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Abstract - We describe some recent themes in the nutritional and chemical ecology of herbivores and the importance of a broad pharmacological view of plant nutrients and chemical defenses that we integrate as “Pharm-ecology”. The central role that dose, concentration, and response to plant components (nutrients and secondary metabolites) play in herbivore foraging behavior argues for broader application of approaches derived from pharmacology to both terrestrial and aquatic plant-herbivore systems. We describe how concepts of pharmacokinetics and pharmacodynamics are used to better understand the foraging phenotype of herbivores relative to nutrient and secondary metabolites in food. Implementing these concepts into the field remains a challenge but new modeling approaches that emphasize tradeoffs and the properties of individual animals show promise. Throughout we highlight similarities and differences between the historic and future applications of pharm-ecological concepts in understanding the ecology and evolution of terrestrial and aquatic interactions between herbivores and plants. We offer several pharm-ecology related questions and hypotheses that could strengthen our understanding of the nutritional and chemical factors that modulate foraging behavior of herbivores across terrestrial and aquatic systems.

Key Words – Aquatic, herbivore, nutrient, pharmacology, plant secondary metabolite, terrestrial.

INTRODUCTION

For several decades now, a central focus in the field of nutritional ecology has been to understand the factors that influence the foraging behavior of terrestrial and aquatic herbivores (Choat and Clements, 1998; Clements et al., 2009; Dearing et al., 2005; Foley et al., 1999; Raubenheimer et al., 2009; Scriber and Slansky, 1981). There is ample evidence from each of these areas that the foraging behavior of herbivores is driven by both nutrients and chemical defenses. Variation in macro- and micronutrients (e.g., protein, carbohydrates, sodium) in foods coupled with the nutritional requirements of herbivores influence intake and food preference (Barboza et al., 2009; Behmer, 2009; Clements et al., 2009; Raubenheimer and Simpson, 1997). However, the presence of plant secondary (or specialized) metabolites (PSMs), which can act as chemical defenses, often constrain intake despite the abundance of critical nutrients in those foods (Appel, 1993; Behmer et al., 2002; Dearing et al., 2005; Foley and McArthur, 1994; Hay and Fenical, 1988; Palo and Robbins, 1991; Paul et al., 2006; Raubenheimer and Simpson, 2009).

Although both nutrients and secondary metabolites influence foraging behavior, there is a general lack of ability to *a priori* predict diet selection even when the nutritional and chemical profiles of available plants are well documented (Clements et al., 2009; Cruz-Rivera and Hay, 2003; Kool, 1992; Sotka et al., 2009; Yeager et al., 1997).

Reasons for this shortcoming are that herbivores often have different nutritional intake targets, and that nutrients and PSMs are too often treated separately (reviewed in Behmer, 2009). Another reason is that researchers tend to regard nutrients as “therapeutic” components that always result in positive consequences whereas PSMs are considered “toxic” components with inevitable negative repercussions. As such,

nutrients and PSMs historically have been studied independently despite proposals for a unified marriage of these components since the 1970s (for review, Horn, 1989; Slansky, 1992). However, the definition of a nutrient versus a toxin is not necessarily clear and is a function of the dose, the herbivore's homeostatic state (Raubenheimer and Simpson, 2009; Raubenheimer et al., 2009) and interactions between nutrients and toxins. In some cases, compounds widely perceived as nutrients, such as amino acids, may be a feeding deterrent in their free form (DeGabriel et al., 2002; Field et al., 2009; Huang et al., 2011; Lokvam et al., 2006). In other cases, animals use PSMs in a manner consistent with them being therapeutic (see Forbey et al., 2009).

Moreover, PSMs can have contrasting effects in that they may be detrimental to some herbivores in some contexts, while having positive effects elsewhere. As an example, low levels of diterpene alcohols produced by Dictyotalean seaweeds deter larger generalist fish and urchins, but are feeding stimulants to smaller amphipods and worms (Hay et al., 1987). In yet other examples, tannins can reduce availability of protein in food for some vertebrate herbivores (Barbehenn and Constabel, 2011; Targett and Arnold, 2001) but may also work as antihelminthics (Min et al., 2003) or as antiviral agents (Appel and Schultz, 1994; Hunter and Schultz, 1993) in other herbivores.

Given that PSMs and nutrients often occur simultaneously in food, there is much to be gained from approaches that investigate the mechanisms by which both nutrients and PSMs act individually and together to influence the foraging ecology, or foraging phenotype, of herbivores. Numerous studies have investigated the relative "value" of these factors by herbivores (Behmer, 2009; Behmer et al., 2002; Bernays et al., 1994; Simpson and Raubenheimer, 2001). In some cases, nutrients are more important than

PSMs (Cruz-Rivera and Hay, 2003; Duffy and Paul, 1992; Felton et al., 2009; Van Alstyne et al., 2009), in others, PSMs are more important (Dearing et al., 2000; Erhard et al., 2007), and in still other cases, these factors are equally important or act synergistically to influence foraging behavior (Duffy and Paul, 1992; Frye et al. in press, Simpson and Raubenheimer, 2001). While approaches, such as the geometric framework, have helped researchers study how herbivores regulate nutrient needs in variable nutritional and PSM environments (Behmer, 2009), what remains poorly understood is *why* certain species respond to specific thresholds of PSMs or nutrients and others do not. If we want to explain and predict variable foraging behavior of herbivores, we need to measure mechanisms that set the tolerance and requirements for PSMs and nutrients.

We propose that the field of pharmacology should be integrated with chemical and nutritional ecology to facilitate the transitions among observations of foraging behaviors in the field relative to PSM or nutrient levels (foraging phenotypes) and pharmacological mechanisms guiding foraging patterns (pharmacokinetics-pharmacodynamics, PK-PD, Figure 1), to ultimately predict patterns of foraging behavior. This approach, linking observation with mechanism to predict pattern, was developed for terrestrial mammals but we argue it can be applied to invertebrate and vertebrate herbivores in terrestrial and aquatic habitats. Although we recognize that not all secondary metabolites consumed by herbivores are derived from plants, we use PSMs throughout to maintain a consistent language.

