MINI REVIEW

CUED IN: ADVANCES AND OPPORTUNITIES IN FRESHWATER CHEMICAL ECOLOGY

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Abstract—We focus this mini-review on how naturally occurring chemical cues mediate ecological interactions, especially interspecific competition and predation in freshwater communities. Although freshwater chemical ecology lags behind terrestrial and marine chemical ecology, we identify recent progress toward: (1) identifying the chemical composition of cues important in food web interactions, e.g., specific glucosinolates, benzyl succinoates, and lignoids as deterrents to herbivory on freshwater macrophytes; (2) employing a nonreductionist approach that tests for emergent responses to suites of multiple chemical cues, e.g., trade-offs in snail refuge-seeking behavior in the presence of chemical cues from both fish and crayfish; (3) investigating how abiotic forces, such as hydrodynamics, impact chemical communication across a broad spatial and temporal scale, e.g., drift responses of mayfly nymphs to whole-stream additions of trout cue; and (4) quantifying the importance of genetic variability, e.g., how chemical cues change selective pressures of local environments. The questions of interest in freshwater chemical ecology cross taxonomic boundaries; traverse broad spatial and temporal scales; demonstrate nonlinear, unpredictable results; and necessitate a multidisciplinary approach for adequate understanding.

Key Words—chemical cue, kairomone, identification, scale, communication, behavior, predator–prey, flow, genetic variability.

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INTRODUCTION

Chemical cues are important signals in aquatic environments, where visual and auditory senses are often ineffective. As we illustrate in this review, chemical communication among freshwater species mediates many aspects of both predation and interspecific competition, forces that play key roles in determining community structure and ecosystem function. Recent advances in aquatic chemical ecology are enhancing our understanding of the connections between individual behavior and ecosystem function. We synthesize results at individual, population, and community levels, emphasizing the importance of investigations at multiple spatial and temporal scales.

Because terminology in the field of chemical communication is often ambiguous (Oldham and Boland, 1996), we explain our use of terms. We use the term "chemical cue" as a synonym for "allelochemical," a cue carrying information that mediates interactions between two organisms (*sensu* Dicke and Sabelis, 1988). Consistent with previous authors, we use the terms "kairomone" to indicate communication between different species and "pheromone" to indicate information relayed within a species (Wisenden and Millard, 2001). In addition, we discuss organisms in terms of their role as signalers versus receivers of infochemicals (*sensu* Dicke and Sabelis, 1988). Throughout the review, we address ecological interactions that are mediated by naturally occurring chemicals (not human-made chemicals). We also try to point out where the exact chemical structure of a cue is known versus generalized knowledge about groups of chemical compounds.

Several recent freshwater-oriented reviews overlap or focus on subsets of our topic: chemical communication (Brönmark and Hansson, 2000); alarm signaling (Chivers and Smith, 1998); predator–prey interactions (Kats and Dill, 1998); benthic interactions (Dodson et al., 1994); and planktonic interactions (Larsson and Dodson, 1993; Tollrian and Dodson, 1999). Rather than attempting to be comprehensive, we rely on these reviews, and emphasize selected publications of the past 5–10 years.

We first present patterns of recent publications that reveal what questions, organisms, and habitats have been studied. Second, we discuss specific advances that involve: (1) quantitative isolation and identification of chemical cues; (2) behavioral trade-offs that occur for organisms responding to multiple chemical cues; (3) abiotic influences that span spatial and temporal scales; and (4) characterization of underlying genetic variability of signalers and responders. Finally, we highlight remaining opportunities and challenges for research in this dynamic field.

