should address a number of key unknowns, including questions regarding disturbance cue chemistry, function, and generality.

Key words: communication; distress; perturbation; pheromones; risk assessment; signals; stress.

INTRODUCTION

Disturbances are physical forces, agents, or processes that cause disruption to an ecosystem (Rykiel 1985). Abiotic examples include climatic events such as flooding or drought, whereas biotic disturbances include aggression from competitors or predators (e.g., Connell 1978, Malmström and Raffa 2000). Exposure to disturbances can result in injury or death, so animals often employ phenotypic changes that allow them to escape these potential threats (Johnson et al. 1992, Schreck et al. 1997). However, such responses are energetically costly and expend time needed for essential activities

such as foraging (Cuthill and Houston 1997). Therefore, natural selection should favor individuals that correctly detect, identify, and respond appropriately to cues indicating disturbances (Dill 1987).

Environmental cues can take a variety of forms including auditory, visual, tactile, and chemical stimuli. Therefore, animals have evolved an array of sensory adaptations, such as the gustatory, olfactory, and vomeronasal organs used for chemoreception in many species (Eisthen and Schwenk 2008, Stevens 2013). Chemical cues are particularly useful in the dark and can travel around visual obstructions (Alberts 1992, Hickman et al. 2004). Unlike tactile cues, chemicals can be detected without being in close proximity to the source. Such cues are particularly useful within aquatic habitats, as diffusion, laminar advection, and microturbulence can each provide valuable information to receivers and can result in a rapid information cascade through social

Manuscript received 13 April 2021; revised 7 July 2021; accepted 8 July 2021. Corresponding Editor: Andrea E. Kirkwood.

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aggregations or subpopulations (Brönmark and Hansson 2000, Webster and Weissburg 2009).

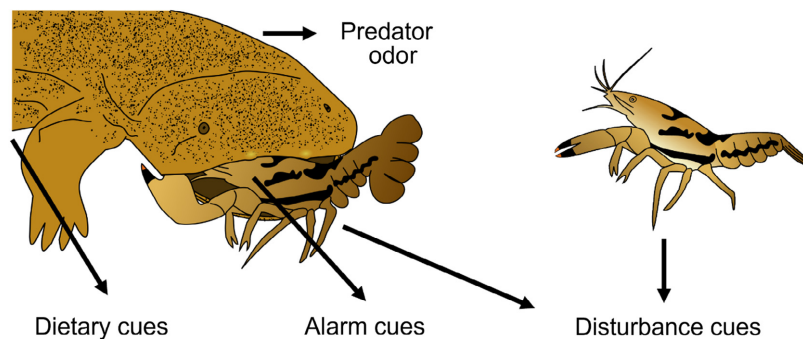
Chemical communication occurs when a chemical released by an *emitter* individual causes a phenotypic change in a *receiver* individual (Brönmark and Hansson 2000, Wisenden 2015b, Mathis and Crane 2017). These chemical messengers are known as *semiochemicals*, which have historically been categorized as intraspecific *pheromones* or interspecific *allelochemicals* (Mathis and Crane 2017). However, a more restrictive definition of pheromones has been widely adopted, in which pheromones are chemicals that have evolved to provide a communication benefit to the emitter (Wisenden 2015b). In this framework, a pheromone is described as a *signal* from a *sender* individual, whereas unmodified chemicals are known simply as *cues* (Maynard Smith and Harper 2003). In contrast, allelochemicals include chemicals that provide fitness benefits to the emitter but not the receiver (i.e., *allomones*), in addition to chemicals that benefit the receiver but not the emitter (i.e., *kairomones*). For example, predator odors are classified as kairomones when received by prey because prey benefit by detecting and responding appropriately to predators, whereas predators lose their ambush advantage over potential prey (Mathis and Crane 2017).

CHEMICAL DISTURBANCE CUES DEFINED

Chemical disturbance cues are characterized as chemical compounds that are released from individuals when *startled but not injured* (Wisenden 2015a). Historically, these cues have also been referred to as disturbance signals, early warning signals, disturbance pheromones, stress pheromones, stress cues, and distress cues (e.g., Hazlett 1985, Wisenden et al. 1995, Chivers and Smith 1998). While the source and chemical nature of disturbance cues remain largely unexplored, a few studies have explored urinary ammonia as the active ingredient (see Section “*Invertebrates*”). The release of corticosteroids and other endogenous correlates of stress may also function as disturbance cues, although there is currently little evidence for such a role (see Section “*Fishes*”).

Disturbance cues are one of several types of chemicals that are available for risk assessment, along with kairomones, injury- or damage-released “alarm cues,” and cues released from prey as they are consumed by predators (i.e., “dietary alarm cues”) (Fig. 1a; Wisenden and Chivers 2006, Wisenden 2015a). While the exact chemistry of alarm cues remains poorly understood, several substances have been identified within species-specific mixtures contained in skin and muscle tissue (von Frisch

(a) Chemical cues used in assessment of predation risk



(b) Cue availability during the predation sequence

	Detection	Attack	Capture	Ingestion
Predator odor:	✓	✓	✓	✓
Alarm cues:			✓	
Dietary cues:				✓
Disturbance cues:		✓	✓	

FIG. 1. (a) Sources of chemical information about predation risk include predator odors (kairomones) and dietary cues released from predators, and alarm cues and disturbance cues released from prey (depicted by a hellbender salamander *Cryptobranchus alleganiensis* and crayfish *Faxonius neglectus*). (b) Temporal availability of these chemical cues during the predation sequence. Modified from Wisenden (2015a).

1938, Brown and Smith 1997, Meuthen et al. 2018). These cues are thought to serve as a reliable indicator of risk to prey because they are released only when prey tissues are damaged, such as during a predator attack. Disturbance cues, however, may represent any number of threatening or non-threatening events and are therefore considered as a less reliable indicator of risk. Although disturbance cues may still be released by injured prey, their release prior to injury may provide earlier warning information in the predation sequence (Endler 1991, Ferrari et al. 2008, Wisenden 2015a) (Fig. 1b), decreasing the chances of being harmed by a disturbance.

There is a “gray area” in categorizing certain cues as disturbance cues vs. alarm cues. For example, many salamanders produce “secretions” that are released upon disturbance without sustaining injury (Mathis and Vincent 2000, Crane and Mathis 2013). However, these secretions appear to be contained within skin glands, similar to the chemical alarm cues of many fishes (Chivers et al. 2007). Therefore, salamander secretions exhibit characteristics of both aquatic disturbance cues and alarm cues. Unfortunately, our limited knowledge regarding disturbance cue chemistry and release mechanisms currently prevents us from using such characteristics for categorization. Further ambiguity in what constitutes a disturbance cue is apparent when considering chemicals that are overtly defensive repellants. For instance, when disturbed, skunks spray noxious secretions (Fisher and Stankowich 2018), squid release colored ink (Wood et al. 2010), some birds defecate in flight (Tillmann 2009), and some grasshoppers regurgitate their gut contents (Sword 2001). It is possible that these defensive cues alert nearby individuals to the threat, but we are not aware of current evidence to support this function. For the purposes of this review, we define disturbance cues as “chemicals that are released by emitters when disturbed but uninjured, eliciting phenotypic changes consistent with predator avoidance in receivers.”

AIM AND SCOPE

While the damage-released chemical alarm cue system has been reviewed many times (e.g., Smith 1992, Chivers et al. 1996, Chivers and Smith 1998, Mathis 2009, Ferrari et al. 2010), disturbance cues have received far less scientific attention (an approximate 20-fold difference). Our goal, here, is to provide the first comprehensive review of disturbance cues in aquatic systems. We conducted a literature search (Web of Science, Google Scholar) for the terms: “disturbance cue,” “disturbance signal,” “disturbance pheromone,” “stress pheromone,” “stress cue,” and “distress cue,” while also performing additional opportunistic searching. In total, we found 55 studies on aquatic disturbance cues, as described above. After first being reported in 1985, disturbance cues had not received much attention until an uptick in recent years (23 studies in the past decade). We categorized studies by the donor and receiver species, the receiver’s

life stage (adult or juvenile), the receiver’s background (being primarily captive or wild), the type of experimental endpoints (e.g., behavioral), and the type of disturbance experienced by donors (e.g., exposure to predator cues), resulting in a total of 108 unique experimental tests. In this review, we discuss the ecological role and other implications of disturbance cues, including the chemistry and release of disturbance cues across taxa, their ecological roles in risk assessment, and their potentially beneficial application in conservation science.