Our goal is to provide examples of how and why pharmacology can advance an understanding of the interactions between plants and herbivores. Researchers focused

on herbivores in one system will benefit from a richer understanding of the pharmacological mechanisms that limit or expand tolerance to nutrients and chemicals by herbivores in other systems. We begin by describing studies that integrate nutritional and chemical ecology to explain herbivore foraging patterns from different systems. We then offer practical approaches from pharmacology to answer ecological questions – termed Pharm-ecology. Pharm-ecology is the study of mechanisms that limit or expand herbivore diet breadth and are therefore useful in predicting foraging patterns. Throughout, we highlight the nuances of using pharmacological approaches in specific systems. However, we emphasize how pharmacology can offer a common approach to address questions posed by ecologists about diet selection in both terrestrial and aquatic systems. Finally, we demonstrate how ecologists can take concepts back into a natural setting to predict foraging patterns. Our hope is to stimulate researchers to perform multi-system comparisons that use pharm-ecology to address questions related to the mechanisms influencing the foraging ecology of herbivores.

In general, the workflow of a pharm-ecological project has three interacting steps. First, the investigator should observe foraging phenotypes that can be compared to other phenotypes (Shipley et al., 2009). Examples of foraging phenotypes include dietary specialists versus generalists, different diets in populations of the same species in different habitats, or diets of animals that shift ontogenetically or seasonally. These comparative systems provide excellent opportunities to understand the mechanisms underlying foraging patterns. Once foraging phenotypes have been identified, behavioral, morphological, physiological, and genetic adaptations that can influence what the body does to PSMs and nutrients (i.e., pharmacokinetics, PK) and what

PSMs and nutrients can do to the body (i.e., pharmacodynamics, PD) can be compared between phenotypes. We recognize that investigating pharmacological mechanisms can be daunting. *In vivo* studies are recommended first to understand how the concentration of PSMs or nutrients influences intake and excretion of these substrates and their metabolites. If animals differ in intake and excretion of foodstuff, there are likely to be mechanisms driving these patterns and more detailed pharmacokinetic and pharmacodynamic approaches such as those outlined below are warranted. We do not provide the details of such studies, but rather provide a general overview of how these pharmacological approaches can be useful in understanding and comparing mechanisms driving variation in foraging phenotypes.

Foraging phenotypes: An integrated view of nutrients and toxins. Much of the early work on plant-herbivore interactions focused on identifying PSMs that constrained intake in herbivores. Specifically, the detoxification limitations hypothesis argued that the ability of herbivores to detoxify ingested PSMs largely determined which plants, and how much, they could eat (Freeland and Janzen, 1974; Marsh et al., 2006a). Although this hypothesis was largely focused on mammalian herbivores, the concepts apply to all herbivores. Threshold intakes of PSMs have been documented in several species of terrestrial (Govenor et al., 1997; Simpson and Raubenheimer, 2001; Stapley et al., 2000; Torregrossa et al., 2012) and aquatic (Demott, 1999; Gross and Bakker, 2012) herbivores.

There are several factors that can influence thresholds to PSMs. Nutrients may interact with PSMs to establish those thresholds. For example, common brushtail possums had higher tolerance to diets rich in benzoic acid when they were provided

with substrates needed for the detoxification and excretion of benzoates (Marsh et al., 2005). In addition, supplemental energy and protein may increase the ability of herbivores to consume foods that contain diverse PSMs (Nersesian et al., 2012; Provenza et al., 2003) by providing the resources needed to pay for high energetic costs of detoxification (Foley and McArthur, 1994; Mangione et al., 2004; Sorensen et al., 2005c).

Ecological and evolutionary experience with PSMs or nutrients also plays a role in shaping foraging phenotypes. For example, previous ecological exposure to PSMs increases tolerance to those PSMs, but may decrease tolerance to novel PSMs (Gustafsson and Hansson, 2004; Sorensen et al., 2005b). Memory of the positive and negative experiences of exceeding thresholds to nutrients and PSMs via conditioned food aversions can also influence foraging phenotypes (Provenza et al., 1998).

Animals form aversions when they associate post-ingestive illness with the taste of food. Olfactory chemoreception is the most ancient sensory cue in animals and may be used for pre-ingestive avoidance of chemicals, whereas taste may be used for post-ingestive limitation of food intake. Although these concepts have been verified using ecologically realistic combinations of flavors and toxins (Lawler et al., 1999), there has been some doubt cast on how well animals can generalize preferences when the foraging choices become more complex both temporally and spatially in natural systems (Duncan et al., 2007; Favreau et al., 2010; Ginane et al., 2005). However, animals can discriminate mixtures of chemicals to remember and track these olfactory cues through space and time (Derby and Sorensen, 2008). There is enormous scope for investigating the role and consequences of previous experience, learning and

associated sensory systems in understanding the foraging phenotypes of herbivores.

PHARMACOLOGICAL MECHANISMS

It has been widely observed that the quality, quantity, and complexity of nutrients and PSMs and the learned responses to these dietary components can mediate the foraging patterns of herbivores (Amsler, 2008; Behmer, 2009; Hay and Fenical, 1988; Paul and Vanalstyne, 1992; Provenza et al., 1998; Provenza et al., 2003; Simpson and Raubenheimer, 2001). Although the general concepts of pharmacology were introduced to ecologists to explain plant-herbivore interactions 38 years ago (Freeland and Janzen, 1974), the empirical use of pharmacology to understand mechanisms driving foraging patterns in any system is limited (Haley et al., 2008; Magnanou et al., 2009; Marsh et al., 2006b; McLean and Duncan, 2006; Sorensen et al., 2006; Sotka et al., 2009). In contrast to the broad knowledge base that exists on the mechanisms required to process nutrients as well as mechanisms used by domestic and laboratory species to metabolize drugs, there is a general lack of understanding of how PSMs are processed by herbivores, especially in the context of a variable nutrient environment (Appel, 1993; Casarett et al., 2008; Gross and Bakker, 2012; Karasov and Hume, 1997). The gap in knowledge related to mechanisms required to process nutrients in marine herbivorous fishes, for example, has only recently begun to close (Choat and Clements, 1998; Clements et al., 2009). Moreover, despite a long history of investigating how aquatic animals process chemical contaminants (Chambers and Yarbrough, 1976; Katagi, 2010; Rewitz et al., 2006; Smital et al., 2004), studies investigating how they process dietary secondary metabolites have only recently been initiated (Gross and Bakker, 2012; Liang et al., 2007; Richardson et al., 2009). An

understanding of pharmacology can fill these gaps in both terrestrial and aquatic systems.