PATTERNS OF RECENT PUBLICATION

With a literature survey using *Current Contents* and *Biological Abstracts* (Winspirs 4.0) from 1992 to 2001, we quantified the temporal trend of publications
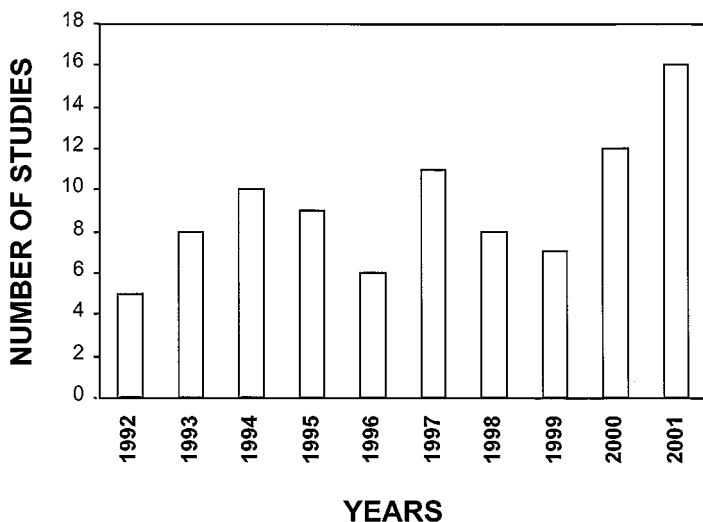
FIG. 1. Number of studies in freshwater chemical ecology from 1992 to 2001 that met our search criteria (in *Biological Abstracts* and *Current Contents*) with keywords: chemical cue, chemical communication, or kairomone.

relating to chemical communication in freshwater habitats. We classified the organisms in these publications as either senders or receivers (Dicke and Sabelis, 1988). Three keyword searches (chemical cue, chemical communication, kairomone) provided limitations for our survey. We limited our search using keywords that we believe freshwater ecologists would most likely use to explore the world of chemical ecology. This delineation limited our search to cues influencing relevant ecological interactions versus identification of nonrelevant or anthropogenic chemicals.

Our restricted search suggests that publications on freshwater chemical ecology are increasing at a slow rate (Figure 1). In addition, striking patterns emerge regarding the types of organisms that act as senders versus receivers of chemical cues (Figure 2). Although our literature search included broad classes of chemical cues, our results suggest that kairomones mediate the majority of species interactions in freshwater systems. Fish and predacious insects act largely as senders, while zooplankton comprise the most studied receivers. Available evidence suggests that some organisms, such as tadpoles, act only as receivers of cues from predators. While other organisms, such as predacious insects, may be both receivers of cues from larger predators as well as senders of their own cues to a lower trophic level, such as zooplankton (especially *Daphnia*).

Our survey confirms Kats and Dill's (1998) assertion that the majority of studies on aquatic chemical communication involve fish–zooplankton interactions. Furthermore, we may only identify some organisms as receivers, when, in
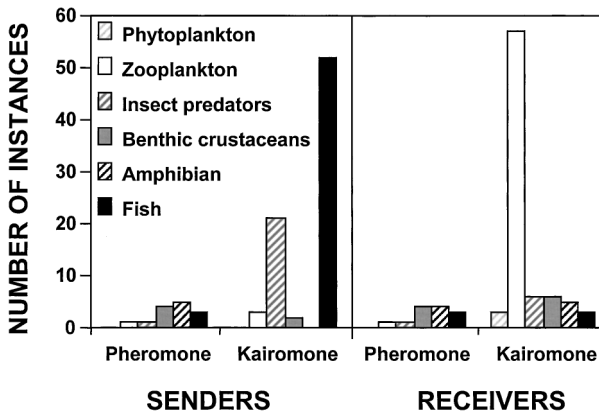
FIG. 2. Number of instances that different taxonomic groups appear as senders or receivers of chemical cues in studies identified from our literature search (1992–2001). Insect predators include both pelagic (Diptera: *Chaoborus*) and benthic (Odonata: *Enallagma*) organisms. Benthic crustaceans include amphipods, isopods, and crayfish. In most instances, fish act as senders and zooplankton serve as receivers.

actuality, those organisms are influencing other inconspicuous organisms in less well-studied interactions. On top of these documented complex patterns, considerable phenotypic plasticity and underlying genetic variability undoubtedly exists in how organisms respond to cues. These complexities indicate that freshwater chemical ecology will develop most quickly with an interdisciplinary approach (Oldham and Boland, 1996).