TYPES OF STUDIES

Tests of adult and juvenile life-stages are each well represented in the disturbance cue literature (Table 1a). In nearly all cases, the life stage of receivers matched that of the donors. One exception is a study that exposed wood frog (*Lithobates sylvaticus*) embryos to disturbance cues from older conspecifics and then tested the embryos once they had developed into free-swimming larvae (I. A. E. Rivera-Hernández et al., *unpublished manuscript*).

The vast majority of disturbance cue studies have documented effects on receivers that were purely behavioral (Table 1b). The metrics used to assess such responses are generally the same as (or consistent with) those known to be indicative of predator-avoidance responses among prey species. Commonly reported behavioral responses to disturbance cues include decreased foraging rates and changes in overall activity/space use. Some postural changes associated with disturbance cue reception have also been observed, such as the “head up” vigilance behavior of Iowa darters (*Etheostoma exile*) (Wisenden et al. 1995) and the increased “neutral” posture of the virile crayfish (*Orconectes virilis*), which represents alertness rather than rest or aggression (Hazlett 1985). Group-living species, such as the guppy (*Poecilia reticulata*), are known to increase aggregation behavior (e.g., shoaling tightness) in response to disturbance cues, and juvenile animals may seek protection near adults, as has been observed in red sea urchins (*Strongylocentrotus franciscanus*) (Nishizaki and Ackerman 2005).

A few studies have explored non-behavioral endpoints, often in addition to behavioral changes (Table 1 b). Most of these studies reported that exposure to disturbance cues causes increased cortisol production (all in fishes) (e.g., Oliveira et al. 2013, Barcellos et al. 2014). Joyce and Brown (2020) explored changes in neural morphology in northern red-bellied dace (*Chrosomus eos*), as relative hypothalamic size and asymmetry are known to correlate with long-term risk exposure in some species (Gonda et al. 2013, Reddon et al. 2018). While repeated exposure to conspecific alarm cues (2 times/day for 14 d) led to smaller and more asymmetrical hypothalami (and significant correlations with individual risk taking tactics), similar exposures to disturbance cues did not differ from controls (Joyce and Brown 2020). Finally, one study explored survival endpoints following exposure to

TABLE 1. Percentage of disturbance cue tests classified by (a) receiver life stage, (b) experimental endpoints, (c) type of disturbance cues used to induce cue release, (d) and taxonomic class of donor individuals.

Category of studies	%
(a) Receiver life stage	
Adult	65.7
Juvenile	34.3
(b) Endpoints used	
Behavioral	88.0
Physiological	11.1
Morphological	0.9
(c) Disturbance type	
Simulated predator chase	55.6
Restraint/handling stress	17.6
Visual and/or chemical predator cues	8.3
Confinement/crowding	5.6
Forced swimming	5.6
Agonistic interactions	3.7
Acidic pulse	0.9
Cortisol exposure	0.9
Food deprivation	0.9
Heat	0.9
(d) Donor taxonomic class	
Actinopterygii	46.3
Malacostraca	22.2
Amphibia	19.4
Mammalia	5.6
Reptilia	2.8
Insecta	1.9
Echinoidea	0.9
Hirudinea	0.9

disturbance cues, documenting higher survivorship in encounters with predators (Mirza and Chivers 2002). In that study, brook charr (*Salvelinus fontinalis*) were exposed to the odor of a predator while simultaneously being exposed to cues from either disturbed or undisturbed conspecifics. Then, in subsequent encounters with a live predator, the charr that were pre-exposed to disturbance cues experienced significantly higher survival (Mirza and Chivers 2002).

Most disturbance cue studies use a simulated predator chase as the disturbance that elicits cue release (Table 1c). However, several other studies have used physical restraint (e.g., handling stress: Bett et al. 2016, Joyce and Brown 2020), agonistic stress from competition (e.g., Hazlett 1985, Briffa and Williams 2006), confinement stress (e.g., crowding at high densities: Manteifel et al. 2005, Fulton et al. 2017), or exposure to visual or chemical cues from live predators (e.g., Jordão and Volpato 2000, Nishizaki and Ackerman 2005). Forced swimming has also been used to obtain aquatic disturbance cues from terrestrial organisms (Abel 1991a), whereas other types of disturbances have received little attention.

A few studies have compared different types of disturbance, although direct comparisons pose logistical challenges (see Sections “Concentrations” and “Experimental

controls”). In zebrafish (*Danio rerio*), disturbance cues that were released upon either a simulated predator chase, an acidic pulse, or food deprivation, each caused receiver avoidance responses, whereas cues from a donor exposed to visual cues from a novel predator did not (Abreu et al. 2016). Hazlett (1985) reported that the disturbance cue responses of virile crayfish were similar regardless of whether donors were disturbed by being chased, aggressing conspecifics, or heat-stress. Giaquinto and Hoffmann (2012) found that spotted sorubim (*Pseudoplatystoma corruscans*) responded more strongly to disturbance cues from chased conspecifics compared with cues that were released in response to physical confinement. Additionally, exposure to cortisol has been used to elicit disturbance cue release, although such treatment had no effect (Bett et al. 2016). We are not aware of studies involving other forms of disturbance, such as noise disturbance or changes in water levels (e.g., pond drying).

Finally, most studies have tested males and females together, rather than distinguishing between the sex of receivers or donors. Bett et al. (2016) found that both male and female sockeye salmon (*Oncorhynchus nerka*) avoided disturbance cues, but only females showed a significant cortisol increase. In general, we might expect that responses to disturbance cues would be weaker among males, as their higher variance in reproductive payoff should lead to more risk taking (Schuett et al. 2010). Moreover, sexually active males may show particularly weak responses to disturbance cues from female conspecifics, as this can potentially be a conflicting situation (disturbance avoidance vs. mating cues) (Hazlett 1985, 1989).

TAXONOMY, CHEMISTRY, AND RELEASE

Invertebrates

Hazlett (1985) conducted the earliest work on disturbance cues, using the virile crayfish in a series of experiments. Since then, disturbance cues have been studied in several other crayfish species, as well as in two other malacostracans (both hermit crabs Hazlett 1990a, Briffa and Williams 2006). While this taxonomic group has received the majority of attention among aquatic invertebrates, two insects, a leach, and an echinoderm have also been tested in response to disturbance cues (Table 1d), as have terrestrial invertebrates (e.g., Landauer and Chapnick 1981, Crane et al. 2013).

Hazlett (1990b) hypothesized that the active ingredient of disturbance cues in virile crayfish was an ammonia/ammonium ion. Indeed, ammonium in the form of NH_4Cl was found to elicit a weak avoidance response in comparison with standard conspecific disturbance cues. Ammonia is primarily released by the gills in crayfish. However, plugging the green glands of the donors prevented this response, suggesting that excretory substances from these glands were responsible for eliciting

the full avoidance response (Hazlett 1990b). Tests on the red swamp crayfish (*Procambarus clarkii*) revealed that gill water from stressed donors did not elicit an avoidance response, whereas urine from stressed donors did (Zulandt Schneider and Moore 2000). Similarly, for the signal crayfish (*Pacifastacus leniusculus*), urine from stressed donors elicited a response (Stebbing et al. 2010). When the donors' nephropores (urinary pores) were blocked, their cues did not elicit responses in receivers. Therefore, in crayfish the active compound(s) in disturbance cues appear likely to be contained in urine and not solely ammonia/ammonium.

Amphibians

A few anuran species have been found to respond to disturbance cues, with wood frog tadpoles being the subject of several contextual studies (e.g., Bairos-Novak et al. 2017, Bairos-Novak et al. 2019a). Kiesecker et al. (1999) found that red-legged frogs produce more ammonia when disturbed, and receivers avoided both conspecific disturbance cues and ammonium (NH_4^+). Similarly, toad tadpoles (*Bufo bufo*) are known to avoid ammonia (NH_3) (Manteifel et al. 2005). Both studies indicate that pulses of ammonia/ammonium may function as disturbance cues for anurans, but other compounds have not been tested. As with crayfish, ammonia/ammonium may be only one of multiple active components of anuran disturbance cues.

