



## SYMPOSIUM

# The Neuroecology of Chemical Defenses

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**Synopsis** Chemicals are a frequent means whereby organisms defend themselves against predators, competitors, parasites, microbes, and other potentially harmful organisms. Much progress has been made in understanding how a phylogenetic diversity of organisms living in a variety of environments uses chemical defenses. Chief among these advances is determining the molecular identity of defensive chemicals and the roles they play in shaping interactions between individuals. Some progress has been made in deciphering the molecular, cellular, and systems level mechanisms underlying these interactions, as well as how these interactions can lead to structuring of communities and even ecosystems. The neuroecological approach unifies practices and principles from these diverse disciplines and at all scales as it attempts to explain in a single conceptual framework the abundances of organisms and the distributions of species within natural habitats. This article explores the neuroecology of chemical defenses with a focus on aquatic organisms and environments. We review the concept of molecules of keystone significance, including examples of how saxitoxin and tetrodotoxin can shape the organization and dynamics of marine and riparian communities, respectively. We also describe the current status and future directions of a topic of interest to our research group—the use of ink by marine molluscs, especially sea hares, in their defense. We describe a diversity of molecules and mechanisms mediating the protective effects of sea hares’ ink, including use as chemical defenses against predators and as alarm cues toward conspecifics, and postulate that some defensive molecules may function as molecules of keystone significance. Finally, we propose future directions for studying the neuroecology of the chemical defenses of sea hares and their molluscan relatives, the cephalopods.

## Introduction

Neuroecology attempts to unify principles from diverse disciplines, by integrating knowledge gained from biophysical properties of nerve and muscle cells to community-wide impacts of trophic interactions (Zimmer and Derby 2007; Ferrer and Zimmer 2009; Derby and Zimmer, in press). As applied to chemical defenses, neuroecology seeks to understand how chemicals act as defenses in inter-individual interactions and as a consequence shape the organization of ecosystems. For any individual or set of chemicals, we are interested in their source and biosynthetic pathways of production and how the molecules may change through trophic levels, the diverse roles that they play in organismal interactions, the mechanisms (molecular, cellular, systems) underlying

and mediating these roles, and the links between all of these processes that lead to structuring of populations, communities, and/or ecosystems. Consequently, as depicted in the Venn diagram in Fig. 1, studies of the neuroecology of chemical defenses unite diverse fields of study, including behavior, chemistry, neuroscience, ecology, and evolution. For example, analytical chemistry is used to identify which molecules are candidate defenses by virtue of their presence in organisms or the environment. Ethology allows evaluation of the behavioral relevance of these molecules. Neuroscience is used to determine what chemicals are detected and the molecular, cellular, and systems level mechanisms underlying their reception and integration. Ecological and evolutionary approaches allow evaluation of

### Neuroecology of Chemical Defenses

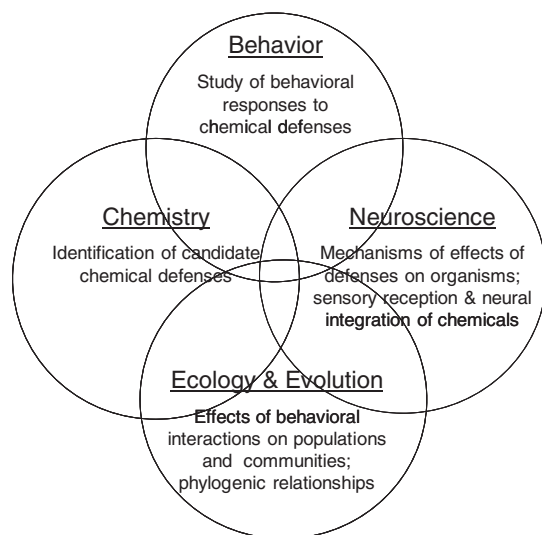


Fig. 1 Neuroecology of chemical defenses.