The patterns illustrated (Figures 1 and 2) represent both standing and flowing freshwater habitats. The duration and orientation of chemical cues may, however, depend partly on the type of freshwater habitat and the organisms present (Figure 3). In lotic systems (i.e., streams), chemical cues from mobile foragers such as trout (McIntosh et al., 1999) or crayfish (Moore and Grills, 1999) may be strongly unidirectional, diluting as they proceed downstream (although see Dahl et al., 1998). In contrast, recycling or renewal of chemical cues by less mobile organisms is more likely in lentic systems (i.e., macrophytes in lakes) (Figure 3). In addition, organisms showing strong site fidelity may also create areas in which cues are recycled. Finally, we might predict chemical cues to be relatively more important in aquatic systems that lack light, such as groundwater or cave environments. However, examples of chemical communication in subsurface organisms are, to our knowledge, nonexistent. We highlight below four promising avenues of recent advances that point the way toward enhanced interdisciplinary research. Future interdisciplinary endeavors will illustrate the importance of chemical ecology in mediating population, community, and ecosystem processes in freshwaters.
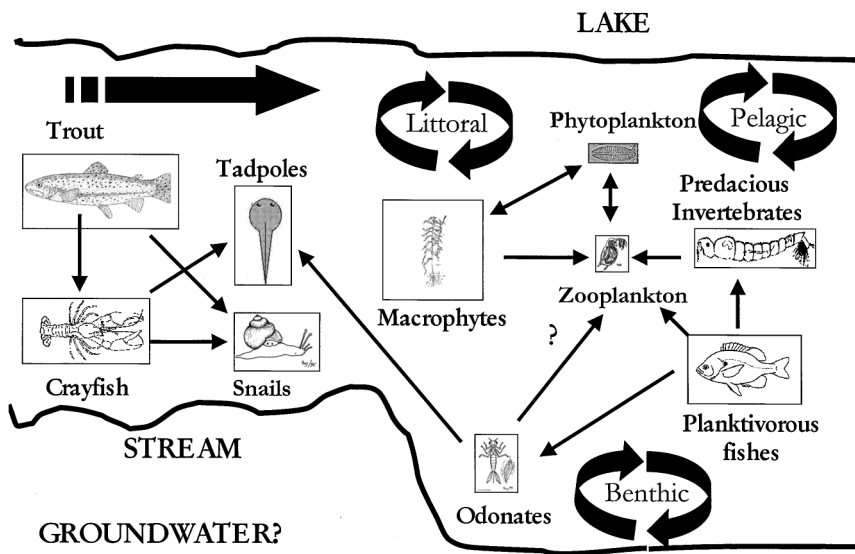
FIG. 3. Schematic of potential interactions mediated by chemical cues in either stream or lake habitats. Large arrows indicate direction of water flow. Curved large arrows suggest that water, and subsequently chemical cues, would circulate or be recycled. Smaller lines with arrows originate with the sender of the chemical cue and point to a potential receiver. Line drawings come mostly from BIODIDAC and a few noncopyrighted images from the Internet. Image of diatom can be found at Bowling Green State University's Algal Images.

RECENT ADVANCES

Quantitative Characterization of Chemical Cues

Probably every ecologist working on chemical ecology would like to know the exact chemical composition of the cue they have documented (Brönmark and Hansson, 2000). Knowledge of chemical structures would advance our insight into the metabolic origins, likely receptors, and the likely persistence and relative importance of different cues (Dodson et al., 1994). However, many ecologists are not trained in biochemistry and do not have the facilities, personnel, or funding necessary to investigate the identity of their chemical cue.

To explore how competition and predation regulate freshwater community structure, ecologists often refer to ecologically relevant chemical cues largely without knowing the chemical structure of the compounds in question. The ecologist places his/her focus on the result of the interaction that takes place when two or more organisms cooccur. A chemist, on the other hand, wants to know what specific chemical environment influenced the result of the interaction. Therefore,

scientists approach the same model system at different scales with different goals. Integrating these approaches, chemical identification with ecological results, is a sizable challenge.

Most compounds that freshwater ecologists suggest act as cues have not been isolated, purified, or structurally identified through complete biochemical assays. Only a few related to freshwater herbivory on macrophytes have been identified or quantified (Newman et al., 1996; Wilson et al., 1999; Kubanek et al., 2001), far fewer than analogous work in terrestrial and marine habitats. Instead, discussion of compounds in freshwater systems is generalized chemically, as groups of compounds (i.e., phenolics) or by chemical properties (lipophilicity, polarity, heat stability, etc . . .). Below we highlight interactions between organisms that show the greatest potential for complete characterization and identification of ecologically relevant chemical cues.