As mentioned previously, many salamander species release secretions commonly referred to as "alarm secretions" (e.g., Watson et al. 2004, Gall et al. 2010, von Byern et al. 2017). Like disturbance cues, these secretions are released upon disturbance but do not require any tissue damage. In the fully aquatic hellbender (*Cryptobranchus alleganiensis*), physical restraint with a net or from human handling is known to trigger the release of the secretion, which can then elicit avoidance responses in conspecific receivers (Crane and Mathis 2011). Hellbender secretion is predominantly protein based (A. L. Crane, *unpublished manuscript*) and appears to be contained in cells in the epidermis, similar to the club cells containing chemical alarm cues in some fishes (Crane and Mathis 2013). These studies collected the secretion in water baths, but whether other sources of chemical disturbance cues (e.g., in urine) were released is unknown. Hazlett (1989) obtained disturbance cues from red-spotted newts (*Notophthalmus viridescens*) by performing a simulated predator chase, with the resulting tank water being fed into tanks containing virile crayfish as cue receivers. Although crayfish avoided the newt disturbance cues, unlike undisturbed newt cues, their responses appeared "qualitatively different" than their responses to disturbance cues from crayfish donors (Hazlett 1989). Although the release pathway and chemical nature of the newt disturbance cues were not determined, Hazlett speculated that they were toxic to crayfish, as this newt species can release a toxin

(tetrodotoxin) stored in the whole body and secreted by skin glands to repel predators (Mebs et al. 2010). Whether newts and other salamanders possess multiple sources (or release different types) of disturbance cues is a topic of future interest.

Fishes

Fishes have received, by far, the most attention in the context of disturbance cues, although only ray-finned fishes (Class: Actinopterygii) have been tested. Most studies have involved freshwater species, but there are also a few marine examples (e.g., Olivotto et al. 2002, Fulton et al. 2017). One study verified the response to disturbance cues in natural populations of Trinidadian guppies (Goldman et al. 2020a) and, to date, this is the only disturbance cue study (fish or not) under fully natural conditions. When a novel heterospecific model was paired with disturbance cues, rather than a stream water control, guppies inspected at lower rates and in smaller groups, while also taking longer to do so. Such changes in inspection behavior are well established as being consistent with an increase in perceived predation risk for this species (e.g., Dugatkin and Godin 1992, Brown et al. 2010).

Among fishes, disturbance cues have been argued to be metabolic by-products, released in urine or across the gill epithelia (Wisenden 2015a). Although most nitrogenous waste is released as ammonia in most fish species, a small percentage is typically released as urea (Smutna et al. 2002, Wilkie 2002, Altinok and Grizzle 2004, Weihrauch et al. 2009). Vavrek et al. (2008) tested the prediction that ammonia may function as an active component of the teleost disturbance cue, as thought for some amphibians (e.g., Kiesecker et al. 1999). When disturbed, convict cichlids (*Amatitlania nigrofasciata*) and rainbow trout (*Oncorhynchus mykiss*) showed no detectable increase in ammonia (NH_4^+) production, and exposure to ammonia at a relevant concentration (0.5 mg/L) caused no detectable change in their behavior (Vavrek et al. 2008). However, in a latter study, reagent-grade urea [$\text{CO}(\text{NH}_2)_2$] was found to elicit avoidance responses in both species, and at an intensity matching their responses to conspecific disturbance cues (Brown et al. 2012). More recently, Goldman et al. (2021) found support for the hypothesis that the disturbance cues of guppies are affected by diet. Receivers responded more intensely to disturbance cues from donors that were fed a protein-rich diet and an overall larger diet. Both findings are consistent with the hypothesis that disturbance cues produced by some species are a by-product of metabolized protein (Lebedeva and Golovkina 1994).

Cortisol may also have the potential to function as a disturbance cue in fishes, being upregulated in response to stress. However, sockeye salmon (*Oncorhynchus nerka*) were found to respond to water conditioned with conspecific disturbance cues but not toward reagent-grade water-borne cortisol (Bett et al. 2016). In another

study, zebrafish did not increase whole-body cortisol when disturbed by visual cues from a predator (Barcellos et al. 2014). However, the tank water from disturbed individuals still elicited receiver responses. Although neither study provided evidence for cortisol as a disturbance cue, further study on other species and other candidate endogenous correlates of stress should be considered (Lebedeva et al. 1994).

Gulf toadfish (*Opsanus beta*) are known to switch between releasing ammonium as their primary nitrogenous waste to releasing pulses of urea across the gills, apparently to communicate reproductive status (Cartolano et al. 2019a,b). Fulton et al. (2017) found that the rate of such pulses increased when exposed to waterborne cues from conspecifics that were crowded in high density, consistent with a reproductive function. Cues from uncrowded (i.e., undisturbed) individuals would be interesting to compare to determine whether such changes might also have a disturbance cue function. In subsequent tests, exposure to ammonium in the form of NH_4Cl caused a small, but significant, reduction in pulse latency, whereas exposure to urea, cortisol, and serotonin each had no effect (Fulton et al. 2017). Therefore, the release of ammonia, rather than pulses of urea, may have a disturbance cue role in this species.

Mammals

Mammals are well known to use a diversity of semiochemicals that play a central role in social behavior (Apps 2013). Although aquatic mammals, to our knowledge, have yet to receive attention in the context of disturbance cues, a few mammals that are predominantly terrestrial have been found to release aquatic disturbance cues (Table 2). Abel (1991a) conducted studies on rats (*Rattus norvegicus*) and mice (*Mus musculus*) that were disturbed in water. After the individuals were removed, the water was found to elicit avoidance responses in receivers, as well as the donors themselves, and this response was not due simply to contamination by feces or urine (Abel and Bilitzke 1990). The active chemical(s) in murine disturbance cues may be cues released either from the perianal region (e.g., 4-methylpentanal, Inagaki et al. 2014) or as urinary metabolites (e.g., biopyrrins, Miyashita et al. 2006). Indeed, urine has been identified as a disturbance cue for some mammal species in terrestrial environments, such as cattle (*Bos taurus*) that decrease foraging and exploration when exposed to urine from stressed vs. unstressed individuals (Boissy et al. 1998). There is also evidence that humans alter their urine chemistry in response to stress (e.g., increasing noradrenaline release: Babisch et al. 2001), although to our knowledge, recognition of such changes has not been tested.

Other taxa

Other taxa have received far less attention in the context of disturbance cues. For instance, many reptile

species are known to release “musk” from the Rathke’s gland when disturbed (Mason 1992), but we are aware of only one test of musk as a disturbance cue (Churilla 2015). In that study, cottonmouth (*Agkistrodon piscivorus*) musk caused receivers to increase chemosensory behaviors and avoidance, therefore providing evidence for musk as a disturbance cue in this species. Hazlett (1990b) collected disturbance cues from another reptile, the painted turtle (*Chrysemys picta*), finding that the cues had no effect on the behavior of virile crayfish. Also receiving little attention are birds, having been largely neglected in a chemosensory context. However, birds are known to respond to odors and produce a variety of odor compounds that could potentially function in intraspecific communication (Roper 1999, Hagelin 2007). Even bacteria communicate between individuals, by excreting chemicals known as “autoinducers” in a process known as “quorum sensing.” For example, streptococci can release a peptide that has been described as an “alarmone” that may act as early warning information, preparing their colony for hostile environmental conditions (Dufour and Lévesque 2013). Plants, too, have been found to release volatiles when disturbed (e.g., by drought), evoking avoidance responses (e.g., stomatal closure) in nearby conspecifics (Falik et al. 2011). While many taxonomic gaps in our knowledge remain, these examples indicate that chemical communication of disturbance is a broad biological theme.