the impact of behavioral interactions on community organization and dynamics. Intersections of some of these four approaches are already standard fields of study. For example, neuroethology combines neuroscience and ethology to yield neural explanations of the behavior of animals. Sensory ecology uses neuroethological approaches with the additional focus on the realistic sensory information that organisms obtain from their environment. Chemical ecology examines the role of identified chemicals in regulating communities and systems. In our view, the value of the term “neuroecology” is that it stresses the value of bringing together all the diverse fields that can lead to a complete understanding, an integration of the parts, and in cases can identify emergent properties that would not be identified by more limited approaches. Neuroecology goes beyond the boundaries of chemical ecology by bringing in more cellular and molecular level mechanistic explanations. It goes beyond neuroethology by considering how those mechanistically-explained behaviors affect the organization of communities. It goes beyond sensory ecology in not being limited to sensory functions. We believe that by integrating more approaches in the study of a given system, we will gain a much greater appreciation for the processes and factors controlling interactions between organisms and the ultimate organization of communities.

In an initial synthesis of the emerging field of the neuroecology of chemical defenses, a virtual symposium of six reviews and an introductory article was published in the December 2007 issue of

“The Biological Bulletin” (Zimmer and Derby 2007). Those articles presented a full range of topics, from identifying natural molecules, their cellular and molecular mechanisms of effect, and their consequences on inter-individual interactions and community structure, and covered most types of organisms and habitats. That volume makes clear that while progress was being made in understanding elements of a diversity of experimental systems—the identity and scaling of molecules, the neurobiological mechanisms of chemosensory systems, the roles of chemistry in mediating ecological interactions, and so on—for each experimental system, both a full range of studies and more importantly the links between these topics was lacking, thus limiting full synthesis. Consequently, despite progress toward the stated goals, an understanding of the full ecological impact of molecular and cellular mechanisms of chemical defenses was incomplete.

This article is an attempt to continue to shine a light on the neuroecology of chemical defenses. It is not meant to be a complete review. Rather, it highlights selected topics that characterize the state of the field and, given the nature of our symposium on which this article is based, focuses on aquatic systems in general and most specifically on the current status and future directions of my group’s research on the neuroecology of the defensive use of ink by marine molluscs.

Most progress in the neuroecology of chemical defenses in the aquatic environment has been made by identifying chemicals and describing their effects on the behavior of consumers. Some behavioral testing has been performed in the field under natural conditions using predators sympatric with the prey of interest, but more often these trials have been based on laboratory assays, usually, but not always, using natural predators. For example, Parker et al. (2007) identified compounds that inhibit feeding on plants by relatively large herbivores such as crayfish and geese, and discussed the implications of the evolution of chemical defenses by plants and of the ecological specializations of smaller and less mobile herbivores. The latter are more abundant in association with chemically defended plants than with nondefended ones, and this may afford them protection from larger organisms. Other compounds that have differential effects are chemicals that deter feeding by generalist feeders but stimulate feeding by specialists. The chemically defended seaweed *Chlorodesmis fastigiata* deters feeding by fishes but is the preferred food of the crab *Caplyra rotundifrons*. This crab does not sequester the deterrent compound but avoids predation by hiding within

patches of seaweed (Hay et al. 1989). Other examples of animals that specialize on compounds that are deterrents for other animals are well known in the aquatic (Hay et al. 1998; Hay 2009) and terrestrial (Chapman 2003) environments.

Another area of the neuroecology of chemical defenses that has received some attention is understanding the consequences of defenses on the organization of communities and ecosystems (Hay 2009; McClintock et al. 2010). An interesting example is that of the interactions between copepods and their phytoplanktonic food. These organisms constitute one of the major trophic levels in marine environments, and the continuously evolving arms race between them has profound effects on the ecosystem. Long et al (2007) showed that phytoplankton *Phaeocystis* can detect the presence of predatory copepods and alter its size from colonies up to 30,000  $\mu\text{m}$  in diameter to single cells 4–6  $\mu\text{m}$  in diameter. The adaptive value of this behavior is that copepods feed more on colonies than on single cells. In another example, waterborne cues from copepods cause phytoplankton to increase their production of toxin as a defensive measure. Interestingly, the amount of toxin produced depends on the identity of the copepod, which means that the composition of the zooplankton is of great importance in determining the toxicity of algal blooms (Bergkvist et al. 2008). This interesting area of inter-specific communication certainly deserves more attention and is critical to the advancement of neuroecological levels of understanding.