Phytoplankton–Zooplankton Interactions. Hessen and van Donk (1993) showed that the presence of the grazer *Daphnia magna* stimulated formation of large colonies in the green alga *Scenedesmus*. Exposure to *Daphnia*-cued water also increases the proportion of fatty acids that occur in *Scenedesmus* (Lürling et al., 1997), a possible mechanism to maintain buoyancy. These colonies are more difficult for daphnids to graze (Lürling et al., 1997) and, thus, potentially change the magnitude of top-down control of algae in lakes.

Von Elert and Franck (1999) characterized the active infochemical released by *D. magna* as an olefinic (i.e., an alkene with only one carbon–carbon double bond), low-molecular-weight (<500 Da) carboxylic acid. Furthermore, bioassays excluded hydroxyl and amino groups as fractions of the infochemical essential for biological activity (von Elert and Franck, 1999). Concentration of 1 liter of *Daphnia*-cued water using C-18 solid-phase extraction (C-18 SPE), followed by desorption with methanol (10 ml), evaporation, and later resuspension (100 ml methanol), produced similar colony formation as occurred when *Scenedesmus* was exposed to nonfractionated *Daphnia*-cued water (Lürling and von Elert, 2001). Collectively, these studies suggest that the daphnid infochemical responsible for colony formation is moderately lipophilic.

Other studies suggested that urea produced by *Daphnia* also induces colony formation in *Scenedesmus* (Wiltshire and Lampert, 1999). However, urea is a polar molecule without lipophilic portions, thus constituting a substantially different molecule from those tested in other bioassays. Consequently, debate continues as to which molecule, or combination of molecules, acts as an infochemical that induces colony formation in *Scenedesmus*. By separating out urea from a more lipophilic substance using C-18 SPE, Lürling and von Elert (2001) suggest that the colonizing-inducing activity of *Daphnia*-cued water is more lipophilic than urea-related. However, the specific identity of the structure(s) remains unresolved.

Stepping beyond *Daphnia* as the model of aquatic chemical communication, measurable quantities of heptadecene-1 exist within swarms of another

zooplankter, *Polyphemus pediculus* (Wendel and Jüttner, 1997). This volatile organic compound was identified using gas chromatography–mass spectroscopy (GC-MS) and may serve as a pheromone to maintain swarms. Wendel and Jüttner (1997) speculate that swarms may reduce mortality from predation. Heptadecene-1 is available as a pure compound, thereby encouraging further experiments on the ecological role of this chemical cue.

Fish Kairomones and Vertical Migration of Daphnia. Although we know that predators prompt diel vertical migration (DVM) of *Daphnia* and other zooplankters, we know relatively little about the chemical cues responsible for this well-studied phenomenon. Similar to other examples, ecologists can only speculate about the chemical nature of the cue(s), as specific molecules are still unknown. Bioassays suggest that the vertical migration-inducing kairomone in *Daphnia* is a freely dissolved compound of low molecular weight (<500 Da), with a lipophilic component, but no carboxyl-, phosphate-, or sulfate groups (von Elert and Loose, 1996). Amplitude of migration is also related to the concentration of this kairomone (von Elert and Pohnert, 2000), illustrating the importance of future tests of natural field concentrations.

Behaviorally or with life history strategies, *Daphnia* often do not distinguish between exposures to different fish species. Ecologists might hypothesize that these generalized responses indicate that kairomones from different species of fish are similar, if not identical (von Elert and Loose, 1996). However, chemists may question whether the active metabolites produced by these different fishes are the same, given that a structure has yet to be determined. This line of thought can only remain speculation until the chemical structures of these cues are actually characterized.

The most recent advancement with the zooplankton–fish system is one in which a hypothesis was rejected by follow-up studies. Boriss et al. (1999) proposed that trimethylamine (TMA) constituted the active component in fish cue, but subsequent investigations showed that daphnids responded only to concentrations of TMA higher than would naturally occur (Pohnert and von Elert, 2000). Furthermore, studies of life history responses of various daphnids to TMA have produced mixed results (Sakwińska, 2000; Lass et al., 2001). Not focusing on any specific compound, Ringelberg and Van Gool (1998) suggested that bacteria produced the kairomone responsible for inducing vertical migration, as antibiotic-treated perch-cued water suppressed migratory responses of *Daphnia*. However, treatment of fish-cued water with antibiotics did not eliminate migration by daphnids. Furthermore, antibiotics would only kill bacteria, not degrade a previously produced compound. Therefore, the origin and identity of the cue(s) responsible for inducing vertical migration remain a mystery.

