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Nutrient Effects on Spring Flora and Fauna

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Summary

Many of Florida's springs and spring runs are enriched in nitrate due to broad-scale contamination of groundwater supplies. This observation fosters two primary and interrelated concerns regarding the effects of nutrients on flora and fauna in spring systems. High nitrate concentrations can affect fauna directly through toxicity. In addition, high nitrate concentrations can promote eutrophication or an increase in organic matter, which drives a diverse array of responses and feedbacks in aquatic systems that comprise a eutrophication progression scheme.

The Literature: What is Known

Florida's springs and spring runs are enriched in nitrate due to broad-scale contamination of groundwater supplies. As a result, the potential for eutrophication becomes a major concern. A eutrophication progression scheme suggests that increased nutrient delivery to aquatic systems favors production of fast-growing algae that ultimately outcompete and displace native vascular plants (Duarte 1995). In some of Florida's spring systems, both the apparent proliferation of nuisance algae and the apparent decline of native vascular plants are consistent with such a progression and a cue that management actions are needed.

In general, nutrients can affect the faunal assemblages in aquatic systems in three primary ways: 1) toxicity, 2) changes to trophic webs and 3) changes in habitats. Elevated concentrations of ammonia and nitrate can lead to increased mortality and sublethal effects. At this time, lethal effects are not an overwhelming concern in most spring systems. However, the potential for sublethal effects should be considered during discussions of management actions, especially given the uncertainty factors suggested for most criteria. The potential for changes in trophic webs and habitats, along with flow-on effects on faunal assemblages does exist. The form and magnitude of any changes are determined by complex interactions among bottom-up and top-down processes, such as grazing pressure, habitat use and predation pressure. The outcomes of such interactions clearly relate to sustainable management of spring systems.

The Literature: What is Not Known

There are few quantitative data that definitively link changes comprising the eutrophication progression scheme to increased nitrate loads in spring systems. In fact, the eutrophication progression scheme predicts that nonlinear responses may have "decoupled" these systems from

contemporary concentrations of nutrients in the water column. Currently, patterns in the distribution and abundance of plants and algae do correlate with other physical and chemical parameters, such as light availability. However, little is known about the individual or combined effects of the various forcing factors or the form and strength of potential feedback mechanisms, including those linked to nutrient concentrations.

At this time, there are no data to support rigorous evaluation of bottom-up and top-down influences on faunal communities. For example, changes in faunal assemblages have not been linked to changes in vegetated communities and changes in grazer abundance have not been linked to changes in floral assemblages. In addition, sublethal effects of nitrate may not translate to effects on populations, assemblages or ecosystems.

Recommendations for Future Research

Management of nutrients in spring ecosystems would benefit from compilation of inventories, collection of baseline data, and completion of diagnostic studies focused on bottom-up and top-down interactions. Ultimately, unforeseeable, emergent properties that arise when management is implemented at real-world, spatiotemporal scales will create a need to manage adaptively.

Efficient and effective research to improve management of Florida's springs and spring-fed systems requires input from multiple stakeholders. Initial suggestions for discussion include:

- inventories and baselines designed to:
  - measure nutrients, oxygen, carbon, light, flow, substrates, grazing, algae, plants, invertebrates, fish, and other key drivers and valued components of spring systems chosen with the best available information and, eventually, with guidance from the results of diagnostic studies
  - target systems where effectiveness of management can be assessed (e.g., systems with total maximum daily loads, minimum flows and levels, pollution load reduction goals, surface water improvement plans or basin management action plans)
  - sample synoptically or at spatial and temporal scales that support rigorous analyses of interactions (note that these inventories and baselines form the platform for adaptive management)
  - balance breadth with depth (e.g., optimize taxonomic detail and replication)
- diagnostic studies that determine assimilation of nutrients by flora as key elements in nutrient budgets for spring systems, including contributions by epiphytes, macroalgae, vascular plants and microphytobenthos or microalgae found in sediments
- diagnostic studies that determine how nutrients and other drivers affect overgrowth, shading and other relationships among periphyton, macroalgae and vascular plants in an effort to determine the need for management actions, including aquatic plant management and restoration of native species
- diagnostic studies designed to:
  - focus on species of algae and plants that are hypothesized or known to play major roles in nutrient assimilation

- extend beyond nitrogen and phosphorus to include other potentially limiting factors and interactions among limiting factors that can significantly influence the outcomes of management (e.g., micronutrients, iron, oxygen, carbon, light, flow and substrate type)
- explore relationships and consequences across multiple levels of biological and ecological complexity, including uptake and other physiological responses, growth and reproduction of individual organisms, and ecological changes in populations or assemblages to promote the success of management actions at the system level (note that some of the key, large-scale relationships form the core of an adaptive management approach)
- elucidate the direction, magnitude and form of limitations (e.g., nonlinear and nonreversible) to identify suitable targets and goals for management actions

- diagnostic studies to identify and elucidate changes in composition and function of faunal assemblages related to changes in habitats in an effort to identify habitats to be protected or restored by management actions

- diagnostic studies to determine palatability of flora to grazers, rates of grazing, and the form and magnitude of predation by primary consumers in an effort to identify flora, grazers and trophic links to be protected or restored by management actions

- diagnostic studies to identify and elucidate the toxic effects of ammonia and nitrate beyond physiological and individual levels of organization (e.g., population and assemblage effects) in an effort to determine threshold levels, safety factors and the need for management actions

INTRODUCTION

Anthropogenic activities have increased the delivery of nutrients in aquatic systems of all types resulting in concerns about the biotic integrity of these systems. In fact, excessive growth of primary producers in water bodies due to high inputs of nutrients, especially phosphorus and nitrogen, has been cited as the most important form of pollution in lentic and lotic systems (Mainstone and Parr 2002; Hilton *et al*. 2006). For example, high levels of nutrients, especially nitrogen and phosphorus, are reported to threaten wadeable streams, the shallow, freshwater systems that represent about 90% of the length of flowing waters in the United States (Figure 1; USEPA 2006). Florida's spring-fed systems share key features with these and other lotic systems because they are often relatively shallow and they rely on primary production that arises from periphyton, algae and vascular plants rather than phytoplankton. Thus, an understanding of the effects of nutrients on shallow, freshwater ecosystems represents a key concern in Florida and beyond.
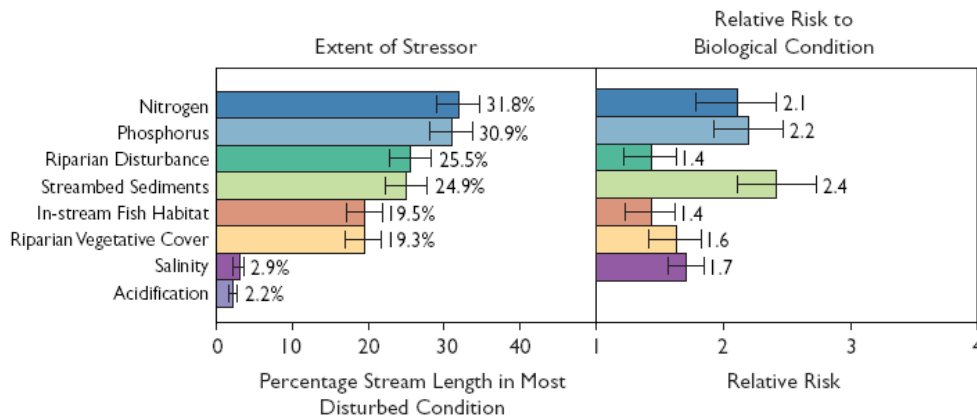

Figure 1 – *Spatial extent of stressors and associated relative risks or relative likelihood of degradation from biological reference conditions in wadeable streams of the United States (USEPA 2006).*

Signs of stress from increased nutrients in aquatic systems have led to development of narrative standards or numeric criteria as important management responses. Nutrient standards and criteria have been set in response to: 1) adverse effects on humans and domestic animals, 2) aesthetic impairment, 3) interference with human use, 4) negative impacts on aquatic ecology, and 5) excessive nutrient transport to downstream systems (Dodds and Welch 2000). These five concerns interact. For example, systems exhibiting negative ecological impacts probably fail to be aesthetically pleasing or support a full range of human uses. In fact, aesthetics and direct human use typically relate to the health of aquatic flora and fauna, e.g., a balance between macrophytes and filamentous algae, the absence of algal blooms, and the presence of healthy fisheries. Flora and fauna also influence the transport of nutrients to downstream systems through processes such as uptake and cycling. Thus, an understanding of nutrient effects on flora and subsequent effects on fauna become important in managing nutrients for almost any purpose.