METHODOLOGY AND EXPERIMENTAL CONSIDERATIONS

Obtaining disturbance cues

The process of obtaining disturbance cues involves exposing donor individuals to a disturbance and then collecting the released cue, often in solution. Typically, water occupied by the disturbed individual is used. However, if the release site is known, a more direct collection (e.g., a catheter) and quantification of the chemicals can also be implemented (Zulandt Schneider and Moore 2000). There is no standard for the duration of the disturbance treatment, although studies typically use less than 10 min, with many using only 30–60 s. However, one important stipulation is that the donor is not injured during the process, which could potentially release chemical alarm cues in addition to disturbance cues. Therefore, when obtaining disturbance cues, researchers should avoid procedures that might impose any tissue damage (e.g., striking the donor during a simulated predator chase).

Concentrations

Researchers seek to use disturbance cue concentrations that are ecologically relevant. The donor environment generally consists of an aquarium filled with water containing one or more disturbed donors. The tank size and water volume depend, of course, on the body size of

TABLE 2. Donor–receiver species combinations tested in studies on responses to disturbance cues.

Donor species	Receiver species	Response	Literature		
Actinopterygii					
<i>Amatilania nigrofasciata</i>	<i>Amatilania nigrofasciata</i>	+	Brown et al. (2012)		
		+	Goldman et al. (2019)		
		+	Jordão (2004)		
		+	Vavrek and Brown (2009)		
		+	Vavrek et al. (2008)		
		<i>Oncorhynchus mykiss</i>	+	Vavrek et al. (2008)	
			+	Goldman et al. (2019)	
		<i>Ambloplites rupestris</i>	<i>Orconectes virilis</i>	+	Hazlett (1990b)
				+	Manassa et al. (2013)
		<i>Amphiprion percula</i>	<i>Amphiprion percula</i>	+	Joyce and Brown (2020)
<i>Chrosomus eos</i>	<i>Chrosomus eos</i>	–	Bryer et al. (2001)		
<i>Cottus cognatus</i>	<i>Diplodus sargus</i>	+	Olivotto et al. (2002)		
<i>Crenilabrus quinqueaculatus</i>	<i>Danio rerio</i>	+	Abreu et al. (2016)		
		+	Barcellos et al. (2014)		
		+	Oliveira et al. (2013)		
<i>Diplodus anularis</i>	<i>Diplodus sargus</i>	+	Olivotto et al. (2002)		
<i>Diplodus sargus</i>	<i>Diplodus sargus</i>	+	Olivotto et al. (2002)		
<i>Etheostoma exile</i>	<i>Etheostoma exile</i>	+	Wisenden et al. (1995)		
		+	Hazlett (1990b)		
<i>Ictalurus natalis</i>	<i>Orconectes virilis</i>	–	Hazlett (1989)		
<i>Oncorhynchus gorbuscha</i>	<i>Oncorhynchus gorbuscha</i>	–	Bett et al. (2016)		
<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	+	Brown et al. (2012)		
		+	Ferrari et al. (2008)		
		+	Toa et al. (2004)		
		+	Vavrek et al. (2008)		
		+	Vavrek and Brown (2009)		
		+	Vavrek et al. (2008)		
		<i>Oncorhynchus nerka</i>	<i>Oncorhynchus nerka</i>	–	Bett et al. (2016)
				+	Fulton et al. (2017)
		<i>Opsanus beta</i>	<i>Opsanus beta</i>	+	Barcellos et al. (2011)
		<i>Oreochromis niloticus</i>	<i>Oreochromis niloticus</i>	+	Jordão and Volpato (2000)
<i>Piaractus mesopotamicus</i>	<i>Pimephales promelas</i>	+	Bairos-Novak et al. (2019b)		
<i>Pimephales promelas</i>	<i>Pimephales promelas</i>	+	Crane et al. (2020b)		
<i>Poecilia reticulata</i>	<i>Poecilia reticulata</i>	+	Goldman et al. (2019)		
		+	Goldman et al. (2020a)		
		+	Goldman et al. (2020b)		
		+	Goldman et al. (2021)		
		+	Goldman et al. (2019)		
		+	Pollock et al. (2021)		
		+	M. S. Pollock et al. (<i>unpublished manuscript</i>)		
<i>Pomacentrus nagasakiensis</i>	<i>Pomacentrus nagasakiensis</i>	+	Giaquinto and Hoffmann (2012)		
<i>Pseudoplatystoma corruscans</i>	<i>Pseudoplatystoma corruscans</i>	+	Barcellos et al. (2011)		
<i>Rhamdia quelen</i>	<i>Salvenius fontinalis</i>	+	Mirza and Chivers (2002)		
<i>Salvenius fontinalis</i>					
Amphibia					
<i>Bufo bufo</i>	<i>Bufo bufo</i>	+	Manteifel et al. (2005)		
<i>Cryptobranchus alleganeinsis</i>	<i>Cryptobranchus alleganeinsis</i>	+	Crane and Mathis (2011)		
		+	Crane and Mathis (2013)		
		–	Gall and Mathis (2010)		
		+	Gall and Mathis (2011)		
		–	Gall and Mathis (2010)		
		–	Gall and Mathis (2010)		
		–	Gall and Mathis (2010)		
		–	Gall and Mathis (2010)		
		–	Gall and Mathis (2010)		
		+	Gall and Mathis (2010)		
+	Gall and Mathis (2010)				
<i>Lithobates sylvaticus</i>	<i>Lithobates sylvaticus</i>	+	G. H. Achtymichuk et al. (<i>unpublished manuscript</i>)		
		+	G. H. Achtymichuk et al. (<i>unpublished manuscript</i>)		

TABLE 2 Continued.

Donor species	Receiver species	Response	Literature
		+	Bairos-Novak et al. (2017)
		+	Bairos-Novak et al. (2019a)
		+	Bairos-Novak et al. (2020)
		+	I. A. E. Rivera-Hernandez et al. (unpublished manuscript)
<i>Pethophylax perezii</i>	<i>Pethophylax perezii</i>	+	Gonzalo et al. (2010)
<i>Rana aurora</i>	<i>Rana aurora</i>	+	Kiesecker et al. (1999)
Echinoidea			
<i>Strongylocentrotus franciscanus</i>	<i>Strongylocentrotus franciscanus</i>	+	Nishizaki and Ackerman (2005)
Hirudinea			
<i>Macrobdella decora</i>	<i>Orconectes virilis</i>	+	Hazlett (1990b)
Insecta			
<i>Ishnura cervula</i>	<i>Ishnura cervula</i>	+	Siepielski et al. (2016)
<i>Paraleptophlebia adoptiva</i>	<i>Paraleptophlebia adoptiva</i>	–	Ode and Wissinger (1993)
Malacostraca			
<i>Calcinus laevimanus</i>	<i>Calcinus laevimanus</i>	+	Hazlett (1990a)
<i>Cambarus diogenes</i>	<i>Orconectes virilis</i>	+	Hazlett (1989)
<i>Cherax destructotor</i>	<i>Cherax destructotor</i>	+	Hazlett and Lawler (2010)
	<i>Euastacus armatus</i>	+	Hazlett and Lawler (2010)
<i>Cherax rotundus</i>	<i>Cherax destructotor</i>	+	Hazlett and Lawler (2010)
<i>Euastacus armatus</i>	<i>Cherax destructotor</i>	+	Hazlett and Lawler (2010)
	<i>Euastacus armatus</i>	+	Hazlett and Lawler (2010)
<i>Orconectes propinquus</i>	<i>Orconectes propinquus</i>	–	Hazlett (1990b)
	<i>Orconectes virilis</i>	+	Hazlett (1985)
<i>Orconectes rusticus</i>	<i>Orconectes rusticus</i>	–	Hazlett (1985)
		–	Hazlett (1990b)
	<i>Orconectes virilis</i>	+	Hazlett (1985)
<i>Orconectes virilis</i>	<i>Orconectes virilis</i>	+	Hazlett (1990b)
		+	Hazlett (1985)
		+	Hazlett (1985)
		+	Hazlett (1989)
		+	Hazlett (1990b)
	<i>Orconectes propinquus</i>	–	Hazlett (1990b)
<i>Pacificatus leniusculus</i>	<i>Pacificatus leniusculus</i>	+	Stebbing et al. (2010)
<i>Pagurus bernhardus</i>	<i>Pagurus bernhardus</i>	+	Briffa and Williams (2006)
<i>Procambus clarkii</i>	<i>Procambus clarkii</i>	+	Zulandt Schneider and Moore (2000)
Mammalia			
<i>Mus musculus</i>	<i>Rattus norvegicus</i>	+	Abel (Abel 1991a)
<i>Rattus norvegicus</i>	<i>Rattus norvegicus</i>	+	Abel (Abel 1991a)
		+	Abel (Abel 1991b)
		+	Abel (Abel 1992)
		+	Abel and Biltzke (1990)
	<i>Mus musculus</i>	–	Abel (Abel 1991a)
Reptilia			
<i>Agkistrodon piscivorus</i>	<i>Agkistrodon piscivorus</i>	+	Churilla (2015)
<i>Chrysomes picta</i>	<i>Orconectes virilis</i>	–	Hazlett (1990b)