Defense Compounds of Aquatic Macrophytes. Many freshwater macrophytes, like terrestrial plants and marine macroalgae, harbor chemical defenses (Lodge et al., 1998; Hay and Lodge, unpublished data). Only a few studies actually identify

the composition of deterrent secondary metabolites (Newman et al., 1996, Bolser et al., 1998, Kubanek et al., 2000). Perhaps more common are studies that speculate that unidentified macrophyte phenolics (benzene-ringed compounds with an —OH group), or subclasses of phenolics including tannins (phenolics that can precipitate proteins) or lignans (structural phenolics), are deterrents to herbivores (Lodge, 1991; Cronin, 1998; but see Dorn et al., 2001) and also to epiphytic algae (Gross et al., 1996).

It is important, however, to emphasize that not all phenolics or tannins are deterrent compounds. For example, Bolser et al. (1998) found that the crayfish *Procambarus clarkii* preferred macrophyte species both with high (*Typha angustifolia*) and low (*Ceratophyllum demersum*) phenolic concentrations, thus contradicting the hypothesis that phenolics always act as a deterrents to herbivory. To our knowledge, no study specifically isolates and identifies a freshwater tannin as a deterrent to herbivory. Furthermore, confusion may occur as to what compounds are being tested, depending on laboratory procedures. Colorimetric assays often used to quantify deterrent compounds do not discriminate among classes of molecules and instead capture total phenolics. Therefore, a danger exists in making generalizations about the deterrent properties of large, variable groups of compounds like phenolics.

Instead, the greatest advances in this field come from studies that have actually identified compounds responsible for deterring herbivory. For example, Newman et al. (1996) demonstrated that glucosinolate deterred multiple herbivores from consuming watercress (*Nasturtium officinale*). In addition, habenariol (bis-*p*-hydroxybenzyl-2-isobutylmalate) deters the crayfish *Procambarus clarkii* from feeding on the freshwater orchid *Habenaria repens* (Bolser et al., 1998; Wilson et al., 1999), and numerous lignoids deter this same crayfish from feeding on lizard's tail, *Saurus cernuus* (Kubanek et al., 2001). Besides influencing herbivory, specific plant compounds may affect competition between submerged macrophytes and phytoplankton. For example, *Myriophyllum spicatum* (Eurasian water milfoil) produces a hydrolyzable polyphenol, tellimangradin II, which deters algal growth (Gross et al., 1996). The possibility that allelopathic compounds play important roles in stabilizing alternative states of lake ecosystems—clear water macrophyte-dominated or turbid algal-dominated (Scheffer et al., 1993)—is an important hypothesis awaiting rigorous testing.

Trade-Offs and Multiple Cues

While identification of active compounds has been part of the advances described above, the multiplicity of cues in many freshwater environments requires that nonreductionist approaches be applied. Testing isolated compounds may often be a poor simulation of natural conditions, and, on practical grounds, we are unlikely to identify all the relevant compounds in the foreseeable future. For

example, extensive bioassays and chemical detective work led to the identification of bis-*p*-hydroxybenzyl-2-isobutylmalate (Bolser et al., 1998; Wilson et al., 1999) and several lignan compounds (Kubanek et al., 2000). However, in both cases, other deterrent metabolites in the water-soluble and lipid fractions remain unidentified (Hay, personal communication). Furthermore, the structural complexity of some molecules makes it difficult to identify pertinent structures, and small stereotypic changes in the molecule may enhance or negate the molecule's "activity" as a cue in communication. Therefore, while collaborating with chemists to identify compounds is recommended, progress in understanding the role of chemicals in community and ecosystem processes does not need to wait on complete chemical characterizations.