The pharm-ecological approach focuses on evolutionary conserved mechanisms that define foraging phenotypes. As highlighted above, pharmacological approaches allow researchers to investigate the mechanisms that drive the fate (what the body does to a chemical, pharmacokinetics, PK) and action (what a chemical does to the body, pharmacodynamics, PD) of PSMs and nutrients in any herbivores. The pharm-ecological perspective views the foraging ecology of herbivores as a dose-concentration-response.

The general outcome of the pharm-ecological mechanisms described, to minimize deficits and excesses to maintain homeostasis, is conserved across species for a range of chemicals and therefore can be applied to a variety of systems (see Behmer, 2009; Sotka et al., 2009). Of the parameters outlined in Figure 1, dose (e.g., total intake), metabolism, excretion and foraging response have received the most attention by ecologists (Haley et al., 2008; McLean and Duncan, 2006; Sorensen et al., 2006). Distribution, specifically associated with the amount and site of sequestered PSMs, is well described in terrestrial insects (Opitz and Muller, 2009; Dobler et al. 2011) and a small number of marine invertebrate systems (Pennings and Paul, 1993; Whalen et al., 2010). The mechanisms of absorption of PSMs and the resultant kinetics (i.e. time course) of secondary metabolites are known in some vertebrate (Boyle et al., 2005; Dziba et al., 2006; Mclean et al., 2007) and invertebrate herbivores (Zangerl et al. 2012). However, detailed pharmacokinetic studies where the concentration-time profile is linked to a specific mechanisms (e.g. metabolizing enzymes, efflux

transporters) are rare for all types of herbivores. Pharmacokinetic data of any kind are even scarcer for aquatic systems (Ibelings and Havens, 2008; Martin-Creuzburg and von Elert, 2009; Pennings and Paul, 1993; Whalen et al., 2010).

Pharmacokinetic mechanisms: Dose. The dose-concentration relationship is primarily dependent on the amount of substrate (nutrient or PSM) orally consumed by an herbivore. Other forms of substrate administration may be important for other taxa, such as dosing through the skin or gills for aquatic species. Although measuring the dose consumed by an herbivore is standard practice, few researchers measure the actual systemic dose or concentration an herbivore experiences during foraging events. Both meal size and frequency influence the peak systemic concentration and exposure (area under the concentration-time curve) of substrates in the body compartment(s) (Figure 2). For vertebrates with closed circulation systems, the most important body compartment is the blood, as this compartment delivers the substrate to all other compartments (e.g., liver, brain, kidney). Within invertebrates, the most important compartments are the gut, hemolymph and fat body (Govenor et al., 1997; Keeley, 1985). Within a species regardless of taxonomic group or morphology, larger, more frequent doses generally result in higher concentrations of the substrate in compartments (Figure 2).

To avoid negative consequences of PSMs, both terrestrial and aquatic herbivores must be able to detect concentrations of key PSMs and regulate intake of food accordingly. The regulation model of dose control (reviewed in Torregrossa and Dearing, 2009) proposes that herbivores regulate daily dosing through modifications to either meal size or the intervals between meals, known as the “intermeal interval”. Several

mammalian herbivores appear to use the strategy of altering meal size as a function of PSM concentration (Boyle et al., 2005; Marsh et al., 2007; Sorensen et al., 2005a; Torregrossa et al., 2011; Torregrossa et al., 2012; Wiggins et al., 2006a; Wiggins et al., 2006b; Wiggins et al., 2003). Of the five species studied to date (three woodrats of the genus *Neotoma* and two marsupials: the koala and brushtail possum), only one exhibited a change in intermeal interval when fed increasing concentration of dietary PSMs (Sorensen et al., 2005a; Torregrossa and Dearing, 2009). Recent studies suggest that generalist herbivores may be better at regulating PSM dose than specialist herbivores (Torregrossa et al., 2012). Specialist herbivores may not need to regulate intake of PSMs since their biotransformation system has evolved to process high doses of PSMs in a limited diet or because they absorb a lower proportion of PSMs consumed (Marsh et al., 2006b; Shipley et al., 2009; Sorensen et al., 2004). The mechanisms through which regulation of meal size and frequency occurs and why they differ between species are currently unknown. The leading proposed mechanisms are detection of PSMs in food and in blood plasma, conditioned learning, and intestinal or gustatory receptors (Foley et al., 1999; Torregrossa et al., 2011; Torregrossa et al., 2012). To date, regulation of PSMs through meal size and intermeal interval has not been studied in aquatic herbivores. However, observations of feeding by free-ranging aquatic herbivores or documentation of intake of PSMs in captive aquatic animals readily lend themselves to investigation of PSM regulation via meal size and meal frequency. There is urgent need to link dose of PSMs with mechanisms of detection to understand how PSMs alter foraging behavior in a variety of systems.

Regardless of mechanism or system, the concentration of PSM is likely to dictate feeding responses of herbivores across systems. As such, observations of temporal and spatial variation in feeding by herbivores could be a result of temporal and spatial variation in concentrations of PSMs. For example, mammalian herbivores select specific trees (Degabriel et al., 2009; Moore and Foley, 2005; Moore et al., 2010) and aquatic herbivorous invertebrates select specific tissue types (Newman et al., 1996) to avoid consequences of toxic PSMs. To add to dietary complexity, feeding by herbivores can influence the concentration and distribution of both chemicals and nutrients. Environmental conditions that change over short (e.g., seasons) and long-time periods (e.g., global climate change) can also influence concentrations of PSMs (Bidart-Bouzat and Imeh-Nathaniel, 2008; Lindroth, 2010) as well as toxicity of PSMs (Dearing, 2012; Dearing et al., 2008). In some cases, changes in concentration of PSMs may differ between terrestrial and aquatic systems. For example, concentrations may vary with fluctuations in hydration and ultraviolet light exposure in water-limited systems (Chen et al., 2011; Turtola et al., 2005), whereas these factors may be less variable in aquatic systems. There is a need for cross-system comparisons of how both qualitative and quantitative variation in chemicals and nutrients influence how herbivores regulate the dose of plants consumed over time.