Note: Plus (+) signs represent significant responses, whereas minus (–) signs represent non-significance.

the species. In many cases with small-bodied species, multiple donor individuals are disturbed together within a shared donor tank, with densities varying (e.g., 1 individual per 10–500 mL of water), again depending on body size (e.g., Vavrek et al. 2008, Gonzalo et al. 2010). Upon disturbance, the donor has the opportunity to release disturbance cues, diluted in the tank water (i.e., “bath” water). In many studies, the water is pumped into

a separate tank containing the cue receiver, with variable flow rates across studies (e.g., 20 mL to 40 L per min) (Hazlett 1990b, Bett et al. 2016). This can occur continuously during the disturbance treatment, or after the disturbance has ended. In contrast with flow-through setups, researchers in many studies simply collect small samples of bath water (e.g., 10–60 mL aliquots) following the disturbance (Bairos-Novak et al. 2019b,

Goldman et al. 2019). When the cues are added to a receiver tank, they will be further diluted according to the volume of water in the receiver tank (usually the same size as the donor tank). The length of time that the disturbance treatment lasts will also influence the disturbance cue concentration. These variables (body size, tank volume and density, test cue volume or flow rate, and length of disturbance) present logistical challenges to comparing the intensity of disturbance cue responses across studies, and unfortunately prevents us from comparing such intensities across species or other categories.

Degradation

The degradation rate of disturbance cue is largely unknown. Hazlett (1985) noticed a trend toward reduced responsiveness to disturbance cues in virile crayfish about 45 min after their release. Bairos-Novak et al. (2017) were concerned about such a tendency after 3 h for wood frog tadpoles. Abel (1991b), however, found that disturbance cues released from rats in water were effective 8 d later. Therefore, longevity of effectiveness is likely to be species specific. We recommend that researchers use disturbance cues as rapidly as possible until the longevity of effectiveness is confirmed in their study species. We know of two studies that used frozen disturbance cues (at approximately -20°C), both finding that the cues were at least partially effective when frozen shortly after release and then used shortly after thawing (Goldman et al. 2020a; I. A. E. Rivera-Hernández et al., *unpublished manuscript*). Theoretically, disturbance cues should not be effective for long after a disturbance, as this would evoke responses by receivers that are no longer correct and would therefore have a net fitness cost.

Depletion

We also know little about whether disturbance cue emitters become “depleted” in their cue production/release following long or repeated disturbances. While we might expect that repeated disturbances lead to a reduction in the quantity or quality of disturbance cues, few studies have addressed this question. Abel’s (1991b) work with rats revealed that repeated disturbance over 1 h did not cause freshly produced disturbance cues to lose their effectiveness. Similarly, wood frog tadpoles maintained their response to disturbance cues from donors that had been disturbed a second time after a previous disturbance 2 h earlier (G. H. Achtymichuk et al., *unpublished manuscript*). We expect that disturbance cues must become depleted at some point, but this has yet to be demonstrated.

Experimental controls

In any disturbance cue study, chemical cues from undisturbed individuals should be used as a control treatment. This allows researchers to confirm whether changes in

receiver responses result from disturbance rather than simply due to the odor of the donor species. Collection of undisturbed cues usually occurs immediately prior to the collection of disturbance cues, using the same individuals. This involves a small temporal confound (undisturbed always collected before disturbed) but controls for individual variation in odor. When collecting undisturbed cues, researchers must be careful not to disturb the donors, or at least keep disturbances to a minimum, so that there is a large qualitative difference in disturbance level when generating the two cues. We recommend at least 1 h of acclimation prior to the gentle collection of undisturbed cues. Bairos-Novak et al. (2017) implemented a gravity-fed hose system to inconspicuously remove and replaced the water. After donor individuals had acclimated in the system for 50 min, the water was gently replaced with clean water to minimize any disturbance cues that were released in response to the stress of being moved into the system. Then, donors were left undisturbed for an additional 10 min before the undisturbed cues were gently removed.

Excretion probably occurs when donors are both disturbed and undisturbed, but stressed individuals typically excrete more than unstressed individuals (Kiesecker et al. 1999, Zulantz Schneider and Moore 2000). Therefore, in some cases, responses to disturbance cues might simply be unpleasant reactions to increased metabolic waste. Controlling for such differences poses major logistical challenges and we know of only one study that has attempted to do so. Zulantz Schneider and Moore (2000) catheterized red swamp crayfish and collected urine from disturbed and undisturbed individuals. The urine from the stressed individuals induced avoidance response in receivers, but the undisturbed individuals did not produce a measurable amount of urine that could be tested. Another treatment in the study consisted of urine from stressed individuals combined with their bath water, resulting in a lower urine concentration and no effect of receivers. Therefore, it remains unclear whether avoidance responses were caused by information about disturbance or simply the unpleasantness of a high concentration of urine. The disturbance cue literature has generally overlooked the potential for a simple effect of the unpleasantness of metabolic waste. However, such an effect would not explain the contextual effects of disturbance cues that have been observed in many studies (see Section “*Ecology of disturbance cues*”). Moreover, responses toward disturbance cues are often predator-avoidance tactics (e.g., shelter use and aggregation) rather than just distancing from the cue.

A positive control (e.g., predator cues) may also be useful in determining whether any disturbance cue response (e.g., increased activity) is indicative of avoidance rather than attraction, and whether such a response matches the intensity of other avoidance responses. We are aware of two studies that have used responses to chemical alarm cues as a positive control treatment to determine whether

disturbance cue responses were similar in intensity. Both studies tested anurans, finding that the behavioral responses to disturbance cues were weaker than those to alarm cues (Gonzalo et al. 2010; G. H. Achtymichuk et al., *unpublished manuscript*). There is also evidence for such a pattern in signal crayfish (Stebbing et al. 2010). However, these studies raise the question of how to use comparable concentrations of alarm and disturbance stimuli. Only by measuring the exact volume of the released chemical would this be possible. We posit that disturbance cues should indeed elicit weaker responses than alarm cues because, as mentioned above, disturbance cues are a less reliable indicator of risk (i.e., their encounter has a weaker correlation with mortal danger).

Disturbance cues may have undesirable (and unintended) effects in some studies, by interfering with treatment stimuli. For example, studies on the chemical ecology of predator/prey interactions often collect predator and non-predator odors by temporarily housing them in tanks with unfiltered water (Ferrari et al. 2010, Mathis and Crane 2017). In such cases, researchers should be careful to avoid housing conditions that are stressful to the donors, as confinement and other factors may induce the release of disturbance cues. Moreover, when obtaining the odors for use, care should be taken to avoid the acute release of disturbance cues. Because some species show generalized responses to metabolic waste products, contamination of species odors with their disturbance cues may have considerable consequences on experimental outcomes.