Our understanding of the sensory and neural mechanisms mediating the behavioral effects of chemical defenses has been rather well studied in terrestrial systems, especially for herbivorous insects (e.g., Chapman 2003) because of their commercial importance or in *Drosophila melanogaster* because of its prominence as a model organism (Weiss et al. 2011), and to a lesser degree for mice and rats for the same reasons (Mueller et al. 2005), but even in this case most of the experiments lack an ecological perspective. In the aquatic environment, the neural underpinnings for the effects of chemical defenses are poorly understood even for model organisms such as fishes and crustaceans.

### Neuroecology of chemical defenses: molecules of keystone significance

Two of the best examples of the successful application of the neuroecological approach to the study of chemical defenses are studies of the guanidine alkaloids saxitoxin (STX) and tetrodotoxin (TTX), and

we summarize these two examples below. STX and TTX have a profound effect on multiple trophic levels in the ecosystems in which they are present, much more than would be expected from their abundance (Fig. 2). Both chemicals are considered molecules of keystone significance (MKS), a concept proposed and developed by Ryan Ferrer and Richard Zimmer (Zimmer et al. 2006; Zimmer and Ferrer 2007; Ferrer and Zimmer 2009). MKS are chemicals that have broad impacts that can lead to profound structural changes in communities. MKS function at multiple trophic levels and mediate a variety of interactions between organisms within a community. Consequently, MKS typically are multifunctional. Because of their central and profound effects, organisms often have evolved sensory receptor cells connected to central neural circuits that allow them to detect MKS and to respond with appropriate behavior. The ability of MKS to bioaccumulate, either within one or across several trophic levels, can be important to their functions. Toxins are strong candidates for MKS because of their potentially pervasive and diverse effects across trophic levels. For example, the toxins STX and TTX can also be chemical cues or signals, antioxidants, and osmoregulatory molecules. However, MKS are not limited to toxins. One example of a nontoxic MKS are proteins associated with barnacles, which can function as a cue for settlement of larval conspecifics and as an attractant of predatory snails; together, these multiple effects can significantly affect the structure of rocky intertidal communities (Ferrier et al. 2011). MKS might also be based on a set of related molecules, such as a parent compound and its metabolites. Dimethylsulfoniopropionate (DMSP) is produced by marine phytoplankton as an osmoprotectant but when released from these cells is metabolized into dimethyl sulfide (DMS) and acrylic acid, which together have cascading and diverse effects that shape the structure and dynamics of marine communities (summarized in Shumway 1990; Hay and Kubanek 2002; Zimmer and Ferrer 2007; Pohnert et al. 2007).

#### STX in the marine environment

STX is a powerful toxin that is produced by dinoflagellates and acts by blocking voltage-gated sodium channels. It can be used as a chemical defense against predators, but once introduced into the environment it takes on other roles (Zimmer and Ferrer 2007). The toxin-producing dinoflagellates (and their STX) are eaten by filter feeders such as clams, which in turn can use the toxin in their own defense (Bricelj et al. 2005, 2010; Connell et al. 2007). These authors