Pharmacokinetic mechanisms: Absorption, distribution, metabolism, and excretion (ADME). The dose consumed does not always translate into a concentration that elicits a response. The concentration-time profile of a substrate in compartments can be modified by herbivores through mechanical and biochemical mechanisms that influence the absorption, distribution, metabolism, and excretion of substrates. These factors are collectively referred to in pharmacological literature as “ADME” and

apply to both terrestrial and aquatic systems (Sotka et al., 2009). Pharmacokinetic studies that determine the concentration-time course may be more informative with respect to predictions of food intake than *in vitro* kinetic approaches that quantify enzyme activities. For example, different individuals consuming the same amount of PSM may not be exposed to the same concentration of PSM due to differences in the amount absorbed (Sorensen et al., 2004). Similarly, animals consuming different amounts of PSMs may have very similar levels of exposure to PSMs. These subtle differences could have major implications for proper interpretation of *in vitro* studies. But both *in vivo* and *in vitro* studies investigating ADME of both PSMs and nutrients will offer insights into understanding the foraging phenotype of herbivores.

Absorption requires that the ingested substrate first be liberated, or released, from the plant biomass. For example, tannin-binding salivary proteins can bind to tannins and increase the availability of protein (Shimada, 2006). In addition, the physio-chemical characteristics of the stomach or the presence of microbes in the gut can influence the release of a PSM or nutrient from the food matrix (Foley et al., 1999; Gross et al., 2008). Once liberated, the substrate must then be absorbed where it can be distributed to the sites of action. The small intestine is the major site of absorption for terrestrial vertebrates, but other tissues can also be important and should be considered (e.g., gills and skin for aquatic species). Regardless of the site of the barrier, absorption can be passive or active depending on the electrochemical properties of the chemical. There are ample reviews on factors such as the length and surface area of the intestine and the concentration and binding affinity of transporters that influence absorption of nutrients (Karasov and Martínez del Río, 2007). In contrast, very few studies have investigated how these factors influence the absorption of PSMs by herbivores

(Sorensen et al., 2006). In addition, only a few studies have investigated how nutrient-PSM interactions influence absorption. For example, some tannins can bind to dietary and endogenous proteins but disrupting these complexes in the gut significantly improves the protein economy of the animal and can improve the reproductive success of animals (Degabriel et al., 2009).

One mechanism to minimize the response to PSMs is to actively transport PSMs out of cells, back into the intestine against a concentration gradient (Sorensen and Dearing, 2006). PSMs that are removed from the cells are then excreted in feces. Whereas some PSMs are substrates for these efflux transporters, others can inhibit or enhance their action (Wen et al. 2006, Zhou et al., 2008) and so influence the body concentrations of other PSMs ingested concurrently. This phenomenon, known as drug-drug-interactions, is well known in human pharmacology when drugs are ingested with other foods or drugs. Identifying transporters that regulate absorption may shed light on herbivore tolerance to PSMs, reveal that herbivores avoid inhibitors or select inducers of these transporters, and explain how animals sequester chemicals in tissues.

Recent studies have highlighted the importance of paracellular absorption of many different compounds and the significant differences among groups of vertebrates in the capacity of this route (McWhorter et al., 2009). For example, paracellular absorption wherein molecules are passively absorbed through the aqueous channel in the tight junctions of adjoining cells appears to be particularly important in birds (Lavin and Karasov, 2008). Although enhanced paracellular absorption in birds relative to most mammals may compensate for shorter intestines of birds (Caviedes-

Vidal et al., 2007), it may also expose birds to significantly greater amounts of water soluble PSMs (McWhorter et al., 2009), which can interfere with digestion and exert potentially toxic effects. Differences in the importance of paracellular absorption can translate to significantly different effects of PSMs on nutrient absorption (Skopec et al., 2010) and offers an opportunity for researchers to investigate mechanisms responsible for nutrient-PSM interactions. Paracellular absorption has not been investigated in fish but given their relatively short intestines, they too may rely on this route of absorption and thus subjected to trade-offs similar to that observed in birds.

Once absorbed, the substrate is distributed throughout the body where it can be metabolized or react with tissues. The liver is the primary site of biotransformation of PSMs for vertebrates. In invertebrates, fat bodies and the digestive system, including the different gut compartments and the digestive gland, are responsible for the biotransformation of toxins (Appel, 1993; Gross et al., 2008; Hyne and Maher, 2003; Keeley, 1985; Rewitz et al., 2006). The enzymes responsible for the metabolism of drugs have been extensively investigated in laboratory and domestic mammals but far less so with respect to PSMs in wild herbivores, particularly aquatic ones (Dearing et al., 2005; Sorensen et al., 2006; Sotka et al., 2009). Drug metabolizing enzymes are extremely diverse and categorized broadly by function into Phase 1 enzymes such as the cytochromes P450 or Phase 2 enzymes such as glutathione S-transferases and glucuronyltransferases (Casarett et al., 2008). In general, metabolizing enzymes act to convert lipid soluble PSMs (and other ingested chemicals) into more water-soluble metabolites that can then be excreted (Casarett et al., 2008; McLean and Duncan, 2006). Individual mammals have hundreds of different drug metabolizing enzymes that are described elsewhere (for reviews see (Casarett et al., 2008; Dearing et al.,

2005; McLean and Duncan, 2006). The cytochromes P450 enzymes of aquatic crustaceans responsible for detoxification differed from those of fish (Koenig et al., 2012), indicating differences between invertebrates and vertebrates. The recent genome sequencing of vertebrates (Margulies and Birney, 2008) and invertebrates, including freshwater (Colbourne et al., 2011) and terrestrial (Adams et al., 2000; Whiteman et al., 2011) species has yielded new insights about the evolution of drug metabolizing enzymes (Asselman et al., 2012). For example, the cytochromes P450 (CYPs) appear to have undergone repeated duplications, resulting in multiple gene copies for similar enzymes (Hu et al., 2008). Such genetic diversity may be critical for herbivores in the process of adapting to toxic or novel diets. Indeed Malenke et al (2011) recently reported putative functional differences related to PSM intake within the subfamily of CYP 2B in herbivorous woodrats. Future comparative genomic studies combined with functional analyses will be fruitful in understanding the evolution and diversity of drug-metabolizing enzymes and their influence on foraging phenotypes across taxa.