In Florida, past and present land uses have resulted in broad-scale nutrient contamination of groundwater (Cohen *et al*. Chapter 2). This nutrient-laden groundwater exists within a very

permeable, karst setting, with myriad pathways between aquifers and surface waters (Knight and Notestein Chapter 1; Cohen *et al*. Chapter 2). In fact, freshwater springs function as conduits that discharge contaminated groundwater into surface waters. Once nutrients, such as nitrogen and phosphorus, are mixed into spring-fed, surface waters, they attain the greatest potential to negatively alter the ecology of aquatic ecosystems. The effects of increased nitrogen in the form of nitrate raise particular concerns for flora and fauna associated with Florida's springs and their downstream receiving waters.

Nitrate as a Compound of Concern

Why the focus on nitrate? Nitrate is a nitrogen compound that occurs naturally in aquatic systems, including Florida's springs. Aquatic plants and algae assimilate nitrate as a way to gain nitrogen, which is one of the elements that contributes to their growth and production. Concerns arise when nitrate concentrations become toxic or an increase in the supply of nitrate or another limiting factor stimulates excess plant and algal growth creating undesirable aesthetic and ecological consequences. Such an increase in plant or algal growth is commonly referred to as eutrophication (c.f. Nixon 1995). The potential eutrophication of Florida's spring systems is a major concern for many resource managers in the state (Knight and Notestein Chapter 1). In fact, the current view among many (if not all) scientists and resource managers is that plant and algal populations in Florida's spring-dominated ecosystems are undergoing major structural and functional changes, due, in large part, to increases in anthropogenic enrichment of nutrient levels in groundwater and the consequent nutrification of spring discharges.

This widely held view is not without merit. There is, in fact, a growing body of literature on the chemistry of spring discharges that supports the conclusion that nutrient concentrations in springs, and nitrate concentrations in particular, are increasing (Cohen *et al*. Chapter 2). The perception that increased algal biomass has led to the displacement of native macrophytes, on the other hand, appears to be based largely on anecdotal accounts and qualitative observations (see Frazer *et al*. 2006b). Nevertheless, the prevalence of such reports is a compelling reason for concern. It is fair to say, however, that there is little direct evidence that either definitively links the observed increases in algae to increased nitrate delivery to spring systems or demonstrates that the purported changes have compromised the ecological health and integrity of these systems. Our goal is to articulate what we know about the factors that affect submersed plants and algae in Florida's spring systems, the relationships among nutrients and spring flora, the habitat associations of spring fauna, and the potential toxic effects of some nitrogen compounds. From this synthesis, we aim to draw guidance or recommendations for policy and management. Where sufficient information is lacking, our goal is to suggest potential remedies.

PRIMARY PRODUCERS IN SPRING ECOSYSTEMS AND FACTORS AFFECTING THEIR ABUNDANCE AND DISTRIBUTION

Florida's springs often are perceived as stable-state ecosystems in which major chemical constituents, temperature and discharge characteristics of individual springs are essentially constant (Odum 1957b). In fact, there is substantial variability in chemical and physical attributes among the state's 700+ springs (Odum 1957a; Jones *et al*. 1997; Scott *et al*. 2002; Strong 2004), and, in some cases, variability within spring systems (Odum 1957b). Patterns in

the abundance and distribution of spring-associated flora and fauna should be expected to vary accordingly. We would argue that spring-dominated ecosystems, like all other ecosystems, are, in fact, dynamic in nature and exist (on some scale) as heterogeneous environments in which various chemical, physical and biological processes interact to determine ecological structure and function. Thus, the inherent complexities in spring-dominated ecosystems must be recognized, and any broad generalizations related to observed patterns and presumed processes should be evaluated objectively.

For example, it is important to recognize that nitrate availability is only one of many factors with the potential to directly influence the abundance and distribution of plants and algae in Florida's spring systems. Other essential nutrients, particularly phosphorus, also are likely to be important determinants of plant and algal abundance (Frazer *et al*. 2001a; Notestein *et al*. 2003; Kurz *et al*. 2004; Frazer *et al*. 2006b; Stevenson *et al*. 2007). In addition, we would be remiss if we failed to acknowledge that other, naturally variable, chemical properties of groundwaters and surface waters influence the vegetative characteristics of Florida's springs and their downstream receiving waters. For example, concentrations of dissolved gases, such as oxygen and carbon dioxide, exert a profound influence on production of submersed aquatic plants and algae (Sand-Jensen 1989, Sand-Jensen and Borum 1991). In addition, vegetative assemblages in several of Florida's spring systems have been shown to vary with specific conductance and salinity (Whitford 1956; Frazer *et al*. 2001b; Hoyer *et al*. 2004; Frazer *et al*. 2006a). One also must consider physical attributes of various spring systems in any attempt to ascertain the relative importance of factors affecting broad-scale or fine-scale patterns in plant and algal abundance. For example, water depth, light availability, stream velocity, flow regimes, and characteristics of substrates all represent important considerations (Butcher 1933, Hynes 1972).

Beyond chemical and physical influences, biological interactions and ecological processes may influence patterns of plant and algal abundance. Grazing on aquatic plants and algae can be significant, and it may serve to moderate the potential negative influences of nutrient over-enrichment (Duarte 1995; Hauxwell *et al*. 1998). In addition, competition for space, light and/or nutrients can alter the species composition of algal and plant assemblages. Changes in these patterns may, in turn, affect higher-order organisms present in the systems, particularly those that use submersed aquatic vegetation when foraging or taking refuge. Thus, the indirect effects of nutrients represent a focus of this chapter and a logical complement to the discussion of direct impacts on spring-associated flora.

Light

Light availability is arguably among the most important factors directly affecting the abundance and distribution of aquatic plants and algae. Without sufficient light, plants and algae cannot utilize nutrients effectively. Light transmittance in aquatic systems is affected primarily by suspended solids and dissolved organic substances (Kirk 1994). Suspended solids include algal cells (Canfield and Hodgson 1983), nonvolatile suspended solids (Canfield and Bachmann 1981; Hoyer and Jones 1983) and detrital material (Buiteveld 1995).

All of the "in-water" constituents that reduce light availability are generally in low concentrations in groundwater serving as the origin of flow for Florida's springs and spring runs. For example, Florida springs with discharges of 5 m^3 s^{-1} or higher (1st magnitude springs) had the

lowest concentrations of dissolved organic matter yet reported (13 ± 1.6 μM C L^{-1}), and springs with lower discharges exhibited only somewhat higher values (Duarte *et al*. 2007). Thus, Florida's springs are not surprisingly among the clearest waters in the world (Odum 1957b; Rosenau *et al*. 1977; Duarte and Canfield 1990a).

Nevertheless, light availability is likely to limit the distribution and production of submersed aquatic vegetation in Florida's spring systems in many instances. For example, Duarte and Canfield (1990b) investigated 31 springs and spring runs in interior central and northern Florida and concluded that shading by riparian vegetation was a primary determinant of primary productivity and macrophyte standing crops in these systems. It is interesting to note that these investigators reported negative rates for daily maximum production in several spring runs devoid of "vegetation," and it was assumed that biochemical oxygen demand exceeded any production by benthic and/or pseudoplanktonic algae in these systems. It was further suggested that oxygen concentrations in groundwater emanating as spring discharge may, in some cases, be too low to support the respiratory demands of aquatic macrophytes (see additional discussion below).

In a separate study of five spring-fed rivers along the Gulf coast, Frazer *et al*. (2001a) suggested that light availability was generally sufficient to support net photosynthesis of submersed aquatic vegetation, but they also noted that terrestrial canopy coverage along some shoreline areas may reduce incident light to a level that restricts the abundance and distribution of aquatic plants and algae. Correlations with the percentage of shoreline canopy cover in the Weeki Wachee River, for example, accounted for 26% of the variation in total vegetative biomass, 21% of the variation in filamentous macroalgal biomass, and 8% of the variation in macrophyte biomass. As the percent canopy cover increased, the total vegetative biomass decreased, which led these authors to suggest that canopy cover in the Weeki Wachee River does influence the distribution and abundance of aquatic plants.

There are few other quantitative studies of the relationship between light and submersed aquatic vegetation in Florida's spring systems, and we did not find experimental investigations that might indicate the magnitude of light limitation in those springs subject to shading by riparian vegetation. This is somewhat surprising as Odum suggested in his seminal work (Odum 1957b) that primary production in Silver Springs (and other spring systems in Florida; Odum 1957a) is linearly related to light intensity.

In addition to shading by riparian vegetation, there is a widely held perception that the accumulation of algae on macrophytes reduces the photosynthetic efficiency of the host plant due to shading. This scenario is, in fact, a crucial element of the argument that nutrification of Florida's spring systems has led to a proliferation of nuisance algae and concomitant decline of native macrophytes. A key to effective management of Florida's springs will be to understand the factors and processes that interact to either accelerate or decelerate this eutrophication progression scheme (see Duarte 1995 and also Valiela *et al*. 1997). At this time, there is no direct evidence that accumulations of periphyton on macrophytes have reduced their growth rates in Florida's spring systems. The potential for such an effect has been inferred from laboratory studies with portions of plants (Sand-Jensen 1977; Chen *et al*. 2007) and relatively few, empirical field studies in estuarine and coastal marine systems (Deegan *et al*. 2002; see also Heck *et al*. 2000). It is likely, however, that the diffusion of carbon dioxide (CO_2) or active uptake of bicarbonate (HCO_3^-) is hindered by increased boundary layer effects created by a layer

of algae. Thus, the effects of changes in light climate will be mediated by stream velocity and the supply of carbon, especially dissolved inorganic carbon (DIC). There is a need to quantify the relationships among light climate, DIC availability, stream velocity, algal abundance and macrophyte production because such relationships have profound implications for management of spring systems.