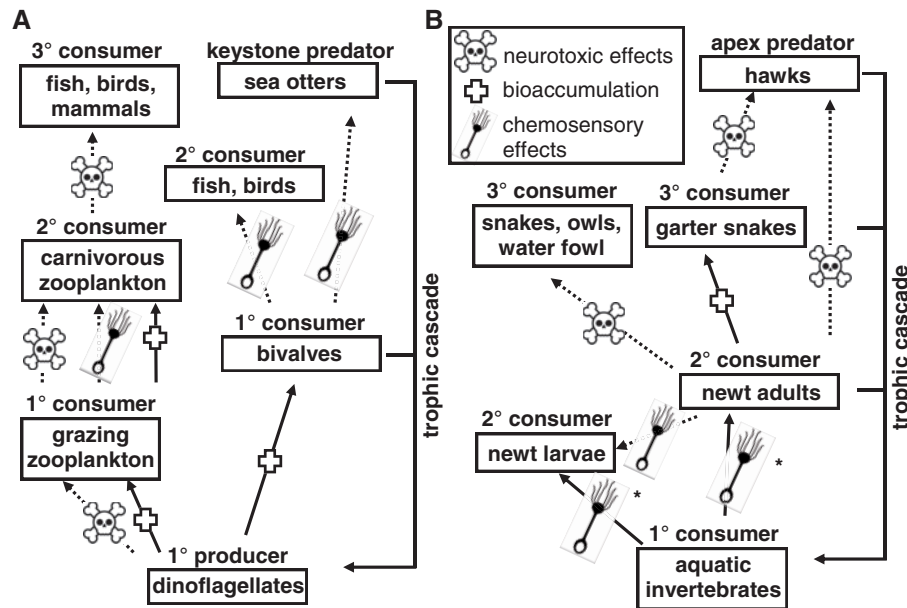


Fig. 2 MKS. (A) Saxotoxin in coastal marine communities. (B) Tetrodotoxin in riparian communities. See text for explanation. From Ferrer and Zimmer (2009).

have shown that STX is a powerful selective agent, capable of modifying the genetic composition of populations exposed to it. Populations of softshell clams, *Mya arenaria*, from areas where red tides occur are more resistant to STX and accumulate it at greater rates than do populations from nonexposed areas (Bricelj et al. 2005). This characteristic is due to modifications in a single amino-acid residue that causes a 1000-fold reduction in the affinity between STX and sodium channels (Bricelj et al. 2005, 2010; Connell et al. 2007). STX also affects the behavior of predators. For example, sea otters, *Enhydra lutris*, which are a keystone marine predator in the Northwestern Pacific, vary their foraging behavior depending on the toxicity of their preferred prey, the softbutter clam, *Saxidomus giganteus* (Kvitek and Bretz 2004). The levels of STX in these clams vary from site to site, and as it increases, the otters first discard the most toxic body parts but eventually avoid these clams altogether and switch to smaller, nontoxic species of clams, which results in significant increases in the abundance and sizes of softbutter clams at sites where they are highly toxic (Kvitek and Bretz 2004). Whether this is due to sensory detection of STX itself or to some other mechanism such as developing learned aversions to other molecules associated with STX-laden clams is not known. Irrespective of the underlying sensory mechanisms, such prey selection can have enormous consequences throughout the whole ecosystem, with the

relative abundance of different species being influenced by the relative amounts of one molecule.

### TTX in the riparian habitats of California

TTX is another potent neurotoxin, and it acts as a chemical defense for newts of the genus *Taricha*, protecting them from predators. Predatory *Thamnophis* garter snakes, however, have evolved resistance to TTX, are able to consume TTX-containing newts, and in fact can acquire enough TTX so as to be in turn defended from their own predators (Geffeney et al. 2002). Williams et al. (2010) showed that newts can be consumed or rejected by snakes, and the outcome depends on the amount of TTX present in the newts' skins. In addition, TTX affects all predators of newts, including those that have not evolved resistance, and owls and waterfowl have been found dead with TTX-containing newts in their digestive tracts (see review by Zimmer and Ferrer 2007). Zimmer and collaborators (Zimmer et al. 2006; Ferrer and Zimmer 2007a, 2007b) showed that TTX is not only used as a chemical defense by adult newts but also that it is detected by larval newts, which use it as a cue indicating the presence of adult newts, which are cannibals against conspecific larvae if their alternative preferred prey is not available. Zimmer et al. (2006) used electrophysiological techniques to show that larval newts use olfaction to detect TTX and that they use these olfactory cue to behaviorally respond to TTX by

seeking shelter. This predator-avoidance behavior is suppressed by the presence of arginine, a cue associated with feeding-associated damage to the adult newts' preferred prey, the worm *Eisenia rosea*. In this scenario, the presence of TTX means that the cannibalistic adults are present, and thus hiding is advantageous, while arginine means that those adults are eating their preferred prey, and thus that the risk of cannibalism is low for the less-favored larval newts.

## Escape by inking: the neuroecology of predator avoidance by inking molluscs

### Defenses of sea hares

Opisthobranchs are soft-bodied snails that are well known for the diversity of defenses that protect them from predators (Johnson and Willows 1999). Their chemical defenses are many and impressive (Kamiya et al. 2006), but they also include cryptic or aposematic coloration, large size, and mechanical protection through mucus and tough skin. Although many opisthobranchs have their defensive chemicals embedded in their skin or other tissues, representing a constitutive defense, some of them release defensive chemicals only when attacked. These chemicals can either be stored ready for release or generated *de novo* from released precursors. A splendid example of such an active, inducible chemical defense is the inking behavior of sea hares (*Aplysia* spp.) (Fig. 3).