Herbivores, however, cannot be treated in isolation of their microbial symbionts. There is increasing evidence that most mammalian herbivores, and many invertebrate herbivores harbor large populations of microbes (bacteria, protozoa and fungi) in their gut that can play a variety of roles including making nutrients more available to the host animal (Broderick et al. 2004; Chandler et al., 2008; Clark et al. 2010; Clements et al., 2009; Janson et al., 2008; Mountfort et al., 2002; Stevens and Hume, 1998). Given the frequency of microbe-herbivore association, microbial communities may influence pharmacokinetics of nutrients and PSMs. From a nutritional perspective, gut microbes likely enhance the digestive and intestinal metabolic processing of nutrients

in most herbivores. For example, gut microbes might provide enzymes such as cellulases, to efficiently digest plant material (He et al., 2009). Aquatic and terrestrial herbivorous *Lepidoptera* larvae harbor distinct communities of bacteria in the gut despite their small body size and the rapid passage of food through gut, and these microbial communities differ depending on the plants eaten (Broderick et al., 2004; Walenciak et al., 2002). Whether these bacteria are involved in the detoxification of PSM remains to be seen. However, bacteria isolated from the gut of aquatic *Lepidoptera* larvae feeding on a tannin-rich aquatic plant were less susceptible to the antibacterial effects of tannins than bacteria isolated from the gut of larvae fed with a tannin-free plant (Walenciak et al., 2002). The importance of gut bacteria in the toxic effects of *Bacillus thuringiensis* kurstaki on *Lepidoptera* larvae has also been demonstrated (Broderick et al. 2006). First attempts to identify the gut bacterial community in zooplankton have been successful (Peter and Sommaruga, 2008), and might aid in determining the role of microorganisms in dealing with toxic phytoplankton. Recent evidence suggests that microbial gut communities of herbivorous fishes are more similar to those of herbivorous mammals than to carnivorous fishes, raising the interesting suggestion that fishes were among the first animals to ferment plant material (Sullam et al. 2012).

Another function of gut microbes might be the detoxification of ingested PSMs (Chandler et al., 2008; Dearing et al., 2005; Foley et al., 1999; Kohl and Dearing, 2012; Kohl et al., 2011). There is at least one spectacular example of this phenomenon in domestic ruminants. Jones and Megarrity (1986) showed that transfer of rumen contents from Indonesian goats to Australian goats allowed the latter to feed on *Leucaena* leaves without negative consequences. *Synergistes jonesii* was isolated

as the microbe responsible for detoxification of the toxic metabolite (goitrogen 3-hydroxy-4(1H) pyridone) generated by the rumen metabolism of the PSM mimosine (Rincon et al., 1998). This bacterium has been readily transferred between individual animals with clear benefits to the host (Jones and Megarrity, 1986). Curiously, none of the players in this system (microbe, plant, herbivore) have a long evolutionary history with one another. In addition to this example, detoxification of PSMs by gut microbes has been suggested for the unique and diverse microbial community found in the gut of a herbivorous rodent, the woodrat (Kohl et al., 2011). Moreover, the diversity of the microbial community appears to be a function of the host's evolutionary experience with PSMs such that more diverse communities are present in herbivores with previous experience to particular PSMs compared to novel ones (Kohl and Dearing, 2012). This microbial diversity may be key reducing the concentrations of PSMs prior to absorption by the host. Recently, the first evidence that microbial detoxification of ingested PSMs occurs in fish was reported (Guan et al., 2009). Nonetheless, there are other examples in domestic ruminants (Majak, 1992) where an increase in toxicity occurs via microbial modification. Although hindgut fermenters are expected to benefit from microbial detoxification of PSMs less than foregut fermenters, microbes inhabit the mucosal lining of the intestine prior to the cecum in many terrestrial (Frey et al., 2010; Yamamoto et al., 2009) and aquatic animals (Ganguly and Prasad, 2011; Mondal et al., 2008). Additional studies are clearly needed to identify the positive and negative influences microbial communities have on herbivores with either complex or simplified digestive tracts such as those found in marine and freshwater invertebrates (Brunet et al., 1994; Freese and Schink, 2011).

The recently developed technique of metagenomics is likely to reveal more about the function of gut microbes and perhaps result in some paradigm shifts (Ley et al., 2008; Ley et al., 2006). For example, the microbial floras of giant and red pandas are exceptional for herbivorous mammals in that they are more similar to that of their carnivorous relatives than to other herbivores (Ley et al., 2008). And a recent meta-analysis suggests that not only do fish have specialized gut microflora, but that the bacterial communities in fish were closely related to those from terrestrial mammals (Sullam et al., 2012). A second frontier in both terrestrial and marine systems is in understanding the impact of nutrient and PSM concentrations on the interactions between gut microflora and the immune function of the host (Hooper and Macpherson, 2010; Perez et al., 2010; Reynaud et al., 2008).

In general, herbivores are predicted to invest in mechanisms that regulate the liberation, absorption, distribution, metabolism, and excretion of PSMs and nutrients to minimize inadequate or surplus concentrations of these dietary components. Investment in particular strategies may be influenced by the extent to which nutrients or PSMs are more important for a particular herbivore. It is likely that many mechanisms are yet to be discovered particularly in non-model organisms.