Carbon and Oxygen Balance in Macrophytes

At saturating light levels, the maximum rate of photosynthetic carbon fixation by submersed vascular plants exceeds the respiratory carbon demand of leaves and belowground tissues (Hemminga and Duarte 2000). This outcome holds only if DIC is in ample supply. Because concentrations of DIC in Florida's springs are naturally high due to microbially mediated remineralization of organic matter in the groundwater system, this situation typically applies (Inglett *et al*. Chapter 3).

Many springs (particularly 1st magnitude springs) are supersaturated with DIC and there is, in fact, a net flux of gaseous CO_2 from the springs to the atmosphere at the point of discharge and for some distance downstream during the equilibration process (Duarte *et al*. 2007). Thus, the supply of inorganic carbon is not likely to limit primary production in spring systems, at least in those areas in close proximity to a groundwater source. Mediation by biological (algal mat formation) or physical (water velocity) processes, however, may result in localized depletions of DIC. At such fine scales, the potential exists for DIC limitation, but we have insufficient data to evaluate the existence or ultimate effect of such limitation.

Oxygen is a byproduct of photosynthesis and a key chemical constituent of all systems that support biological communities. Any factor or combination of factors that reduces primary production in spring systems will necessarily reduce the availability of dissolved oxygen. The potential consequences of reduced oxygen availability on faunal assemblages may be intuitively obvious, but the consequences of low oxygen concentrations on flora are less clear.

Rooted aquatic plants require dissolved oxygen to support aerobic respiration, especially in belowground tissues. Reduced photosynthetic capacity compromises the supply of oxygen, and such a change may negatively affect plants by disrupting their carbon balance (see Hemminga and Duarte 2000). Oxygen deficits might be expected to occur where macrophytes are highly epiphytized or covered by extensive macroalgal growth because of increased respiration and/or boundary layer effects on diffusion. We reiterate, however, that there are no experimental studies clearly demonstrating a negative effect of epiphytes or macroalgae on vascular plants in Florida's spring systems, although there are a number of empirical investigations that are suggestive of such a cause and effect relationship (e.g., Frazer *et al*. 2006b; Jacoby *et al*. 2007). In those spring systems that exhibit chronic hypoxia, one might expect rooted macrophytes to be more susceptible to the potential effects of algal overgrowth. It is interesting to note that Stevenson *et al*. (2004) reported a significant negative relationship between dissolved oxygen concentrations and algal cover based on a broad survey of springs in Florida. The nature of this relationship merits further investigation, especially in light of recent information that suggests a broad-scale decline in dissolved oxygen concentrations in Florida's springs (Heffernan *et al*. unpublished data).

Stream Velocity and Substrate

Water movement can affect the gas exchange, physiology and growth form of plants and algae, particularly periphyton. In addition, stream velocity can determine the characteristics of the substrate, which, in turn, influence the viability of plants and algae.

For example, a stream/river bed consisting of bare rock or large stones that are continually being rolled or scoured will have little submersed aquatic vegetation, while a river bed that is comprised largely of mud, silt and sand has the potential to support abundant aquatic vegetation (Butcher 1933; Allan 1995). Systems with flow rates greater than 0.60 m sec^{-1} tend to have heavy rock substrates, and systems with progressively slower flow have light gravel, sand, silt and mud substrates that are more favorable for aquatic plant growth.

All of Florida's spring runs are considered low gradient streams, and they seldom exhibit flow rates in excess of 0.60 m sec^{-1}. Therefore, substrates within these systems generally are conducive to the colonization and subsequent production of many types of submersed aquatic vegetation (see Frazer *et al*. 2001a) if nutrients, light and other conditions are suitable. However, sections of spring systems may not support all types of submersed aquatic vegetation. For example, exposed limestone is prevalent in the immediate vicinity of spring vents, and outcrops of limestone are distributed patchily throughout many spring runs. Rooted macrophytes will not colonize these areas, although algae does grow successfully near spring vents and limestone outcrops are colonized by algae and bryophytes (Whitford 1956; Frazer *et al*. 2001a; Kurz *et al*. 2003 and 2004; Hoyer *et al*. 2004; Stevenson *et al*. 2004 and 2007).

In the context of this review, stream velocity will be a concern primarily because of its effects on the periphyton assemblages associated with macrophytes. Although submersed vascular plants and large macroalgae in springs draw considerable attention, epiphytic microalgae also are an important component of spring systems. In fact, epiphytic algae and benthic mats can be major, or even dominant, elements of the primary producer community, in terms of both productivity and biomass (Quinlan *et al*. 2008). The composition of algal communities, however, varies considerably between different spring ecosystems (Whitford 1956), and it might be expected to vary temporally due to seasonal differences in light availability (see above) or more stochastic variations in flow regimes that occur as a consequence of climate variability or anthropogenic demands on source water (Odum 1957b).

In systems with relatively high flow rates, periphyton associated with macrophytes or other substrates are comprised mostly of low-lying mucilaginous diatoms that are resistant to sloughing even at high velocities (Biggs *et al*. 1998). Long, filamentous algal species are much less resistant to high velocity environments. Indeed, for such a species, Biggs *et al*. (1998) reported a linear decrease in ash-free dry mass of periphyton as a function of near-bed velocity. To our knowledge, there are no rigorous experimental studies in Florida spring systems that quantify the effects of flow on periphyton biomass or species composition. There are, however, several empirical investigations in which both flow and periphyton abundance on macrophytes have been measured. For example, Frazer *et al*. (2001a) found no significant relationship between flow rates and periphyton associated with macrophytes in any of the five spring-fed, coastal rivers they investigated. In a subsequent study of these same systems, however, statistically significant decreases in periphyton associated with macrophytes in the Weeki

Wachee River coincided with an increase in flow (Frazer *et al*. 2006b). Kurz *et al*. (2004) found no significant relationship between periphyton abundance on macrophytes and flow in the main stem of the Ichetucknee River. It should be noted, however, that periphyton abundance on macrophytes was significantly higher in several feeder springs where flow rates were significantly less (Kurz *et al*. 2004; see also Dormsjo 2007; Politano unpublished data).

These findings are counter-intuitive because reduced flows are expected to increase boundary layer thickness. Boundary layers determine, in part, the availability of nutrients (or other chemicals such as dissolved oxygen and free dissolved inorganic carbon) to the periphyton. When boundary layers are thick, as in areas with slow-moving water, nutrients and other chemicals must diffuse or be actively transported across large distances. Thinner boundary layers facilitate contact between cells and nutrients or other chemicals. The steepness of the concentration gradient across the boundary layer determines transport rate, per Fick's law:

$$J \; = \; -D \, \frac{\partial \phi}{\partial \chi}$$

where J is the diffusion flux in, for example, moles $m^{-2} \, s^{-1}$, D is the diffusion coefficient or diffusivity in $m^2 \, s^{-1}$ (determined by temperature and the properties of both materials – here, water and cell cytoplasm), ϕ is the concentration gradient per unit length in moles m^{-3}, and χ is the length in m of the boundary layer. Depletion of nutrients in a slow-moving system decreases the concentration gradient, $\partial \phi / \partial \chi$, so periphyton come to have limited access to nutrients. Even minute increases in stream velocity (< 0.1 cm/s) have been shown to increase photosynthesis of aquatic macrophytes (Westlake 1967), presumably by maintaining a steep concentration gradient of nutrients near the plants' surfaces or by reducing the concentration of metabolic by-products that might impair photosynthesis. In general, periphyton biomass increases as velocity increases until shear reaches a level that initiates sloughing (Biggs and Hickey 1994; Biggs *et al*. 1998). Clearly, the relationship between water velocity and periphyton dynamics in Florida's spring systems merits further investigation.

Nutrients

Although modified and influenced by other factors, the availability of nutrients plays a major role in governing productivity in natural systems. In freshwaters, phosphorus generally is considered the nutrient that most frequently limits the production of plants and algae (Wiebe 1931; Schindler 1971). Nitrogen, however, can and does limit primary production in many instances (see Vitousek and Howarth 1991). Other macronutrients and micronutrients (e.g., potassium, calcium, magnesium, sulfur, iron, manganese, copper, zinc, molybdenum, sodium, cobalt, chlorine, bromine, silicon, boron, and iodine) also can limit plant and algal production depending on their relative abundances and the particular physiologies of the taxa present in the system. In some situations, plants and algae may be limited by the availability of dissolved inorganic carbon, although there is little indication that this might be the case in any of Florida's springs, except perhaps in algal mats. Due to their import, our focus here is on nitrogen and phosphorus and their potential to limit the production of submersed aquatic vegetation in Florida's springs and spring runs.