### Inking behavior

The inking behavior of sea hares has been noted at least since Aristotle (Carefoot 1987), and it has long been thought that this has a defensive function. Indeed, toxic or defensive properties of ink have been demonstrated frequently in the literature; however, most of these studies have focused on the ink's antimicrobial effects, which may be important in the context of preventing infection from attack-induced wounds, or on the ink's repellent properties against species unlikely to be of ecological relevance to sea hares. Furthermore, it was not until 1995 that there was an experimental demonstration that ink actually protects sea hares during encounters with natural predators—giant sea anemones (Nolen et al. 1995). Since then, there have been other demonstrations of the ink's protective effects against vertebrate and invertebrate predators (Kicklighter et al. 2005; Nusnbaum and Derby 2010a, 2010b).

## Mechanisms of the action of ink

The mechanisms whereby ink protects sea hares from predators are diverse, as are the chemicals mediating these effects (Derby 2007). The ink's defensive effects can be both direct and indirect; i.e. ink can act directly on predators, but it can also act as an intra-specific alarm cue that indicates to neighboring sea hares that a conspecific has been attacked. We summarize below what is known about the molecules and mechanisms behind both of these defenses.

### Inter-specific effects

#### Aversion

Inter-specific effects of ink—by acting on attacking predators—can be mediated by several mechanisms. The most intuitively obvious and best studied in that ink contains deterrent chemicals that are distasteful, aversive, or possibly even toxic, to predators. Ink is actually the combined product of two co-released glandular secretions: a purple ink from the ink gland, and a sticky whitish opaline from the opaline gland. For most predators (with spiny lobsters being a noteworthy exception), ink is much more aversive than opaline. Although many antimicrobial compounds have been identified in sea hares' ink (e.g., Kamiya et al. 2006), relatively few compounds aversive to predators have been identified. We describe here two examples of aversive compounds. The first are compounds that are diet-derived and used without any major alteration—phycoerythrobilin and aplysiocyanin (Kamio et al. 2010a, 2010b). Sea hares eat red algae with its red, photosynthetic pigment phycoerythrin, then through digestion cleave the protein part of the molecule from the red chromophore phycoerythrobilin. Sea hares methylate a hydroxyl group of phycoerythrobilin to produce aplysiocyanin, which they concentrate and store in the ink gland. Aplysiocyanin acts through the olfactory system to deter the approach phase of attack by fishes and to affect the consumptive phase in both fish and crustaceans by being unpalatable (Kamio et al. 2010b; Nusnbaum 2011; J. Aggio, unpublished data). A second set of chemicals that act directly against predators are not diet-derived but are related to a protein produced in the sea hares' ink gland. This protein—escapin—is an oxidase enzyme that uses L-lysine as a substrate and produces a complex set of compounds that include hydrogen peroxide, ammonia, an equilibrium mixture of  $\alpha$ -keto-acids, and reaction products of hydrogen peroxide and this equilibrium mixture (Kamio et al. 2009). Hydrogen peroxide is a deterrent against fish and crustaceans (Aggio and Derby 2008; Nusnbaum and



Fig. 3 Inking sea hare. Courtesy of Genevieve Anderson.

Derby 2010a, 2010b), and some components of the equilibrium mixture (the escapin intermediate products) are also deterrents against fish (Nusnbaum and Derby 2010a, 2010b). In addition, the acidity of ink secretion may potentiate its effects, either acting by itself or by enhancing the effects of other molecular defenses (Shabani et al. 2007).

#### Phagomimicry

Spiny lobsters, especially, have been shown to be affected by the sea hares' phagomimetic defense. This arises from the presence in ink of an extraordinarily high (100 s of millimolar) concentrations of free amino acids (Kicklighter et al. 2005; Johnson et al. 2006). Because amino acids are powerful stimulants for feeding in many marine animals, including spiny lobsters, they are likely candidates for mediating the observed phagomimicry of ink, whereby lobsters drop the inking sea hare and attend instead to its ink (Kicklighter et al. 2005). This phagomimetic defense is reminiscent of tail-autotomy by some species of lizards when disturbed; but secretion of ink has the advantages of being less energetically costly and more quickly reversible (sea hares can replenish their ink within 2–3 days of total release, provided they have access to red algae) than is autotomy of the tail. It also is reminiscent of the use of glandular secretion by male cockroaches to lure females into a pre-copulatory position by exploiting a sensory bias in the female's feeding response (Nojima et al. 1999).