The influences of multiple chemical cues may not always be additive (Turner et al., 1999), and predictions based on exposure to only one cue at a time may be incorrect (Sih et al., 1998; Hazlett, 1999). Testing whole suites of chemical cues, in combination with single cue tests, may allow the researcher more power in extrapolation (Peckarsky et al., 1997). For example, a natural suite of (unidentified) littoral zone chemicals caused *Daphnia magna* to grow and reproduce more slowly than when grown in spring water (Burks et al., 2000). Laboratory experiments confirmed that daphnids altered their life history traits in response to cues from both macrophytes (waterweed, *Elodea canadensis*) and fish (roach, *Rutilus rutilus*). This result partially explains the paradox between historical studies documenting *Daphnia* avoidance of macrophytes, and recent studies illustrating that macrophytes can provide a refuge for *Daphnia* against predation by fishes (Burks et al., 2001). Without predation risk, daphnids avoid macrophytes because a reproductive cost comes with exposure. This cost may be outweighed by benefits of reduced mortality from fish predation in vegetated habitats.

Behavioral or life history trade-offs may require multiple chemical cues. For example, predator-induced diapause (production of ephippia) in *Daphnia magna* requires cues from both crushed-up conspecifics and kairomones from fish (Slusarcyzk, 1999). Therefore, organisms need to possess the ability to distinguish among multiple chemical cues. Dytiscid beetles (*Acilius sulcatus*) discriminate between chemical cues from hungry and satiated perch (*Perca fluviatilis*) and between perch satiated on fish versus those satiated on dytiscids (Åbjörnsson et al., 1997). Damselflies (*Enallagma sp.*) consistently decreased their movement and foraging in the presence of cues from crushed-up sympatric organisms (pike, *Esox lucius*, fed fathead minnows, *Pimephales promelas*). However, damselflies only responded to cues from biologically unrelated organisms (i.e., mealworms, *Tenebrio molitor*) with previous exposure to an actively foraging predator (Chivers et al., 1996). Even neurologically simple organisms such as flatworms (*Dugesia dorotocephala*) recognize fish odor as an indication of risk based on a single, simultaneous exposure to crushed conspecific and predator cues and do not respond to fish odor without the conspecific cue (Wisenden and Millard, 2001). Thus,

even apparently simple prey exhibit complex, chemically mediated responses to predators.

The presence of multiple predators may also elicit nonlinear, or unpredictable responses. When exposed to chemical cues from crayfish (*Orconectes rusticus*), physellid snails avoided covered habitats in contrast to seeking refuge when exposed to cues from pumpkinseed sunfish (*Lepomis gibbosus*). However, exposure to chemical cues from both predators produced an intermediate response (Turner et al., 1999, 2000), instead of the predicted response to the most dangerous predator. Identifying which cues are dominant controls in species interactions can be a challenging task. Trout cues suppress the impact of stonefly cues in inducing drift by stream-dwelling *Baetis* mayflies (Peckarsky and McIntosh, 1998), and cues from planktivorous fishes override the repellency of macrophytes in *Daphnia* (Lauridsen and Lodge, 1996; Burks et al., 2001).

Bullfrog tadpoles (*Rana catesbeiana*) exhibited nonadditive responses to multiple predators (i.e., dragonfly *Anax junuis* and bluegill *Lepomis macrochirus*), as only small tadpoles decreased their swimming with both predators and significantly more so with dragonflies (Eklöv, 2000). In testing responses of six larval anurans (wood frogs, leopard frogs, American toads, bull frogs, green frogs, and grey treefrogs) to five different predators (mudminnows, dragonfly larvae, newts or salamanders, dytiscid beetles, and belostomatid water bugs), Relyea (2001) found that the six prey species exhibited different responses to the same predator and that each prey species responded differently to the assorted predators. Clearly, the natural environment is full of predators and cues. Studies examining interactions among multiple cue effects will increase our understanding of the role of chemical ecology in structuring natural freshwater communities.

Abiotic Influences That Span Spatial and Temporal Scales

In many cases, the impact of chemical cues depends on the abiotic context, including light, temperature, and hydrodynamics. Chemical cues from light and predators are known to act in concert to prompt vertical migration of *Daphnia* (Brewer et al., 1999). *Chaoborus* only migrated toward light in the presence of chemical cues from fish occurring in combination with a moonlight gradient (O'Bryan and Forrester, 1997). More studies that identify proximate versus ultimate cues will add to our understanding of ecological interactions.