Rooted vascular plants, e.g., *Vallisneria americana* and *Sagittaria kurziana*, are generally assumed to acquire nitrogen and phosphorus largely from sediment porewaters (Chambers *et al*. 1989), although some research suggests that many plants (some of which are present in Florida's spring systems) are capable of nutrient uptake through their aboveground tissues (see DeBrabandere *et al*. 2007 and references therein). There has been no experimental work to demonstrate unequivocally that any submersed vascular plant in a Florida, spring-fed system is limited by either nitrogen or phosphorus. Moreover, empirical data generated in a large number of spring systems suggests that there is no significant linkage between surface water nutrients and production of macrophytes.

For example, Canfield and Hoyer (1988a and b) carried out an intensive investigation of 17 inland streams (many of which were spring-fed) to determine if the abundance of aquatic macrophytes (composite category including both rooted vegetation and macroalgae) was related to nutrient enrichment. Standing crops of macrophytes in these systems were not correlated with in-stream concentrations of total phosphorus or total nitrogen. Furthermore, in a subsequent investigation, Duarte and Canfield (1990b) sampled 31 spring runs and similarly found that standing crops of submerged vegetation were not significantly correlated with either total nitrogen or total phosphorus.

Frazer *et al*. (2001a) investigated the relationship between nutrient concentrations in surface waters and characteristics of submersed aquatic vegetation in five spring-fed coastal rivers subjected to increasing nitrate delivery. They found that statistically significant, positive relationships between nitrate and standing crops of either macrophytes or filamentous macroalgae were evident only in the Chassahowitzka and Homosassa Rivers (Table 1). Mean nitrate concentrations declined markedly at downstream sites in both rivers, which is indicative of rapid nitrate removal. Subsequent research in the Chassahowitzka River suggests, however, that the vast majority of the nitrate removal occurs as a consequence of denitrification (Saunders 2007; see also DeBrandere *et al*. 2007) rather than uptake and assimilation of nitrate by submersed aquatic vegetation. Denitrification also may be operating in the Homosassa River where nitrate concentrations decline markedly along the river's length even though little vegetation remains in the system (Frazer *et al*. 2006b). It is noteworthy that Frazer *et al*. (2001a) reported that soluble reactive phosphorus was correlated with macrophyte and macroalgal biomass in the Chassahowitzka and Homosassa rivers, and they suggested that phosphorus might be a more important determinant of vegetative biomass than nitrate in these two rivers. Notestein *et al*. (2003) subsequently determined that phosphorus does in fact limit algal growth in the Chassahowitzka River. The addition of soluble reactive phosphorus resulted in significantly higher periphyton accumulation on artificial substrates relative to nitrate amended treatments or controls (no nutrient addition). Frazer *et al*. (2001a) reported that soluble reactive phosphorus was correlated with macroalgal biomass in the Weeki Wachee River as well, so phosphorus also may limit the growth of algae in this river.

Table 1. Pairwise Pearson Product-Moment correlations between $\log_{10}$–transformed annual mean nutrient concentrations and $\log_{10}$–transformed summer mean biomass for macroalgae, all vegetation, macrophytes and periphyton from ten transects in four coastal rivers during 1998, 1999 and 2000 (n = 30). Data for the Withlacoochee River were not shown because plants were largely absent from the system below the dam at Inglis where the substrate (largely rock) was unfavorable (from Frazer *et al*. 2001a).

Nutrient	Biomass	Correlation coefficient	Probability
CHASSAHOWITZKA			
Ammonium	Macroalgae	-0.31	0.09
Nitrate	Macroalgae	0.63	< 0.05
Soluble reactive phosphorus	Macroalgae	0.55	< 0.05
Total nitrogen	Macroalgae	0.21	0.27
Total phosphorus	Macroalgae	-0.19	0.31
Ammonium	All vegetation	-0.46	< 0.05
Nitrate	All vegetation	0.79	< 0.05
Soluble reactive phosphorus	All vegetation	0.68	< 0.05
Total nitrogen	All vegetation	0.29	0.12
Total phosphorus	All vegetation	-0.31	0.10
Ammonium	Macrophytes	-0.38	< 0.05
Nitrate	Macrophytes	0.70	< 0.05
Soluble reactive phosphorus	Macrophytes	0.61	< 0.05
Total nitrogen	Macrophytes	0.33	< 0.05
Total phosphorus	Macrophytes	-0.20	0.29
Ammonium	Periphyton	-0.34	0.07
Nitrate	Periphyton	0.28	0.14
Soluble reactive phosphorus	Periphyton	0.23	0.23
Total nitrogen	Periphyton	0.36	0.06
Total phosphorus	Periphyton	-0.42	0.83
CRYSTAL			
Ammonium	Macroalgae	-0.22	0.24
Nitrate	Macroalgae	0.38	< 0.05
Soluble reactive phosphorus	Macroalgae	-0.46	< 0.05
Total nitrogen	Macroalgae	-0.48	< 0.05
Total phosphorus	Macroalgae	-0.43	< 0.05
Ammonium	All vegetation	-0.13	0.48
Nitrate	All vegetation	0.23	0.22
Soluble reactive phosphorus	All vegetation	-0.33	0.07
Total nitrogen	All vegetation	-0.70	< 0.05
CRYSTAL (continued)			
Total phosphorus	All vegetation	-0.28	0.14
Ammonium	Macrophytes	-0.12	0.52
Nitrate	Macrophytes	0.21	0.26
Soluble reactive phosphorus	Macrophytes	-0.32	0.09
Total nitrogen	Macrophytes	-0.69	< 0.05
Total phosphorus	Macrophytes	-0.26	0.16
Ammonium	Periphyton	-0.44	< 0.05
Nitrate	Periphyton	0.18	0.40
Soluble reactive phosphorus	Periphyton	0.10	0.63
Total nitrogen	Periphyton	-0.34	0.11

Nutrient	Biomass	Correlation coefficient	Probability
Total phosphorus	Periphyton	0.01	0.97
HOMOSASSA			
Ammonium	Macroalgae	-0.34	0.07
Nitrate	Macroalgae	0.60	< 0.05
Soluble reactive phosphorus	Macroalgae	0.44	< 0.05
Total nitrogen	Macroalgae	0.34	0.07
Total phosphorus	Macroalgae	-0.55	< 0.05
Ammonium	All vegetation	-0.37	< 0.05
Nitrate	All vegetation	0.64	< 0.05
Soluble reactive phosphorus	All vegetation	0.49	< 0.05
Total nitrogen	All vegetation	0.25	0.19
Total phosphorus	All vegetation	-0.66	< 0.05
Ammonium	Macrophytes	-0.31	0.09
Nitrate	Macrophytes	0.43	< 0.05
Soluble reactive phosphorus	Macrophytes	0.32	0.09
Total nitrogen	Macrophytes	0.02	0.92
Total phosphorus	Macrophytes	-0.62	< 0.05
Ammonium	Periphyton	0.44	< 0.05
Nitrate	Periphyton	0.12	0.53
Soluble reactive phosphorus	Periphyton	-0.25	0.20
Total nitrogen	Periphyton	0.57	< 0.05
Total phosphorus	Periphyton	-0.37	< 0.05
WEEKI WACHEE			
Ammonium	Macroalgae	-0.47	< 0.05
Nitrate	Macroalgae	0.20	0.30
Soluble reactive phosphorus	Macroalgae	0.54	< 0.05
Total nitrogen	Macroalgae	-0.12	0.53
Total phosphorus	Macroalgae	-0.36	< 0.05
Ammonium	All vegetation	-0.48	< 0.05
Nitrate	All vegetation	-0.02	0.90
Soluble reactive phosphorus	All vegetation	0.29	0.12
Total nitrogen	All vegetation	-0.23	0.21
Total phosphorus	All vegetation	-0.24	0.20
Ammonium	Macrophytes	-0.33	< 0.05
Nitrate	Macrophytes	-0.22	0.25
Soluble reactive phosphorus	Macrophytes	0.04	0.82
Total nitrogen	Macrophytes	-0.30	0.10
Total phosphorus	Macrophytes	-0.13	0.51
Ammonium	Periphyton	0.43	< 0.05
Nitrate	Periphyton	-0.46	< 0.05
Soluble reactive phosphorus	Periphyton	-0.32	0.12
Total nitrogen	Periphyton	-0.32	0.13
Total phosphorus	Periphyton	-0.04	0.85

Kurz *et al*. (2004) reported no statistically significant, positive relationships between nutrients and coverage or biomass of submersed aquatic vegetation in the main stem of the Ichetucknee River. There was a weak, but statistically significant, negative correlation between terrestrial canopy cover and submersed aquatic vegetation cover suggesting that, in some cases, light may limit the distribution of macrophytes. In a complementary analysis, these investigators related a suite of chemical and physical parameters to periphyton abundance on macrophytes in several small spring runs adjoining the main river. Periphyton abundance was not correlated with either nitrate or total nitrogen concentrations, but it was significantly correlated with soluble reactive

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phosphorus concentrations suggesting phosphorus limitation.