Phagomimicry is a dominant feature of the purple-ink component of the ink secretion against spiny lobsters, but it seems less important against other predators that we have examined (sea anemones; fish; other crustaceans), perhaps because the purple ink is highly aversive to these other predators but less so to spiny lobsters.

#### Sensory disruption

Sensory disruption is another mechanism of defense linked to the amino-acid components of ink. Because ink is sticky, it can cover parts of the body surface of predators that interact with it, and this has indeed been observed in the case of the anterior sensory organs of crustaceans (Kicklighter et al. 2005). Having an amino-acid-laden covering on sensory organs is likely to influence their ability to function normally, e.g., interfering with the animal's ability to acquire accurate information regarding its chemical environment. This mechanism has not been demonstrated to function in any animal, but we are currently examining it in spiny lobsters, using behavioral and electrophysiological assays, and preliminary results indicate that ink does indeed disrupt chemosensory input (T. Love-Chezem and J. Aggio, unpublished data).

#### Why have so many effects?

One explanation for the diversity of effects of sea hares' ink is that sea hares have a diversity

of predators. These predators may vary by species, life-history stage, physiological state, such as hunger, and learned aversions and preferences, and other factors. This diversity brings with it different chemical sensitivities, chemosensory responses, and chemosensory-guided behaviors. As a result, it should be advantageous to have a broad array of defensive molecules capable of affecting as many putative predators as possible. A second reason for the diversity of mechanisms is that ink can always function through mechanisms involving diet-independent chemicals, but the effects can be augmented by adding diet-dependent chemicals and mechanisms when they are available. Finally, the combination of effects of several mechanisms acting on a single animal may be more powerful than anyone acting alone.

#### Intra-specific effects: alarm cues

Ink not only defends sea hares from predators as described above, but it also cues neighboring sea hares to perform defensive behaviors such as avoidance and fleeing (Fiorito and Gherardi 1990; Kicklighter et al. 2007). Both ink and opaline contain alarm cues, although the molecules mediating their effects are very different. As with the effects operating directly on predators, these alarm cues include both diet-independent and diet-dependent compounds. The alarm cues in the ink portion of the secretion are diet-independent: three nucleic acids and nucleosides—uracil, uridine, and cytidine (Kicklighter et al. 2007). The active compounds in opaline are all diet-dependent: mycosporine-like amino acids (MAAs) (Kamio et al. 2011). MAAs are especially interesting because they are multifunctional molecules, potentially acting as sun screens, anti-oxidants, and/or anti-foulants (Karentz 2001; Shick and Dunlap 2002). In sea hares, MAAs concentrated in the skin may act as sunscreens (Carefoot et al. 2000). We hypothesize that these diet-derived MAAs evolved as intraspecific chemical cues from a prior function as sunscreens, which might include antimicrobial, antifouling, or anti-predatory functions.

#### Molecules of keystone significance in ink?

Are inks' defensive compounds MKS? There are some preliminary indications that this may be possible. For example, phycoerythrobilin is already known to operate at two trophic levels and with more than one function: it is a chromophore in red algae used in photosynthesis and, after being

acquired by sea hares through their diet, an aversive compound (together with its slightly modified form, aplysiocyanin) released as a component of ink (Kamio et al. 2010a, 2010b; Nusnbaum 2011). The prevalence of red algae in the diet of marine herbivores, together with the demonstrated bioactivity of phycoerythrobilin as an aversive deterrent, raises the possibility that other animals besides *Aplysia* may use phycoerythrobilin, or derivatives of it, as chemical deterrents, thus spreading its potential as a key molecule in other trophic interactions. Even more generally, as this is the first demonstration of a photosynthetic pigment being used as a chemical defense, it raises the possibility that other diet-derived photosynthetic molecules may function in trophic or other interactions in other species. Along these same lines, the prevalence of MAAs in plants and microbes and their known use by many animals that eat MAA-containing organisms (Shick and Dunlap 2002), together with our demonstration that MAAs are used as intraspecific cues by sea hares (Kamio et al. 2011), show that these molecules can have diverse functions across trophic levels in an unaltered form. It deserves to be explored whether MAAs might be used as cues, signals, or defenses by other organisms, leaving open the possibility that some of these algal-derived compounds may have even broader effects across the community. Still lacking toward assessing whether or not these molecules might be MKS is an analysis of their community-wide impacts such as how they may affect abundances and distribution of organisms.