Pharmacodynamic mechanisms. Compared to our understanding of pharmacokinetic mechanisms, we know even less about pharmacodynamic (PD) mechanisms. The concentration-response relationship, referred to as pharmacodynamics, describes how a known concentration of a substrate elicits a behavioral or physiological response in herbivores (Figure 1). In general, it represents the extent to which a chemical reacts with the body to cause an observable response. Variation in food intake has been the

response measured most commonly in herbivores (Behmer, 2009; Ibelings and Havens, 2008; Martins and Vasconcelos, 2009; Torregrossa and Dearing, 2009). Other responses such as locomotor activity (Sorensen et al., 2005c), metabolic rates (Bozinovic and Novoa, 1997; Sorensen et al., 2005c), thermoregulation (McLister et al., 2004), organ damage (Fu et al., 2004), water balance (Dearing et al., 2002; Mangione et al., 2004) and acid-base homeostasis (Foley et al., 1995) have also been investigated. Only a single study has examined the effect of blood concentration of PSMs on intake in a wild mammal (McLean et al., 2007), which may be critical to predicting foraging phenotypes in nature. The link between intake, blood (or other body compartment) concentration, and more specifically, distribution to specific tissues deserves attention if we want to know the mechanism of action of PSMs. Given the difficulty in collecting repeated blood samples for PSM concentrations for most animals, an alternative and potentially more effective pharmacodynamic approach could be to measure efficacy (the maximum response for a given dose), potency (the dose needed to produce a response), slope (how much or how little the difference is between the dose that causes no effect and one that causes a maximum effect) and variation of the dose-response curve within and between species administered different doses of PSMs (Figure 1).

Identifying a mechanism of action that elicits a response is far more challenging than investigating enzyme metabolism. Some PSMs are known to have highly specific targets such as the inhibition of Na^+/K^+ ATPase by cardenolides (Petschenka et al. 2012) and cardiac glycosides (Holzinger et al. 1996) and inhibition of succinate dehydrogenase by papyriferic acid from birch (Forbey et al., 2011; McLean et al., 2009). Other PSMs interact with nutrients to cause broad pharmacological responses

such as oxidative stress (Aucoin et al., 1995) or work synergistically with other PSMs to elicit responses (Guillet et al., 2000; Wen et al. 2006). Others may exert their effects indirectly through multiple pathways. For example, jensenone, a formylated phloroglucinol compound in *Eucalyptus* whose intake is closely regulated by folivorous marsupials, exerts its effects by binding to amine groups on critical molecules in the gastrointestinal tract, leading to a loss of metabolic function (McLean et al., 2004), followed by the release of 5-hydroxytryptamine (5HT), which in turn may mediate an emetic response leading to a conditioned aversion (Lawler et al., 1998). Not surprisingly, species differ in their dose-response curves (Forbey et al., 2011; Majak, 1992). Moreover, the molecular targets of most PSMs that elicit the behavioral or physiological consequences (e.g. basal metabolic rate, energy excretion, weigh loss, increased body temperature) are not always apparent. Emerging advances in molecular modeling provide ecologists with *in silico* techniques that can reveal the most likely molecular targets of specific PSMs with known structures (Forbey et al., 2011). Coupling molecular modeling with *in vitro* and *in vivo* pharmacodynamic studies will facilitate understanding how differences in the interactions between nutrients, PSMs and target receptors explain foraging patterns of herbivores.

PREDICTING FORAGING PATTERNS

Chemistry-herbivore patterns. Although pharm-ecological approaches have largely been applied in controlled laboratory settings, the ultimate aim is to use the insights from this framework to predict foraging of herbivores in the field. Free-ranging animals have many more choices, broader diets and other competing costs such as predation than animals in captivity. For example, the ability of herbivores under laboratory conditions to regulate dose through frequent, smaller meals may be

constrained in the field if animals cannot meet biomass needs from a single plant and expend energy or have greater predation risk when moving between foraging patches (Nersesian et al., 2011; Wiggins et al., 2006b). Consequently, we should not necessarily expect simple correlations between the occurrence of one or several PSMs or nutrients and diet choice from field studies even when there are very strong relationships between particular PSMs, nutrients and intake in captive studies.

Moreover, animals will rarely, if ever, encounter the full range of variation in intraspecific variation of PSMs in a species. Most PSMs are synthesized through the action of multiple genes and this results in concentrations of PSMs being normally distributed amongst different plants (Andrew et al., 2007). Consequently, animals frequently encounter the median concentrations but rarely the high and low extremes. Thus, the power to detect relationships between feeding and PSM or nutrient concentrations will be low unless a large number of observations are made and a large number of plants examined. Doing so requires high-throughput techniques such as near infrared reflectance spectroscopy (NIRS). This analytical technique relies on establishing relationships between traits of interest (e.g., nitrogen, specific PSM) and the NIR spectrum of the sample. Once this relationship is defined statistically, the traits can be predicted in a large number of additional samples by collecting the spectrum alone (Foley et al., 1998; Stolter et al., 2006). Although NIRS has mostly been applied to terrestrial plants it also works well with aquatic plants such as seagrass (Lawler et al., 2006) and brown macroalgae (*Sargassum*, Hay et al. 2010). The biggest benefit is being able to predict complex multi-dimensional traits such as the overall palatability of a plant to herbivores. With this approach, Moore et al. (2010) used NIRS to map the palatability of multiple home ranges used by koalas in

an area of forest and found that the palatability measure predicted feeding better than measuring PSMs and nutrients separately. NIRS thus allows measurement of complex traits of many plants and over large areas and a more comprehensive evaluation of the nutritional and chemical landscape. These traits can then be linked to mechanisms employed by populations or species with exceptionally low or high tolerance to these traits.

In addition to high throughput approaches, genetic modification holds great promise for predicting foraging phenotypes of free-ranging herbivores (Kessler et al., 2004; Kessler et al., 2008; Wu and Baldwin, 2009). For example, silencing of the jasmonate cascade in *Nicotiana attenuata* improved the performance of specialist herbivores and allowed non-adapted generalist herbivores to attack (Kessler et al., 2004). Insect transgenesis has been used to manipulate agricultural and medical insect pests (Fraser, 2012) and could also be used to observe how foraging phenotypes of herbivores are influenced by control of pharmacokinetic (e.g., detoxification enzymes) and pharmacodynamic (e.g., target receptors) mechanisms. We suggest that genetic manipulations of free-ranging species offer exciting opportunities for examining the underlying mechanism of herbivore responses to variable diet quality.