The issue of nutrient limitation of algal production can be viewed at several levels of organization, from individual algal cells or filaments to complex mat communities. For example, the nitrate half saturation constants for the growth of the common filamentous green alga *Cladophora* range from 100–200 µg l^{-1} (Lohman and Priscu 1992), and recent research on growth saturating concentrations of nitrate for *Lyngbya wollei* suggests values between 100–200 µg l^{-1} (Stevenson *et al.* 2007). Saturation levels for the growth of natural populations of benthic algae in Sycamore Creek, Arizona (Grimm and Fisher 1986) and Saline Creek, Missouri (Lohman *et al.* 1991) were 55 and 100 µg l^{-1}, respectively. Thus, the limited literature listing levels of nitrate that saturate the growth rates of natural, benthic, algal mats suggests that nitrate concentrations currently observed in the water column of many springs are not limiting. However, this does not preclude the possibility of nitrogen limitation in large, well-developed algal mat communities, where growth may be impacted by diffusion rates and nutrient gradients within the mat. In fact, Stevenson *et al.* (2007) showed that water column concentrations limiting growth may be considerably higher for mats than for exposed, individual algal cells or filaments. Half saturation constants for phosphorus exhibit similar variability. Constants have been reported to vary from 9 to 45 µg l^{-1} for the green alga *Spirogyra* (Borchardt *et al.* 1994) and from 15 to 240 µg l^{-1} for *Cladophora* (Lohman and Priscu 1992). Lower values of 0.6 to 6 µg l^{-1} were reported for mixed periphytic diatoms (Bothwell 1985). Half saturation constants for natural, benthic, algal mats ranged from 7 to 50 µg l^{-1} (Wuhrmann and Eichenberger 1975; Welch *et al.* 1988; Horner *et al.* 1990).

In contrast to the widespread and dramatic increase in nitrogen levels in many Florida springs, phosphorus concentrations are reported to have remained similar to historic levels (Stevenson *et al.* 2007; Munch *et al.* 2006). Current phosphorus concentrations vary considerably among springs, from low levels in systems like the Weeki Wachee River (Frazer *et al.* 2001a and 2006b) to high concentrations in systems like Silver Springs and the Ichetucknee River and springs (Munch *et al.* 2006; Kurz *et al.* 2003 and 2004). Some springs exhibit concentrations that fall below the reported saturation levels for growth of benthic algae. The possibility that phosphorus may be a limiting factor for algal growth in some springs has been further highlighted by recent observations of high N/P ratios in plant and algal samples from several Florida springs (Stevenson *et al.* 2007). However, some springs with very low phosphorus concentrations in the water column have extensive benthic algal mats (Frazer *et al.* 2001a and 2006b). We hypothesize that the mobilization of phosphorus from sediments may provide sufficient nutrients to sustain this biomass. This hypothesis is supported by observations of increasing phosphorus levels in the pore water of some benthic algal mats (Stevenson *et al.* 2007). The absence of sufficient information on nutrient dynamics within algal mat communities in spring ecosystems hampers our ability to predict the outcomes of changes in nutrient loads.

Community Responses to Increased Nutrients

Plant and algal communities appear to exhibit qualitatively similar responses to increased nutrient loads. The primary responses and the mechanisms by which algae replace submersed vascular plants have been reviewed in depth by Duarte (1995), and we reiterate the key points from his significant contribution.

Interactions between supplies of nutrients and light availability, two resources that most often limit the production of plants and algae, are at the root of observed changes in floral assemblages (Duarte 1995). Because both plants and algae require the same basic resources (nutrients and light), they are likely to compete (intra-specifically and inter-specifically) for the resource that is in the shortest supply (Liebig's Law of the Minimum). At low nutrient concentrations in the water column and under favorable light conditions, slow-growing, rooted vascular plants are superior competitors owing to their relatively low nutrient requirements, their capacity to efficiently recycle nutrients internally and their ability to access sedimentary nutrient stores. In contrast, algae become more abundant when nutrient limitation is released, in large part, by capturing light more efficiently.

In the eutrophication progression scheme laid out by Duarte (1995), faster growing macroalgae and microflora, i.e., periphyton, can ultimately overgrow long-lived and relatively slow-growing plants and algae, e.g., rooted vascular plants and macroalgae with thick fronds. This change exacerbates light limitation and reduces scope for growth for the vascular plants and "thick" algae. Ultimately, these long-lived forms are outcompeted and extirpated from the system. In extreme cases of nutrient over-enrichment, the fast-growing, benthic algae will be replaced by planktonic microalgae via the same mechanism. The short residence times for water in Florida's springs and most spring runs make this final stage in the progression unlikely.

A critical point in Duarte's (1995) review, however, is that a change in the structure of the vegetative community is not a continuous, gradual process that runs parallel to the rate of increased nutrient delivery. Rather, changes occur abruptly. Sudden shifts in the community, in many cases, are not tightly coupled to increased nutrient loading, and once a shift has occurred, the system can become decoupled from existing concentrations of nutrients in the water column. Thus, indirect effects of nutrient inputs and complex feedback mechanisms must be involved. For example, fast-growing algae tend to put less energy into creating structural tissues so they tend to be subject to higher grazing rates. Increased algal respiration during blooms may create hypoxic or anoxic conditions that kill grazers, which decreases grazing pressure on the fast-growing algae and creates a positive feedback in the eutrophication progression. Thus, there are likely to be nonlinear responses and thresholds that need to be fully understood in order to predict the longer-term consequences of nutrient over-enrichment for vegetative communities in Florida's various spring systems.

One potentially key shift in the floral communities in Florida's springs is from palatable species to unpalatable species, especially *Lyngbya wollei*. Recent evidence indicates that this cyanobacterium may have the capacity to produce neurotoxins that affect ecosystem structure and function, as well as human health (Carmichael *et al*. 1997; Onodera *et al*. 1997; Teneva *et al*. 2003; Berry *et al*. 2004). For example, it has been hypothesized that heavy infestations of *Lyngbya* on the skin of manatees in springs may be related to recent observations of health problems (Bledsoe *et al*. 2006). From a human health perspective, it is unclear whether *Lyngbya* toxins are associated with recent reports of contact dermititus by recreational users of springs.

A final point relates to the recovery of submerged aquatic vegetation communities following nutrient remediation and reduction. Recovery is likely to be a very slow process, in large part, because it involves the replacement of fast-growing species with relatively slow-growing species. Duarte (1995) employed a simulation model that predicted recovery times of centuries

for systems originally occupied by slow-growing seagrasses. It is worth noting here that many of the historically dominant vascular plants in Florida's spring systems (e.g., *Vallisneria americana* and *Sagittaria kurziana*) share morphologies and life histories with these seagrasses. Expectations regarding responses to nutrient reduction should be tempered by these considerations.