ECOLOGY OF DISTURBANCE CUES

Disturbance avoidance

Disturbance cues allow receivers to assess potential risks in their environment, providing opportunities to make informed behavioral decisions. According to the threat-sensitive predator-avoidance hypothesis (Helfman 1989), greater threats should elicit more intense avoidance responses, and such “threat-sensitivity” has been reported in studies that varied the concentrations of disturbance cues (i.e., higher concentrations elicited stronger behavioral responses) (Abel 1991b, Vavrek and Brown 2009). Ferrari et al. (2008) explored the idea that disturbance cues might serve as an early “warning signal” during the early stages of a predation sequence (Fig. 1b). In that study, rainbow trout were pre-exposed to either disturbance cues or undisturbed cues before a subsequent exposure (5 min later) to different concentrations of alarm cues. The two cues had an additive effect, in which trout that were pre-exposed to disturbance cues showed elevated responses to alarm cues. A similar additive cascade of information has previously been shown in glowlight tetras (*Hemigrammus erythrozonus*) (Brown et al. 2004). Tetras exposed to very low concentrations of alarm cues did not show a quantifiable increase in avoidance behavior, but they did exhibit an

increased response to a conspecific visual alarm display, suggesting that the “subthreshold” concentration primed them to respond to the visual threat. Similarly, disturbance cues, detected early in the predation sequence could “prime” prey to respond more intensely to information detected later.

As risk assessment is influenced by the overall risk level of a particular environment (Brown et al. 2013), several recent studies on disturbance cues have explored how high levels of background predation risk affect both donors and receivers (i.e., both production and response). Typically, repeated exposures to alarm cues are used to simulate high background risk (Brown et al. 2013, Crane et al. 2020a). In wood frog tadpoles, high background risk increased the responses of receivers to disturbance cues, and receivers with low background risk showed stronger responses to disturbance cues that were produced by high-risk donors (Bairos-Novak et al. 2017). However, the background risk exposures for receivers and donors were not additive (i.e., only one was required to elicit the maximum response) (Bairos-Novak et al. 2017). Similarly, guppies exposed to high background risk responded more strongly to disturbance cues from high-risk donors compared with low-risk donors, whereas low-risk receivers did not discriminate (Goldman et al. 2020a). This same pattern was also observed in guppies from natural populations experiencing variable background predation risk (Goldman et al. 2020a), as well as in situ (Goldman et al. 2020b).

High environmental risk can also be simulated by repeated exposures to disturbance cues. (I. A. E. Rivera-Hernández et al. *unpublished manuscript*) exposed wood frog embryos repeatedly to disturbance cues from conspecific tadpoles. After hatching, the tadpoles that had been exposed to disturbance cues as embryos showed weaker avoidance responses to disturbance cues compared to tadpoles that had not experienced the cues as embryos. This suggested that tadpoles had habituated to the cues as embryos. However, unlike unexposed individuals, the tadpoles that had been exposed to disturbance cues as embryos became neophobic, showing avoidance responses toward a novel odor (I. A. E. Rivera-Hernández et al., *unpublished manuscript*). Such phenotypically plastic neophobia in response to repeated disturbance cue exposure is of interest and is consistent with a predation threat signaling function of disturbance cues (see Section “*Disturbance signaling*”).

Within aquatic environments, there is likely to be a continuous and variable presence of nitrogenous waste products, either from the passive release of waste by animals or the decomposition of biological materials. Such background levels of nitrogenous waste may limit the detection and functionality of disturbance cue pulses. Brown et al. (2012) tested this “background noise” hypothesis (Fig. 2) by acclimating juvenile convict cichlids to varying levels of nitrogenous waste (urea). Cichlids responded to levels of urea (used as a proxy for disturbance cues) only at concentrations that exceed the

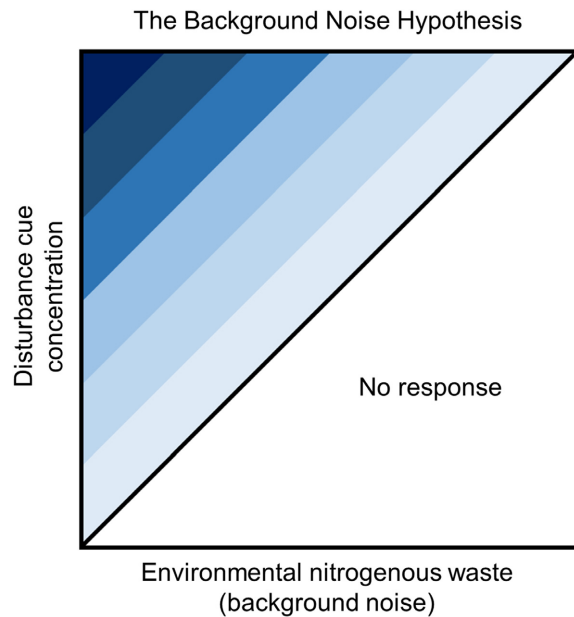


FIG. 2. The Background Noise Hypothesis predicts that the intensity of a receiver's response to disturbance cues (depicted by darkening shading) is driven by the concentration of disturbance cues relative to the background level of nitrogenous waste in the environment (Brown et al. 2012).

ambient level. Pollock et al. (2021) extended this hypothesis to marine environments, where disturbance cues appear to be released less frequently and at higher concentrations than in freshwater systems.

A prerequisite for avoiding conspecific disturbance cues, however, is that they are correctly recognized. Whether such recognition is innate or learned has not been directly tested as far as we know and would be particularly challenging given that receivers would need to be reared in isolation from conspecifics. We can, however, conclude that having experience in the natural environment is not necessary for disturbance cue responses. Our review revealed a greater frequency of responses to conspecific disturbance cues when test subjects had no experience in the natural environment, compared with test subjects that had experience in the wild before being tested in a laboratory environment (96 vs. 73%; test of proportions: $Z = 2.49$, $P = 0.006$, Table 3a). This may suggest that experience in the natural environment could influence receivers to discount disturbance cues in some situations.

Disturbance signaling

The initial discovery that disturbance cues could be modulated, either in quantity or quality, by risk levels (Bairos-Novak et al. 2017) raised the question of whether these cues could be released voluntarily as a "signal" (Maynard Smith and Harper 2003, Wisenden 2015b). Bairos-Novak et al. (2019b) found that fathead

TABLE 3. Frequency of significant responses to disturbance cues reported across studies, categorized by the donor–receiver relationship (conspecific vs. heterospecific), the receiver's background experience (wild vs. laboratory only), and whether the donors and receivers are sympatric.

Donor–receiver relationship	Wild experience	Sympatry	Response
(a) Conspecific			
Total			65 of 74 (88%)
	No	Yes	46 of 48 (96%)
	Yes	Yes	10 of 26 (73%)
(b) Heterospecific			
Total			23 of 34 (68%)
	No	Yes	4 of 6 (67%)
		No	4 of 4 (100%)
Subtotal, no wild experience			8 of 10 (80%)
	Yes	Yes	15 of 24 (63%)
		No	No data
Subtotal, wild experience			15 of 24 (63%)

minnows (*Pimephales promelas*) responded more strongly to disturbance cues released from donors within a group of other donors, compared with the cues produced by a donor alone (i.e., an audience effect) (Fig. 3). Similarly, guppies and cichlids showed stronger responses to cues produced by donors in a larger group, compared with the cues produced by donors in a smaller group (Goldman et al. 2019). These effects were not due to differences in cue concentration, which was standardized (g/L) across densities. Therefore, these studies provide evidence that fishes might modify disturbance cue release for coordinating group defense (shoaling) (Wisenden 2019). Further evidence for such signaling comes from studies manipulating the relationships among disturbance cue donors. Minnows show stronger responses toward disturbance cues produced by donors that are familiar with one another (Bairos-Novak et al. 2019b). The same effect has been observed in guppies, but only among individuals from high-predation environments (Crane et al. 2020b). In contrast, wood frog tadpole do not discriminate between disturbance cues from familiarized vs. unfamiliarized donor groups (Bairos-Novak et al. 2020), nor does familiarity between the donor and receiver have an effect (Bairos-Novak et al. 2019a). These studies also found that disturbance cue responses were unaffected by kin relationships. Therefore, wood frog tadpoles do not appear to use disturbance cues as a signal, suggesting that perhaps only more social species do so.