#### Future directions in studying the neuroecology of chemical defenses of inking molluscs

Our work has focused more on the neuroethology and chemical biology of sea hares' ink defenses, but there are still important directions we have not yet explored. For example, except for studies of the involvement of amino-acid-sensitive chemoreceptor neurons on the antennules and mouthparts of spiny lobsters in the phagomimetic defense (Kicklighter et al. 2005), little is known about the neuronal basis for detecting chemical defenses in this system. Spiny lobsters, blue crabs, and sea catfish are promising neural animal models for use in how chemical defenses such as hydrogen peroxide, aplysiocyanin, phycoerythrobilin, and MAAs are detected and processed by the nervous systems.

Even less work has been done on ecological topics in the neuroecology of sea hares' ink. For example, are molecules in sea hare ink, such as

mycosporine-like amino-acids or phycoerythrobilin, MKS? Clearly, these molecules occur at multiple trophic levels, being present in the algae and microbes that produce them, and the consumers that acquire and accumulate them through diet. The molecules are also known to function in a variety of ways across trophic levels, thus satisfying one of the criteria for MKS. How broadly distributed and multifunctional across the community they are currently unknown and will require further study. But also critical in determining if they are MKS is to know if they play critical roles in structuring communities.

Crustaceans and fish are key predators in marine environments. Their presence and selection of food can profoundly influence community structure. For example, spiny lobsters may have major impacts on trophic webs by preying on foundation keystone species. This also has been shown for sea urchins (Tegner and Levin 1983; Shears and Babcock 2002), which play a key role in regulating kelp forests (Harrold and Reed 1985), as well as for mussels (Robles et al. 1990). Field studies in the marine reserves of Catalina Island, California, have demonstrated that spiny lobsters do prey on sea hares, probably because their high abundances there have led to a depletion of their favorite foods, resulting in hunger-induced acceptance of less-preferred, chemically-defended prey such as sea hares (William Wright, personal communication). Whether predator-prey interactions between spiny lobsters and sea hares and other chemically defended opisthobranchs can influence structure and dynamics of marine communities is unknown. However, it is known that sea hares can affect algal populations differentially, depending on the defensive compounds they contain. Sea hares and other opisthobranchs choose their dietary algae in part because of the algal chemical defenses (Paul et al. 2007; Verges et al. 2008; Baumgartner et al. 2009). Opisthobranchs feeding on invasive algae may in fact aid in spread of the algae by cutting them into tiny fragments capable of dispersion and regeneration (Gianguzza et al. 2007 Žuljevic et al. 2001). The presence and density of opisthobranchs may also influence the biomass of cyanobacterial blooms (Capper and Paul 2008; Geange and Stier 2010). In this way, predation (or the lack thereof) on sea hares could modify algal population structure and consequently other trophic levels.

Cephalopods are better known than sea hares as inking molluscs, yet very little is known of whether or how ink protects them in attacks by predators. In laboratory experiments, inking by squid is correlated with changes in the attack behavior of predatory

fishes that are advantageous to the squid: it caused bluefish to increase startle behavior and abandon attacks, and it caused flounder to misdirect attacks (Staudinger et al. 2011). Ink may also protect squid against French grunts because ink introduced between the fish and its food delayed attack by the grunts, an effect in which the chemical senses are very likely to play a part because squid's ink is distasteful to grunts (Wood et al. 2010). As is the case with sea hares, squid's ink can also modify the behavior of conspecifics, thus functioning as an alarm cue (Wood et al. 2008). These initial results suggest that there is a great deal of promise in future explorations of the neuroecology of cephalopod chemical defenses.

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