EFFECTS OF NUTRIENTS ON FAUNA

Florida's 700+ springs represent important aquatic habitats for fauna that are subject to stress by increased nutrients. Key elements of spring systems resemble key elements of many lotic or flowing water systems (Figure 2; Vannote *et al.* 1980; USEPA 2006). Therefore, effects of nutrients can be elucidated from science conducted in these springs and by extracting key principles drawn from beyond Florida and from other aquatic systems.
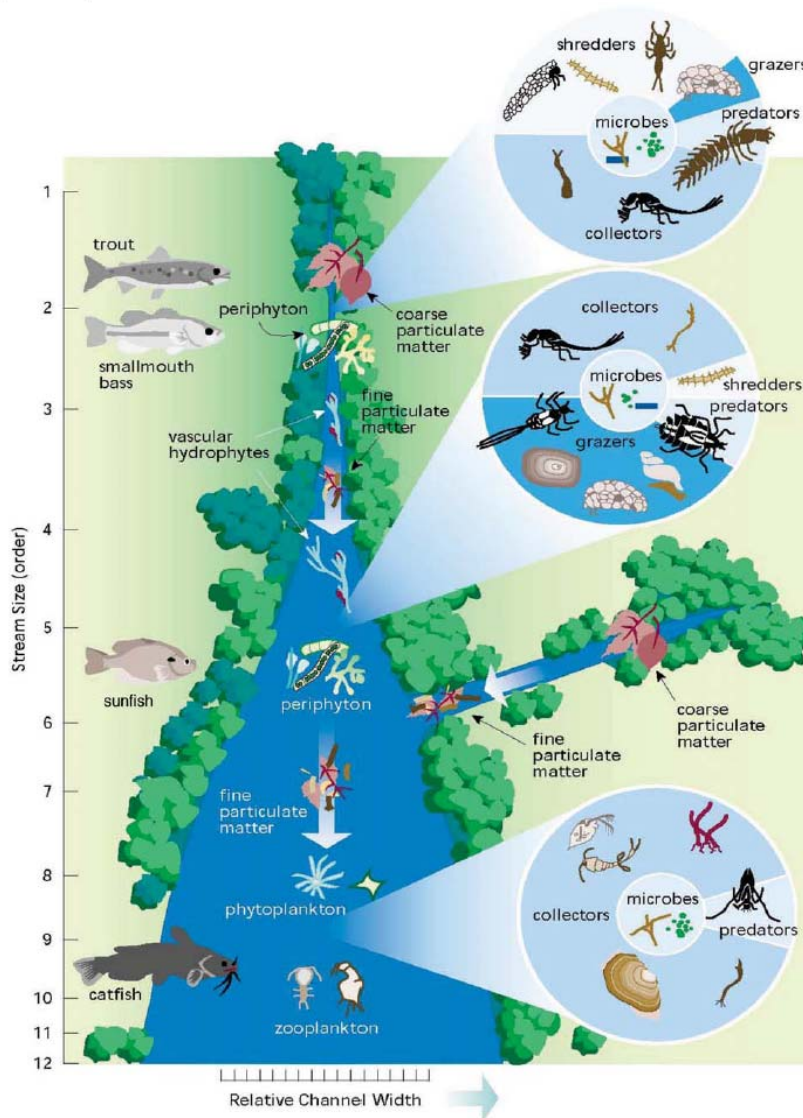
Figure 2 *– Key components of lotic ecosystems (USEPA 2006).*

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Components of Spring Ecosystems

In general, phytoplankton and zooplankton represent minor components of lotic systems until they are relatively large, deep and slow flowing; therefore, the focus falls on benthic organisms and trophic links. The base of trophic webs in flowing systems includes allochthonous material, or external inputs such as leaves from terrestrial vegetation, and autocthonous material that is produced in the system. Autocthonous material becomes more important as shading by riparian vegetation decreases and in-stream primary producers photosynthesize more effectively. Key primary producers in flowing systems include macroalgae, vascular macrophytes and periphyton, with these algal species growing on almost all biotic and abiotic surfaces. Primary producers supply food to primary consumers dominated numerically by a variety of invertebrates, especially snails and larvae of aquatic insects. Primary and secondary consumers include invertebrates and fish, with piscivorous fish representing the major tertiary consumers.

Past and recent surveys of faunal assemblages in Florida's springs documented species that fill the expected ecological niches. For example, invertebrates from five functional feeding groups were found in the Wekiva and Ichetucknee Rivers (Warren *et al*. 2000; Steigerwalt 2005). These groups comprise grazers on attached algae or microbiota, shredders that consume coarse particles of living or dead plant material, gatherers that collect fine particles of organic detritus, filterers of suspended particulate organic matter, and predators feeding on live animal prey. Similarly, fish in the Wekiva River spanned the expected trophic roles, including detritivores, herbivores that often feed on algae, planktivores, invertivores and piscivores of various sizes (Warren *et al*. 2000).

Karst habitats in Florida support cave fauna or stygobiota and obligate fauna in springs that are not found in other types of aquatic systems (Walsh 2001; Shelton 2005). Nearly one third of stygobites have recorded distributions limited to single cave systems, and 67% of these taxa have been found in 10 systems or less. Therefore, a high percentage of these species are listed as of special concern, rare, threatened or endangered. Obligate fauna of springs include hydrobiid and pleurocerid snails with highly restricted ranges leading to about eight species being considered endangered, threatened or of special concern (Walsh 2001). For example, the upper St Johns River basin holds ten species of rare and endemic snails, with *Aphaostracon monas*, *Floridobia alexander*, *Floridobia petrifons* and *Floridobia wekiwae* found only at a single spring and *Aphaostracon pycnum* found at two springs (Shelton 2005).

In aquatic systems, nutrients exert effects on animals primarily through changes in primary producers or direct toxicity. Changes in primary producers flow through ecosystems to affect animals primarily by altering habitats or trophic interactions. This section focuses on these indirect effects and direct toxicity.

Relationships Between Nutrients and Invertebrates in Florida Springs

Nutrients can affect the type and amount of flora found in spring systems. In turn, changes in vascular plants, attached macroalgae and mats of filamentous algae can alter the food and refuge available to invertebrates in Florida springs. However, few studies have assessed invertebrate assemblages in different types of vegetation to obtain a synoptic view.

A survey of the Homossassa and Weeki Wachee Rivers found differences in the number of species and relative densities of certain insects along the length of the waterways (Sloan 1956). Habitats were classified according to the areal coverage of dominant macrophytes. Our reanalysis of the data showed that numbers of species did not correlate with variations in coverage from 10% to 70%, and the relative density of mayflies was positively correlated with increasing cover of macrophytes in the Homosassa River but not in the Weeki Wachee River. In general, the insect assemblages appeared to vary primarily according to concentrations of dissolved oxygen near spring boils and salinity near the estuarine endpoints of the rivers.

In Sulphur Springs Run, quantitative samples taken in bare sediment and algal mats with a petite Ponar dredge showed no significant differences in numbers of individuals and no obvious differences in assemblage composition (Allen *et al.* 2001). An introduced snail, *Tarebia granifera*, dominated all samples, and evenness and diversity indices indicated assemblages dominated by few species.

A more in-depth survey of four key habitats in the Wekiva River yielded similar and statistically equal numbers of invertebrates, i.e., 20,000–34,000 individuals m^{-2} (Warren *et al.* 2000). Sampling methods were designed specifically for each habitat; however, the comparative efficiencies of the different methods were not assessed. Therefore, the data support general comparisons of faunal assemblages among habitats, but detailed quantitative comparisons may prove unreliable.

The relative abundance of different functional types varied among habitats (Figure 3; Warren *et al.* 2000). Gatherers were found in all habitats, and they dominated bare sediments. The relative importance of grazers increased in floating mats composed primarily of *Hydrocotyle umbellate* (water pennywort) and beds of *Vallisneria americana* (tape grass). Predators were found in all habitats in similar relative abundances. Filterers were most common in beds of *Vallisneria americana* and essentially absent from floating mats, which was attributed to the increased flow measured at sites with *Vallisneria americana*. Shredders, which are considered key components of most shallow freshwater systems, represented less than 1% of the organisms sampled. Overall, this survey indicated that altering the areal coverage and species compositions of habitats might lead to changes in invertebrate assemblages.
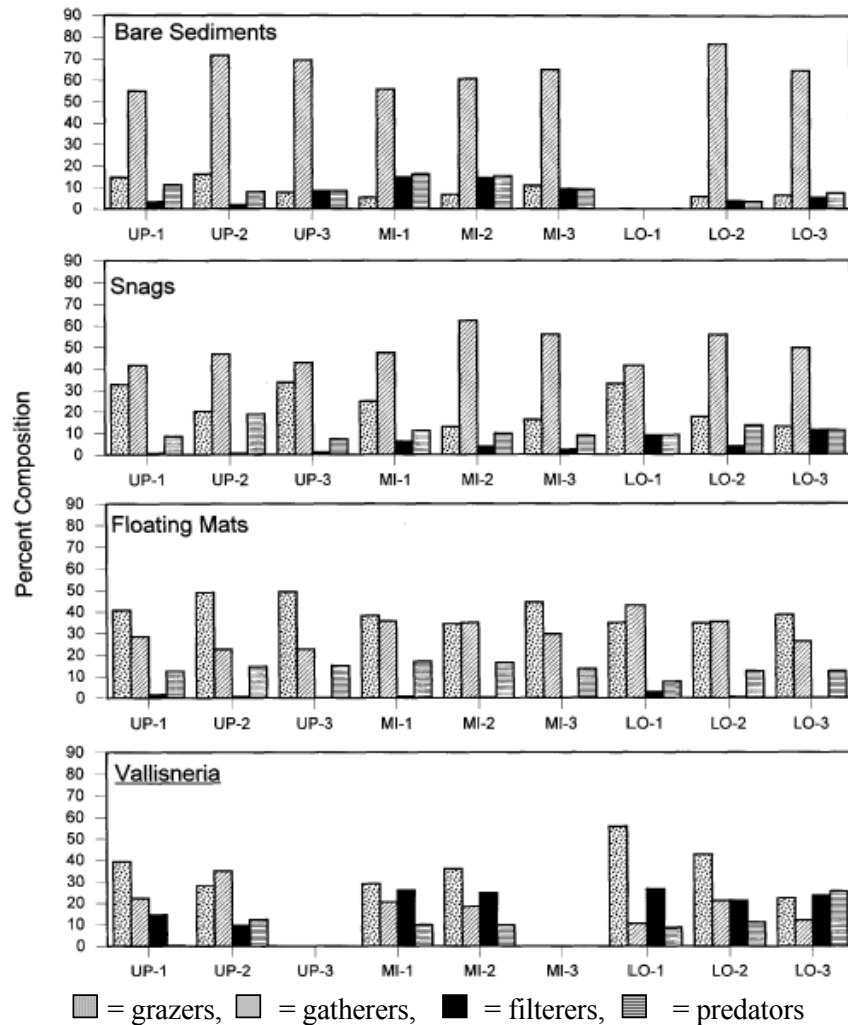
Figure 3 *– Relative abundance of functional groups of aquatic invertebrates in May/June and October samples taken at multiple sites in the Wekiva River (Warren et al. 2000).*

Invertebrate assemblages in lotic systems are used as indicators of ecological health (e.g., USEPA 2006). For example, Florida has two biotic indices, the Stream Condition Index (SCI) that relies on laboratory processing of samples and the more rapid Biological Reconnaissance (BioRecon) that uses sorting in the field (Fore *et al*. 2006).