Understanding which components of the diet an animal is regulating, is important in applying pharm-ecological frameworks in the field. One particularly useful approach is the geometric modeling framework of Simpson and Raubenheimer (Raubenheimer and Simpson, 1997; Simpson and Raubenheimer, 2001; Simpson et al., 2010). This graphical approach allows preferences for one nutrient or PSM to be evaluated against multiple others while taking into account the animal's current nutritional state. An

animal feeding on a nutritionally balanced food that contained a manageable concentration of PSMs could eat sufficient amounts of that food to meet requirements for multiple nutrients (e.g., protein and energy), whereas an imbalanced food might require ingesting too much of one nutrient or PSM to obtain sufficient quantities of another. The geometric framework then becomes a very powerful tool for observing tradeoffs and compromises and is equally applicable to examine tradeoffs between multiple nutrients, nutrients and PSMs or even nutrients and medicines (Raubenheimer and Simpson, 2009). Moreover, it can be used to identify how genetic modification or chemical inhibition or induction of pharmacokinetic or pharmacodynamic mechanisms influence tradeoffs.

The geometric framework has been most widely applied to captive species (Fanson et al. 2009; Jensen et al. 2011; Mayntz et al. 2009; Miller et al. 2009). However, it has been successfully used in free ranging herbivores as well (Wright et al. 2003). A recent study in wild primates showed that contrary to prevailing views, spider monkeys prioritized acquisition of protein not bound by tannins over energy across many days of individual foraging (Felton et al., 2009). In contrast, similar studies of wild gorillas found no prioritization of protein (Rothman et al., 2011). This framework should be applied more broadly in multiple taxa to continue bridging the gap between a functional understanding of foraging in individual animals to that of whole communities (Simpson et al., 2010).

CONCLUSIONS AND PHARM-ECOLOGICAL QUESTIONS

The majority of studies in plant-herbivore interactions have focused solely on the effects of PSMs or nutrients on total intake, such that nutritional demands result in an increase in total intake of the required nutrient, whereas toxicity of PSMs result in a

decrease in total intake. We propose that the variable patterns of intake observed in herbivores cannot be fully explained unless we understand the mechanisms by which dose, concentration, and response are related and how they can be modified by herbivores. We also propose that a pharm-ecological approach can overcome and actually capitalize on differences between aquatic and terrestrial systems to answer how chemistry, ecology and taxonomy explain foraging phenotypes. Although all animals use highly conserved mechanisms to regulate the dose-concentration-response relationship, each animal will likely have a unique pharmacokinetic and pharmacodynamic phenotypes that are dependent on various combinations of genetic, behavioral and biochemical mechanisms. Understanding the mechanisms driving similarities and differences between taxonomic groups of plants and herbivores will advance our understanding of the evolution of plant-herbivore interactions. We offer some of the many questions that could be addressed through collaborations between ecologists studying aquatic and terrestrial systems:

1. Does the physio-chemical environment influence foraging phenotypes? Although terrestrial and aquatic plants provide similar resources (e.g., protein, fats and carbohydrates) to herbivores and share many of the same broad classes of secondary metabolites (see Sotka et al., 2009), there are some notable differences. For example, cyanogenic glycosides, glucosinolates or tetraterpenoids have either not been reported or are not produced by marine algae. How might terrestrial and aquatic herbivores respond to novel classes of PSMs only found in the other system? Do aquatic herbivores have specialized mechanisms to deal with more water soluble PSMs generated in the lower oxygen availability in marine environments (Kong et al., 2010) than terrestrial herbivores? Are there differences in receptors for PSMs between

aquatic and terrestrial herbivores depending the sensitivity to detect water or lipid-soluble PSMs? Because herbivores in freshwater systems experience a gradient from dry to wet, do they possess PK-PD mechanisms that are functional in both marine and terrestrial systems? Are aquatic herbivores more likely to experience synergistic chemical consequences when they than terrestrial herbivores? Have ecologists overlooked the consequences of inadvertently consuming cryptic toxins from small epiphytes living on larger plants (Cruz-Rivera and Hay, 2003; Sotka et al., 2009) by focusing only on host plant traits (Porrás-Alfaro and Bayman, 2011)?

2. Does the ecological extent of herbivory influence PK-PD mechanisms and foraging phenotypes? Do species that transition between carnivory, omnivory and degree of specialized herbivory during their life span have more generalized or plastic PK-PD mechanisms than species without these transitions? For example, terrestrial vertebrates, aquatic, and particularly marine herbivores, are largely generalists and rarely specialize on particular foods (Poore et al., 2008; Shipley et al., 2009).

Moreover, herbivorous fishes are often carnivores as juveniles and transition to herbivory with age (Horn, 1989). Is there a relationship between the expression and diversity of PK mechanism and dietary transition points that is consistent among taxa? Certainly, shifts in generalism and omnivory will make linking laboratory pharm-ecological studies to field foraging patterns difficult as the link between diet and response is clearer in specialist herbivores. However, broadscale approaches such as state-of-the-art genomic and proteomic techniques can compare the diversity of detoxification mechanisms (Browning et al., 2010; Eyckmans et al., 2012; Glenn et al., 2010; Itokawa et al., 2010; Shawahna et al., 2011; Thiyagarajan and Qian, 2008; Whalen et al., 2008) and microbial communities (Langille et al., 2012; Masahira et al., 2009; Matteotti et al., 2011; Weinstock, 2012) relative to dietary diversity. These

emerging approaches offer enormous potential to compare and contrast pharm-ecological mechanisms and foraging phenotypes across wide taxonomic groups

3. Does phylogenetic diversity influence PK-PD? Relative to terrestrial herbivores, there is a broader phyletic diversity of herbivores in the ocean than on land. While herbivory is largely the domain of two terrestrial groups (insects and mammals), there are at least six phyla that consume benthic seaweeds: vertebrates (fish, reptiles and manatees), arthropods (insects, amphipods, isopods, crabs), echinoderms, annelids, pycnogonids, and mollusks (chitons, snails, sea slugs, abalone). Moreover, herbivory arose independently within dozens of clades of more recent origin (Vermeij and Lindberg, 2000). Given the phylogenetic diversity within marine systems, do marine herbivores as a group, use a broader suite of mechanisms to ‘deal’ with the challenges of consuming chemically-defended and nutritionally-poor foods relative to terrestrial consumers?