Temperature may also change the costs and benefits of responding to kairomones from predators (although see Sakwińska, 1998). For example, helmet size of *D. ambigua* reared with *Chaoborus* kairomones decreased with decreasing temperatures below 23°C (Hanazato, 1991). Furthermore, mortality of large helmeted daphnids increased at high temperatures (above 28°C). Thus, exposure to predator chemical cues reduced the tolerance of *Daphnia* to high water temperatures that commonly occur during summer.

Hydrodynamically different freshwater environments also influence the duration and properties of chemical cues. In streams, we generally assume that chemical cues originate upstream and influence taxa downstream. However, Dahl et al. (1998) demonstrated that *Gammarus pulex* uses chemical cues from downstream predators (trout) to assess drift behavior. Thus, eddies may play an important role in dispersing cues.

In freshwater systems, the best demonstration of the effect of flow involves crayfish foraging and orientation, analogous to the well-studied effects of flow and chemical cues on blue crabs and lobsters in marine systems (Zimmer and Butman, 2000). Crayfish (*Orconectes rusticus*) located food more quickly in streams with cobble (turbulent flow) versus sand substrate (more laminar flow) (Moore and Grills, 1999). Cobble breaks up the plume and increases dispersion, while laminar flow localizes the cue in parts of the stream not occupied by crayfish. The spatial distribution of complex odor cues (food + predator presence) also significantly impacted crayfish foraging (Tomba et al., 2001) when predator cue occurred in the middle of an artificial stream. Under this scenario, crayfish spent more time using side shelters and less time finding the food source. Thus, orientation to food and predator cues in changing flow regimes may strongly influence community interactions and ecosystem function. Experiments should test responses to chemical cues across a range of natural field conditions, including diverse spatial and temporal scales.

Studies that address larger spatial and temporal scales may discover patterns not apparent or predictable from extrapolation of smaller scales (Peckarsky et al., 1997). For example, McIntosh et al. (1999) pumped "trout-cued" water into a trout stream and monitored drift response of the mayfly, *Baetis bicaudatus.* Minimal drift occurred during the day in streams containing either background trout cues or added cues, but significantly less drift occurred at night in the stream where additional trout cues were added (McIntosh et al., 1999). Thus, mayflies quickly responded to the presence of trout cues. This group also added cued water into fishless streams and found that size at maturity of *Baetis* declined in streams where trout cues occured (Peckarsky et al., 2002). To test how quickly naive prey fishes respond to a predator, Brown et al. (1997) added juvenile pike to a 4-acre pond filled with fathead minnows. Minnows recognized chemical cues from pike after only 2 days, whereas fright responses to a visual cue did not occur for up to 8 days. Potential prey show differential responses to chemical cues, depending on experience. Lake-dwelling *Chaoborus* species (that coexisted with fish) exhibited no preference among ovipositing sites, while pond species showed an ovipositing preference for fish-free water (Berendonk, 1999). These results suggest that ovipositing behavior, influenced by chemical cues from fish, contributes to landscape-level patterns in *Chaoborus* distribution.

Looking for geographic patterns in predator avoidance, Covich et al. (1994) found that two species of physellid snails responded equally to crayfish taken from

their own habitat or from another geographic region. This result suggested that some generality exists in the crayfish kairomone. However, crawl-out responses of six snail species exposed to a single crayfish suggested species-specific responses that depended on microhabitat use (Covich et al., 1994). Thus, more studies investigating the generality of cues are needed to establish a contextual basis for prey response.

In addition to conducting research on large spatial scales, investigating time scales at which responses occur and cues last is also a topic ripe for study. De Meester and Cousyn (1997) suggest that behavioral responses of *Daphnia* to fish kairomones are rapid and reach their maximum after only a few hours. After exposure to chemical cues from fish, daphnids immediately sought refuge in macrophytes and maintained that response for at least 6 hours (Lauridsen and Lodge, 1996). Hazlett (1999) also found the behavioral effect on crayfish of predator odors from snapping turtles lasted only about 2 hours. Life history traits, on the other hand, may take longer to respond to chemical cues depending on genetic differences (see below) and may be enhanced in response to low food concentrations (Weber, 2001). Therefore, when addressing the generality of chemical cues, ecologists must explicitly consider the spatial and temporal scales relevant to the whole suite of chemical cues offered in a particular habitat or set of connected habitats. The relevance of chemical ecology to community and ecosystem ecology will be enhanced by larger and longer studies.