Indices combine various metrics derived from standardized sampling of invertebrate assemblages, e.g., number of taxa, abundances of specific taxa, and relative abundances or percentage dominance of selected taxa. The concept that invertebrate taxa exhibit some consistency in their response to disturbances underpins biotic indices. In Florida and elsewhere, biotic indices were developed empirically using samples of invertebrates collected from locales chosen to represent different levels of disturbance. The levels of disturbance used to classify locales combined multiple factors, such as habitat quality, one or more water quality parameters, and evaluation of land use. Overall, these indices provide indications of differences or changes in assemblages; however, the causes of such differences or changes typically are derived from correlations with auxiliary data on water quality, habitat quality, or other putative forcing factors.

Interpreting correlations between biotic indices and forcing factors may be less than straightforward. For example, over a 14-year period, invertebrate abundance was positively correlated with nitrate concentrations in the waters of the Suwannee River basin (Hornsby *et al*. 2004). In contrast, number of taxa, diversity, and evenness were negatively correlated with nitrate concentrations (Hornsby *et al*. 2004). These results were interpreted as indicating that higher nitrate concentrations led to an increase in the absolute number of invertebrates and a decrease in the biodiversity of the invertebrate assemblage. However, 17 of the 40 taxa that were common in these samples have been classified as indicative of clean water in the Florida Department of Environmental Protection's Florida Index, a metric for surface water quality based on invertebrate assemblages.

In Florida, assessments have been conducted in various streams. Analysis of assessments conducted after recalibration of the Stream Condition Index (SCI) showed that SCI values were not significantly correlated with nitrate–nitrite concentrations or habitat assessment scores (Table 2; Figure 4). In nine springs with very poor to poor SCI values, nitrate–nitrite concentrations ranged from 0.02 to 1.90 mg L^{-1}. Concentrations in four springs with fair SCI values ranged from 0.06 to 1.60 mg L^{-1}, which is a nearly identical range. Correlations with nitrate–nitrite concentrations and habitat assessment scores accounted for less than 1% and 16% of the variation in the data, respectively. In contrast, SCI values were significantly correlated with dissolved oxygen concentrations, with a positive correlation that accounted for 36% of the variation in the data (Table 2; Figure 4). Such results illustrate the complexities associated with interpreting biotic indices, and they provide little insight into the form and magnitude of ecological responses to nutrient concentrations.

Table 2. Results from sampling in springs after recalibration of the Stream Condition Index (Agharahimi 2006a and b; Deck 2005a and b; Deck 2006a, b and c; Patronis 2006a, b, c, d, e and f). VP = very poor; P = poor; F = fair; < DL = below detection limit; < PQL = below practical quantitation limit; O = optimal; SO = sub-optimal; LO = low optimal; I–F = impaired-fair; I = impaired; F = fair.

| Spring | Sampling date (DD-MM-YY) | Stream Condition Index | | NO$_x$ | NH$_3$ | TP | DO | Habitat Assessment | | Overall rank |
		Score	Rank		(mg L^{-1})			Score	Rank	
Rock (upper)	25-05-06	11	VP	1.40	< DL	0.11	4.2	134	O	I–F
Alexander	07-06-06	17	VP	0.04	< DL	< PQL	3.7	119	SO	I
Volusia Blue	26-04-05	17	VP	0.57	< DL	0.08	2.5	105	SO	I
Wakulla	11-04-06	20	P	0.37	< DL	< PQL	6.6	129	O	I
Manatee	21-06-06	21	P	1.90	< DL	< PQL	2.6	98	SO	I–F
Ponce de Leon	22-06-06	32	P	0.25	< DL	< PQL	7.0	114	SO	I
Silver Glen	08-06-06	32	P	0.02	< DL	< PQL	6.8	118	SO	I
Wekiwa	26-04-05	35	P	1.10	< DL	0.13	3.6	117	SO	I–F
Blue Hole	22-06-06	36	P	0.42	0.02	< PQL	6.9	111	LO	I–F
Ichetucknee	18-04-06	40	P	0.57	< DL	< PQL	4.5	145	O	F
Silver River	07-06-06	46	F	1.30	< DL	< PQL	6.3	129	O	F
Rainbow	25-05-05	47	F	1.60	< DL	< PQL	—	140	O	F
Rock (lower)	25-08-06	47	F	1.40	< DL	0.10	5.4	126	O	I–F
Juniper	08-06-06	51	F	0.06	< DL	< PQL	8.7	129	O	F
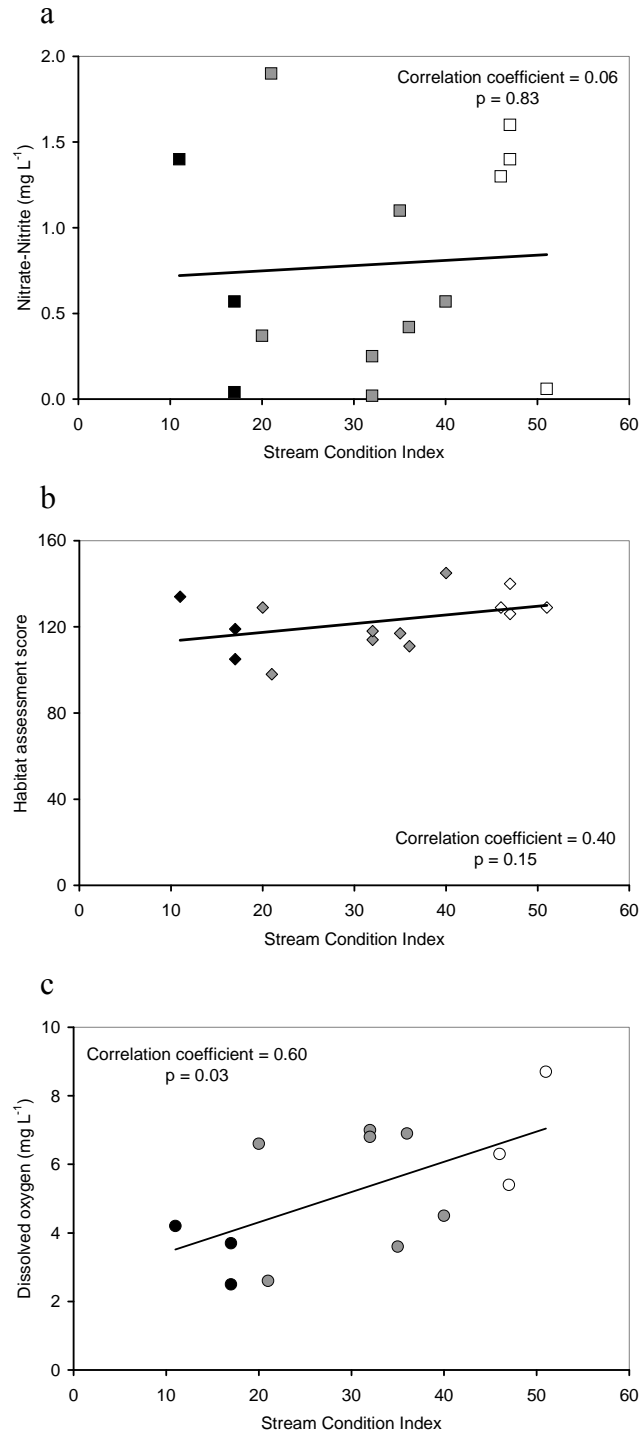
Figure 4 – Correlations between Stream Condition Indices (SCI) and (a) nitrate–nitrite concentrations, (b) habitat assessment scores and (c) concentrations of dissolved oxygen (Agharahimi 2006a and b; Deck 2005a and b; Deck 2006a, b and c; Patronis 2006a, b, c, d, e and f). black squares, triangles and circles = very poor SCI values; gray squares, triangles and circles = poor SCI values; white squares, triangles and circles = fair SCI values; lines provided to illustrate the correlations

Relationships Between Nutrients and Fish in Florida Springs

By affecting the flora of springs and other aquatic systems, nutrients also can affect fish that rely on this flora for food and shelter, including fish species that provide considerable economic value. Florida's lotic systems contain 289 species, with 113 species native to North America and 16 species endemic to Florida and adjacent portions of Alabama and Georgia (Bass 1991). Unfortunately, most studies, such as a broad survey of fish in streams throughout Florida, lack detailed information about assemblages in specific habitats (Bass and Cox 1985).