Disturbance-recognition learning

A few studies have explored whether disturbance cues can facilitate learned recognition of a specific disturbance, where the disturbance cues function as an unconditioned stimulus (i.e., classical conditioning). To our

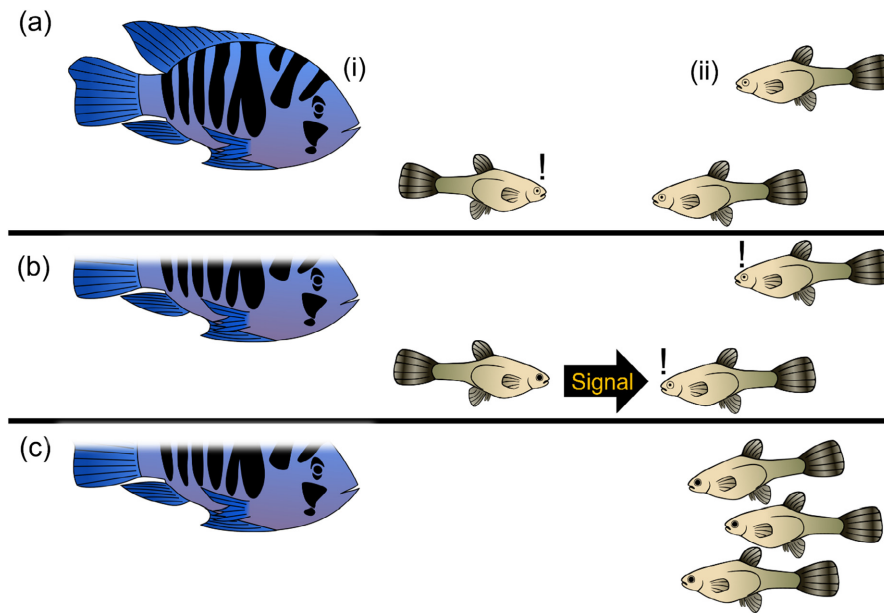


FIG. 3. An alarm signaling system, proposed by Bairos-Novak et al. (2019b) with a test of fathead minnows *Pimephales promelas* and then corroborated with guppies *Poecilia reticulata* from high-predation populations (Crane et al. 2020b). First, a signaling context (a) occurs via: (i) perceived external stimuli (e.g., the presence of a cichlid predator), and (ii) a nearby target audience of familiar individuals. Then, (b) receivers control the release of disturbance cues to induce (c) receivers to respond with increased shoaling, thereby increasing the chances of survival of both the signaler and receiver(s). Figure modified from Bairos-Novak et al. (2019b).

knowledge, such disturbance-recognition learning has only been tested in the context of learning a predator species. All of these studies were conducted on either fishes or amphibians, and with different outcomes. A study on wood frog tadpoles found no evidence for predator-recognition learning after experiencing disturbance cues paired with a novel predator odor (I. A. E. Rivera-Hernández et al., *unpublished manuscript*). However, such a pairing did promote learned responses in Iberian green tadpoles (*Pelophylax perezi*), but the learned information was not retained for long compared with alarm cue learning (~2 vs. 9+ d) (Gonzalo et al. 2010). Studies on hellbender salamanders also support learning from disturbance cues, although as mentioned previously, these cues may be more akin to alarm cues (Crane and Mathis 2011, 2013).

In studies on fishes, rainbow trout and an anemonefish (*Amphiprion percula*) showed no evidence of learning from disturbance cues (Ferrari et al. 2008, Manassa et al. 2013), whereas brook charr and blue-scribbled damsel (*Pomacentrus nagasakiensis*) did (Mirza and Chivers 2002; Pollock et al. 2021). In Mirza and Chivers' (2002) survival study, exposure to disturbance cues had an effect 6 h later (and in a new tank) and, therefore, the disturbance either caused a long-lasting behavioral effect or the fish had learned the identity of the predator species. However, the preponderance of evidence suggests that disturbance cue responses are relatively short (i.e., prey quickly return to baseline activity levels in the absence of subsequent threats), therefore supporting learned predator recognition in this study. Overall,

learned responses and their retention can be enhanced by having multiple learning opportunities (i.e., conditionings) (e.g., Crane et al. 2017), but this has not been tested in the context of disturbance cues.

Recognition of heterospecific cues

Disturbance cues may not only be recognized by conspecifics, but also by heterospecifics that can benefit from detecting the nearby disturbance. Studies on heterospecific disturbance cues have primarily used receiver species that were closely related to the donor species (e.g., congeners), or species that share an ecological role (i.e., prey guild members) (Table 2). Therefore, avoidance responses toward heterospecific disturbance cues (and not undisturbed cues) may indicate that receivers have been alerted to potential danger from a disturbance, rather than from a direct threat of the heterospecific donor. However, such avoidance might also occur if heterospecific disturbance cues are irritating or toxic. As mentioned previously, Hazlett (1989) found that virile crayfish showed a stress response toward disturbance cues from red-spotted newts, suspecting that the simulated predator chase caused the release of a toxin (Mebs et al. 2010) that was a direct threat to the crayfish.

A pulse release of disturbance cues might also be recognized by predators, either alerting them of the disturbance, attracting them to vulnerable prey, or even deterring their foraging. Although numerous studies have investigated the role of undisturbed cues in

predator/prey interactions, disturbance cues have rarely been used. Gall and Mathis (2010, 2011) exposed seven fish species to food soaked in disturbance cues from a potential prey species (hellbender salamanders). Most of the fishes were unaffected by the salamander cues, whereas one species was repelled and two were attracted. Notably, one of the attracted species, the banded sculpin (*Cottus caroliniae*), showed avoidance of the cues when tested at a smaller body size, revealing an ontogenetic shift in their response. These smaller sculpin may have been vulnerable to the same predation threats as the salamanders, or they were potential prey for the salamanders themselves (Gall and Mathis 2011). In contrast with predator responses to prey disturbance cues, we are not aware of any studies on the responses of prey to predator disturbance cues or any disturbance cue studies involving conspecific predators (i.e., cannibals).

Heterospecific disturbance cues might be learned by receivers, and if so, we would expect more frequent and stronger responses when receivers have ontogenetic experience with the donor species. However, we are not aware of any studies testing such. Across studies, significant responses actually tend to be less common when receivers have experience in the wild (63 vs. 80%; test of proportions: $Z = 0.59$, $P = 0.28$; Table 3b). Therefore, like conspecific disturbance cues, recognition of heterospecific disturbance cues does not necessarily require experience in the natural environment, which may instead lead to discounting certain disturbance cues. If heterospecific disturbance cues are indeed recognized innately, we might expect evolutionarily naïve receivers to respond less. Although data are too limited for statistical comparison, we know of four disturbance cue tests involving allopatric species (Vavrek et al. 2008, Goldman et al. 2019). Perhaps surprisingly, each test revealed a significant response to heterospecific disturbance cues, despite the donor and receiver species being phylogenetically distant (different taxonomic families) in each test. This innate recognition of heterospecific disturbance cues in the absence of recent evolutionary experience supports the hypothesis that at least some disturbance cues are composed of non-specific compounds (e.g., common metabolic by-products), which then elicit generalized response patterns in heterospecifics.

Species specificity

Significant responses to conspecific disturbance cues are more commonly observed than significant responses to heterospecific disturbance cues (88 vs. 68%; test of proportions: $Z = 2.24$, $P = 0.013$; Table 3). The strength of such responses has also been found to differ. For instance, Abel (1991a) demonstrated that rats showed significant responses toward the disturbance cues of mice, but at a weaker intensity than their conspecific responses. The same pattern was found for convict cichlids in response to rainbow trout disturbance cues (Vavrek et al. 2008). Goldman et al. (2019) found

that convict cichlids and guppies responded to each other's disturbance cues, but discrimination based on donor intragroup size occurred only when the donors were conspecifics. These results indicated that, at least for some species, disturbance cues are a mixture of specific and non-specific compounds. However, rainbow trout were found to respond similarly to conspecific and heterospecific disturbance cues, indicating that this species does not use species-specific disturbance cues (Vavrek et al. 2008). Interestingly, a study on the common yabby (*Cherax destructor*) reported responses to heterospecific disturbance cues that were actually stronger than their conspecific responses (Hazlett and Lawler 2010). While the cause for the reversed pattern remains unclear, such an outcome does indicate species specificity.