The pharm-ecological questions and approaches provided here allow researchers to explain and predict how intake, dose, and herbivore morphology, physiology and taxonomy interact to influence kinetic and dynamic interactions between plants and herbivores. The true value of the pharm-ecological approach requires interdisciplinary collaboration among aquatic and terrestrial ecotoxicologists or physiologists who focus solely on mechanisms and ecologists who look primarily at ecological patterns and processes. Pharm-ecology provides a common language and approach to meet a future objective: scaling a functional understanding of nutrient acquisition in individual organisms to the more complex interactions at higher levels of biological organization and comparing these scales across diverse systems.

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Figure Captions

Figure 1. Pharm-ecological concepts explaining the dose-concentration-response in plant-herbivore interactions. Animals consume diets containing plant secondary metabolites (PSMs) and nutrients. The doses of PSMs and nutrients ingested are dependent upon the amount of plant material ingested and concentration of these chemicals in the plant. The animal's mechanisms of absorption, distribution, metabolism and excretion (ADME) determine the concentrations of the PSM or nutrient in body compartments (e.g. circulation, tissues) over time. The concentration-time course of chemicals is called pharmacokinetics (PK). Circulating or distributed PSMs or nutrients can interact at one or multiple sites within the body through dynamic mechanisms of action in the body and elicit a physiological or behavioral response. The concentration-response relationship is called pharmacodynamics (PD). This response can directly impact the subsequent intake of food. Occasionally, the resultant metabolites of metabolism are more bioactive than the original chemical and can have pharmacodynamic responses. Herbivores may detect PSM and nutrient concentrations in the environment (via volatile chemicals), after tasting the diet or perhaps in the circulation and adjust intake (dose). ADME mechanisms and sensitivity to mechanisms of action ultimately influence the response to PSMs and nutrients. But see (McLean and Duncan, 2006; Sorensen et al., 2006; Sotka et al., 2009) for additional details.

Figure 2. A schematic of the relationship between meals (grey bars), concentration of a PSM or nutrient in the blood after a meal (solid line) and time. The upper dashed line (A) represents a theoretical maximum threshold of intake above which animals experience negative effects due to a surplus of PSMs or nutrients. The lower dashed

line (B) represents a minimum threshold of intake below which animals are in a nutritional deficit or do not derive the potential positive benefit of PSMs. If meal sizes are too large (C), blood concentrations of PSMs or nutrients are in surplus, whereas if meal size is too small (D), there is a nutritional deficit or lack of potential therapeutic benefit from PSMs. Small meals consumed more frequently will allow animals to reach a “steady state”, or balance, between deficiencies or surplus of PSMs or nutrients and this can then be maintained with a constant meal size and frequency interval (E). However, even small meals consumed too frequently will result in concentrations of PSMs or nutrients above maximum thresholds. The terminal slope of the concentration curves for each meal is constant to indicate a non-saturable elimination of the PSM or nutrient from the blood.

Figure 1.

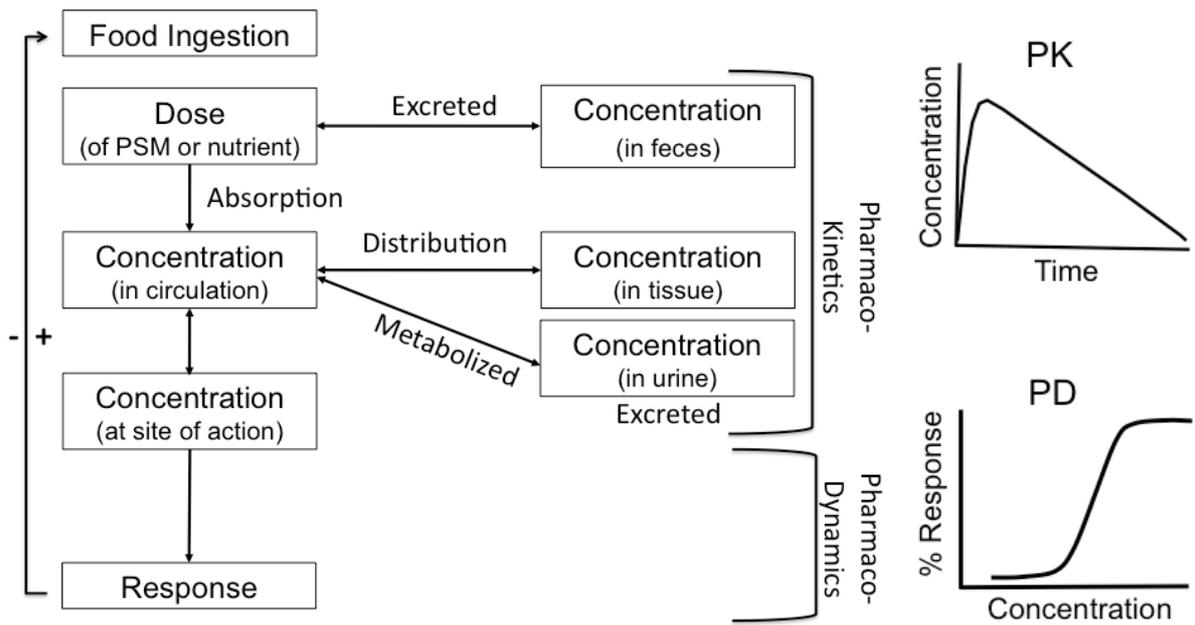


Figure 2