Chemical Communication within a Genetic Context

As the field of chemical ecology advances, studies not only need to identify important morphological and behavioral responses elicited by chemical cues, but also contrast phenotypic plasticity with underlying genetic variability. Increasing numbers of studies use *Daphnia* (which are parthenogenic most of the time) as a model to test the role of phenotypic and genetic variability and the broader evolutionary significance of chemically mediated responses (Lass and Spaak unpublished). Expression of ecologically relevant traits varies both within and between *Daphnia* populations (Stirling and Roff, 2000; for review, see De Meester, 1996a; Lass and Spaak unpublished). The presence of chemical cues from predators changes the selective pressures of local environments. For example, De Meester (1996b) measured the phototactic swimming behavior of ten *Daphnia* clones isolated from three different lakes (fishless, few fish, and numerous fish). Phenotypic plasticity differed among clones, with some altering their behavior more than others in the presence of fish. Furthermore, *Daphnia* clones from habitats with fish responded significantly more strongly to fish kairomones than daphnids from fishless systems (Boersma et al., 1999).

Genetic diversity may depend, in part, on the connectedness of the aquatic system. The self-contained nature of lentic systems may set boundaries for gene

flow and encourage local adaptation (De Meester, 1996a). However, for a lotic system, Storfer and Sih (1998) argue that gene flow between stream salamander populations (*Abystomas barbouri*) swamps local adaptation to chemical cues emanating from permanent streams with fish. Studies that begin to quantify the genetic variation associated with responses to chemical cues will add much insight to a growing field of interest.

OPPORTUNITIES AND CHALLENGES

Our review highlights several areas of freshwater chemical ecology poised for rapid progress. Research that identifies and quantifies chemical cues, such as the multifaceted approach used to characterize the *Daphnia–Scenedesmus* system, serves as a model for the study of inducible defenses (Tollrian and Harvell, 1999; van Donk et al., 1999; Lürling and van Donk, 2000). Recent identification of defensive compounds in freshwater macrophytes show the promise of this area to serve as a new arena in which to test theories of plant–herbivore interactions developed for terrestrial and marine systems. Further advances in freshwater inducible defenses, plant–herbivore interactions, and other areas will require greater collaboration of natural product chemists and ecologists to establish standard bioassays and chemical identification techniques that are practical and ecologically relevant.

Whether chemical cues are identified or not, much can be learned quickly with additional experiments using the natural suites of compounds existing in different habitats within lakes and streams. While cruder chemically, these sorts of experiments lead to rapid identification of larger scale patterns of trade-offs involving multiple cues. Identifying such patterns is essential to a more complete understanding of the organization of freshwater communities and the function of aquatic ecosystems. New studies should explore the importance of chemical communication in light-limited aquatic systems, such as groundwater or within caves. Such studies are relevant ecologically and provide rich material for subsequent chemical characterization.

In order to provide maximum insight into natural interactions, experiments must include realistic mimics of the complex abiotic milieu (flow, temperature, light, etc.) in which chemical cues are perceived by organisms (Moore et al., 2000). Comparisons of lake and stream environments may be especially fruitful. Freshwater environments provide excellent model organisms for the study of phenotypic and genetic responses to chemical cues and chemically mediated trophic interactions. Rapid progress can be made in this area, in a way that emphasizes the importance of chemical ecology to evolutionary ecology.

Overall, freshwater chemical ecology lags behind terrestrial and marine chemical ecology but provides vast immediate opportunities for interdisciplinary work of high relevance to chemistry, ecology, and evolution. Combining ecologists' desire to "assign ecologically relevant biological activity to natural products" with

chemists' desire to "unravel inter-organism communication at the molecular level" (Oldham and Boland, 1996) will lead to rapid progress of broad significance. These "ecologically relevant" interactions involve multiple species, vary within species, and take place within contextual environments dictated by flow, abiotic influences, and scale. Thus, questions in the field should increasingly attract other disciplines, including geneticists, physicists, and hydrologists. We must integrate these multiple perspectives and become "cued" into the diverse disciplinary cues.

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