In contrast with disturbance cues, chemical alarm cues are characterized by a high degree of species specificity (e.g., Mirza and Chivers 2001, Mitchell et al. 2012). Kelly et al. (2006) argued that different proportions of carrier compounds associated with a nitrogen-oxide molecular trigger account for the well documented heterospecific alarm cue responses by closely related taxa (e.g., congeners). Brown et al. (2010) observed responses to alarm cues in Trinidadian guppies that differed based on the donor population, suggesting that local diet specializations might drive population-specificity in alarm cue production. Similarly, Goldman et al. (2021) found that diet quality and quantity influence disturbance cue production in guppies, which could similarly shape responses to conspecific vs. heterospecific disturbance cues.

ETHICAL IMPLICATIONS

Studies on risk assessment have often involved the use of alarm cues, but we may be able to ask many of the same questions using disturbance cues, which are less invasive to obtain. Because alarm cues are released upon tissue damage, animal research guidelines dictate that alarm cue donors must first be euthanized. However, to obtain disturbance cues, no physical harm is necessary. Therefore, replacing alarm cues with disturbance cues may be a more ethically satisfying option for studies of risk assessment, particularly when research involves threatened or endangered species (e.g., in pre-release training, Crane and Mathis 2011). However, one major caveat that must be considered is whether disturbance cues serve a similar ecological function as alarm cues. For example, as mentioned previously, some species do not learn from disturbance cues or even respond to disturbance cues. Therefore, pilot studies determining whether disturbance cues elicit responses should be considered before larger implementation. Another consideration is that obtaining a concentration of disturbance cues that elicits a response may require more donor individuals compared with obtaining alarm cues. For example, in recent research on responses to disturbance

cues by fathead minnows, one donor individual was used to provide disturbance cues for 12 receiver trials (Bairos-Novak et al. 2019b). However, a single alarm cue donor of this species can provide effective cues for hundreds of trials after serial dilution of the initial tissues (e.g., Crane and Ferrari 2016). Therefore, the experimental use of disturbance cues may require substantially more donors. As animal research guidelines are continually refined, researchers should consider whether their study objectives can be adequately met by using disturbance cues in place of alarm cues and how such a choice might affect the total stress/harm inflicted upon the animals.

CONSERVATION IMPLICATIONS

Disturbance cues may play an important role in biodiversity and conservation science because they provide information about threats and induce stress responses. For instance, studies on the role of disturbance cues in invasion ecology may be useful for management decisions. In the Gall and Mathis (2010, 2011) experiments involving disturbance cues from an endangered prey species, two out of five native predators were significantly attracted to the cues, as was one of two non-native species that were continuously being introduced. Disturbance cues of native and invasive competitors is also of interest. Hazlett and Lawler (2010) investigated the common yabby, a species undergoing a range expansion. This species was found to use the disturbance cues of competitor species more broadly than the native species, perhaps indicating that generalized recognition of disturbance cues can be a pathway to range expansion for some species. A few recent studies have demonstrated that conspecific alarm cues can be used to control the movement patterns of invasive species. Di Rocco et al. (2016) reduced the number of migratory sea lamprey (*Petromyzon marinus*) entering tributary streams by deploying chemical “curtains” of lamprey alarm cues. Similarly, Johnson et al. (2019) demonstrated that lamprey alarm cues, coupled with visual cues led to increased trap efficiencies. However, the use of alarm cues can entail the sacrifice of large numbers of animals to produce sufficient stimuli, rendering such an application impractical on a large scale. Future studies could examine the potential use of disturbance cues to regulate movement or trapping efficiencies of unwanted or invasive taxa.

Experimentation on disturbance cues may also be useful in understanding sublethal effects of environmental changes on cognition and behavior. M. S. Pollock et al. (*unpublished manuscript*) found that blue-scribbled damselfish showed a significantly weaker response to conspecific disturbance cues in degraded coral habitats due to coral bleaching, compared with habitats with healthy corals. However, the impacts of other types of environmental change on disturbance cues, as far as we know, have yet to be explored.

FUTURE DIRECTIONS

Despite the recent growth in the study of disturbance cues, there are multiple avenues of research that require attention and should provide valuable information regarding the evolution, functions, mechanisms, and potential applications of disturbance cues. Additional work is required to understand the chemical composition of disturbance cues and the potential differences within and between taxonomic groups. There are likely to be multiple constituent compounds, leading to critical questions regarding species specificity vs. generalization of disturbances among ecologically interacting species. Similarly, we are only just starting to grasp the role of individual metabolism in shaping the production of disturbance cues, which probably shifts slightly (qualitatively or quantitatively) when animals are disturbed. Metabolomics is an emerging field for studying endogenous metabolites in biofluids (e.g., urine), organs, and whole organisms (Samuelsson and Larsson 2008, Zhang et al. 2012). It can be used for metabolite profiling and to detect changes due to stressors. Weissburg et al. (2016) used this approach in characterizing the urine of blue crabs (*Callinectes sapidus*), finding shared spectral features (aromatic compounds, amino acids, and lipids) that differed in concentration due to diet. Research on disturbance cues would benefit tremendously from adopting metabolomics on a broad scale, assessing heterospecific differences and changes due to evolutionary experience and anthropogenic pollutants.

Secondly, while we have a wealth of data regarding the highly sophisticated and complex learning mechanisms that shape predator recognition (i.e., Brown et al. 2011, Crane and Ferrari 2013), we have a poor understanding of the role of disturbance cues in ecologically relevant learning mechanisms. For example, we do not know if disturbance cues facilitate learning in taxa other than amphibians and fishes. Similarly, no studies to date have explored any potential role of disturbance cues in the cultural transmission of acquired information, as is the case for alarm cues (Suboski et al. 1990).

Third, the majority of studies to date have been based on the implicit assumption that the main function of disturbance cues is risk assessment and avoidance. An intriguing possibility, however, is that disturbance cues may have multiple functions within communities. For example, disturbance cues may contain information that plays a role in the mediation of local foraging or mate choice competition (e.g., sex pheromones, Belanger and Corkum 2009, Stacey et al. 2009). We might predict that the increased activity associated with the active defense of a limiting resource could result in the release of signals that reduce the short-term costs of competition.

Fourth, the functional benefits of releasing and detecting variation in disturbance cues might vary across species depending on their degree of sociality. We predict that disturbance cues might provide a more reliable and abundant source of risk assessment for species that are

social, either throughout their life histories (e.g., guppies) or at particular ontogenetic stages (e.g., juvenile convict cichlids). Therefore, social species should show increased discrimination of differences in disturbance cues and experience greater benefits from their use. However, we currently lack sufficient data regarding the function of disturbance cues among social vs. non-social taxa to make such a comparison.

Fifth, disturbance cues have been investigated in relatively few taxa compared to other chemical cues, and further comparative studies are needed to address critical questions regarding the chemistry, function, and generality of disturbance cues across taxa and ecotypes. For example, the musk of many reptile species should be tested for a disturbance cue function. Similarly, the various semiochemicals found among mammalian and avian taxa could also function as disturbance cues. Clearly, additional research is required to establish the generality of disturbance cues across underrepresented taxa. Related to this point, is the diversity of habitats in which disturbance cues might function. For example, water chemistry differences between freshwater, brackish water, and marine ecosystems would be expected to drive differences in the production of and reliance on disturbance cues across these systems. Due to the increased osmoregulatory demand placed on marine fishes, a higher proportion of nitrogenous wastes are converted and released as urea (Wilkie 2002), possibly increasing the reliance on urea as a disturbance cue between marine vs. freshwater species. Therefore, broadening the taxonomic and ecological diversity of studies is likely to improve our understanding of disturbance cue production across species as well as habitats.

Finally, to date, only a single study, with a single species, has demonstrated that disturbance cues function under fully natural conditions (Goldman et al. 2020a). Additional studies are required to provide ecological validation for the function of disturbance cues. Studies conducted under fully natural conditions will also allow us to test the potential roles of microhabitat complexity and local prey guild diversity in deriving disturbance cue function(s). Similarly, future studies should assess the effects of anthropogenic impacts on the production and response to disturbance cues across habitats.

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