For example, the fish community in Silver Springs has been examined in two studies separated by approximately 50 years (Odum 1957b; Munch *et al.* 2006). The assemblages were reported to be similar, with the primary difference being reduced numbers of mullet and catfish ascribed to disrupted migratory patterns following construction of a dam. However, neither study focused on differences in fish assemblages among habitats.

In contrast, two habitat classes were included in a survey of 16 Florida streams (Canfield and Hoyer 1988a and b). These streams were sampled with blocknets and electrofishing gear as part of a study assessing the effects of nutrients from treated sewage effluent on the Wekiva River. Habitat classes were limited to open and closed forest canopy, and sampling was biased towards fish with total lengths of over 100 mm. Regardless, higher standing stocks and biomasses were found in reaches with an open forest canopy, which coincided with reaches where macrophytes were more abundant.

Detailed data on fish assemblages in specific habitats comes from a second study of the Wekiva River (Warren *et al.* 2000). Throw traps and a combination of blocknets and electrofishing were used to sample fish in four specific habitats: bare sediment, *Hydrocotyle umbellate*, *Nuphar advena* subspecies *lutea* (spatterdock) and *Vallisneria americana*.

Throw traps and blocknets collected forty species of fish from various reaches, with more species collected from vegetated habitats (Figure 5). *Hydrocotyle umbellate* yielded the highest mean density of fish, followed by *Vallisneria americana*, *Nuphar advena* and bare sediment (Figure 6). In contrast, samples from *Vallisneria americana* tended to yield the greatest biomass because these samples contained more sunfish in the family Centrarchidae (Figures 7–11). Samples from *Hydrocotyle umbellate* were dominated by small, livebearing Poeciliidae, and samples from *Nuphar advena* and bare sediment contained large numbers of small minnows in the family Cyprinidae and killifishes in the family Fundulidae (Figures 8–11). Mullet also were found in samples taken from within blocknets set over bare sediment in the Lower Wekiva River (Figures 10 and 11). These differences in fish assemblages were attributed predominantly to interactions among characteristics of the habitats and the biology and ecology of fish species. Similar to comparisons for Silver Springs, broad comparisons between the two studies of the Wekiva River indicated that the fish assemblage had changed little between 1985–1987 and 1997 (Canfield and Hoyer 1988a; Warren *et al.* 2000). Thus, we do not have data linking changes in fish assemblages to changes in nutrient concentrations in springs at this time.
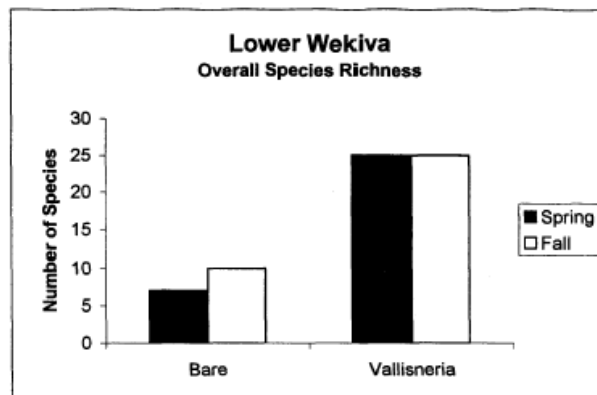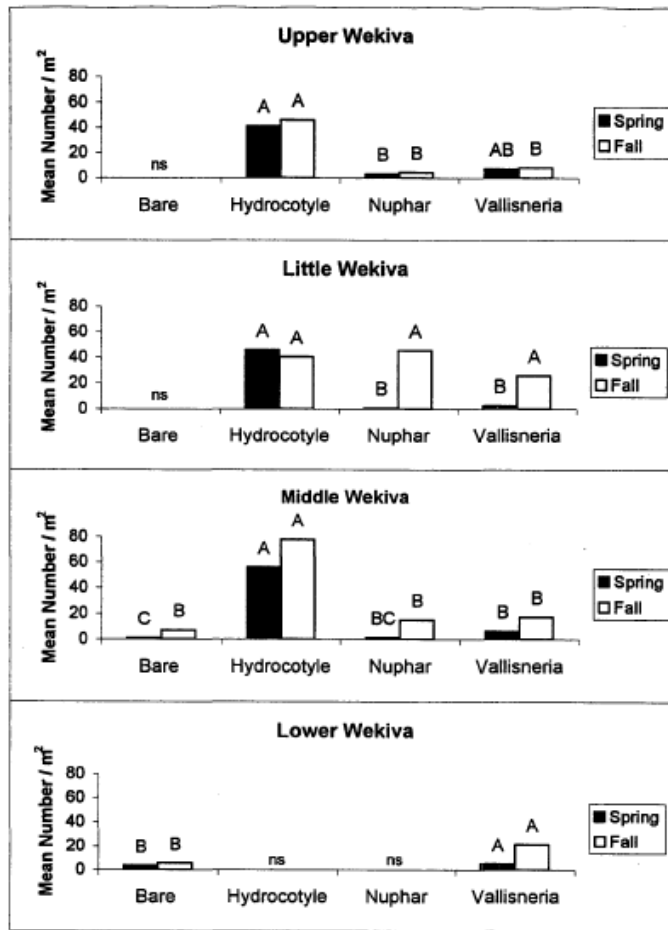
a
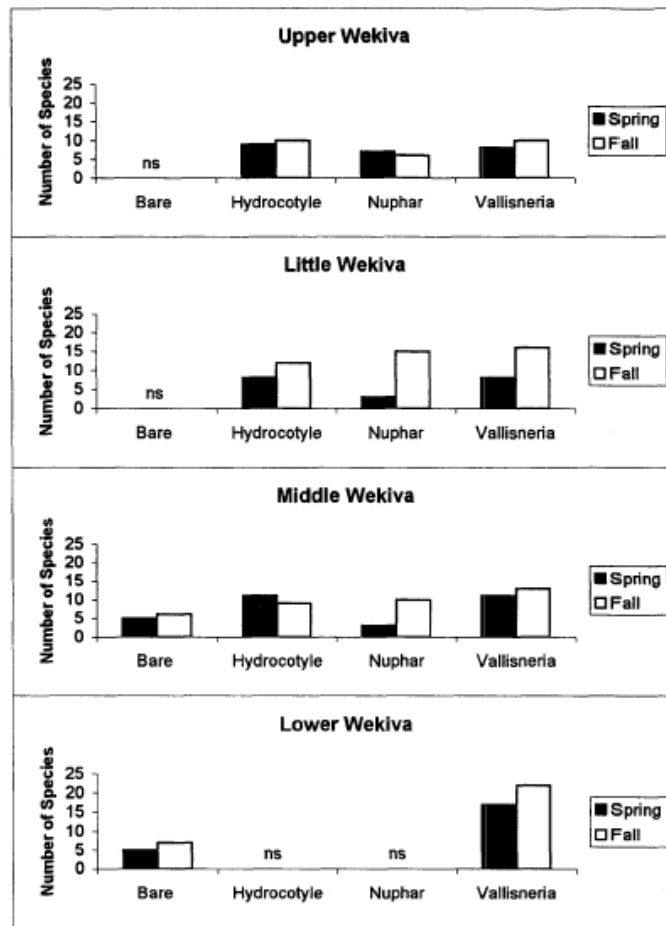
b

Figure 5 – *Species richness in (a) throw trap and (b) blocknet samples (Warren et al. 2000).*
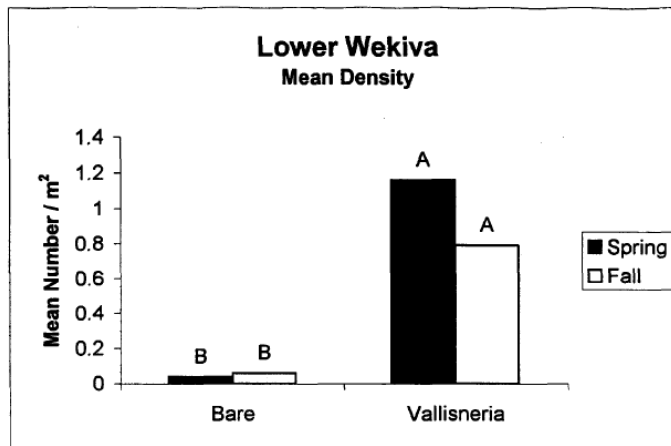
a

b

Figure 6 – *Densities of fish in (a) throw trap and (b) blocknet samples (Warren et al. 2000).*
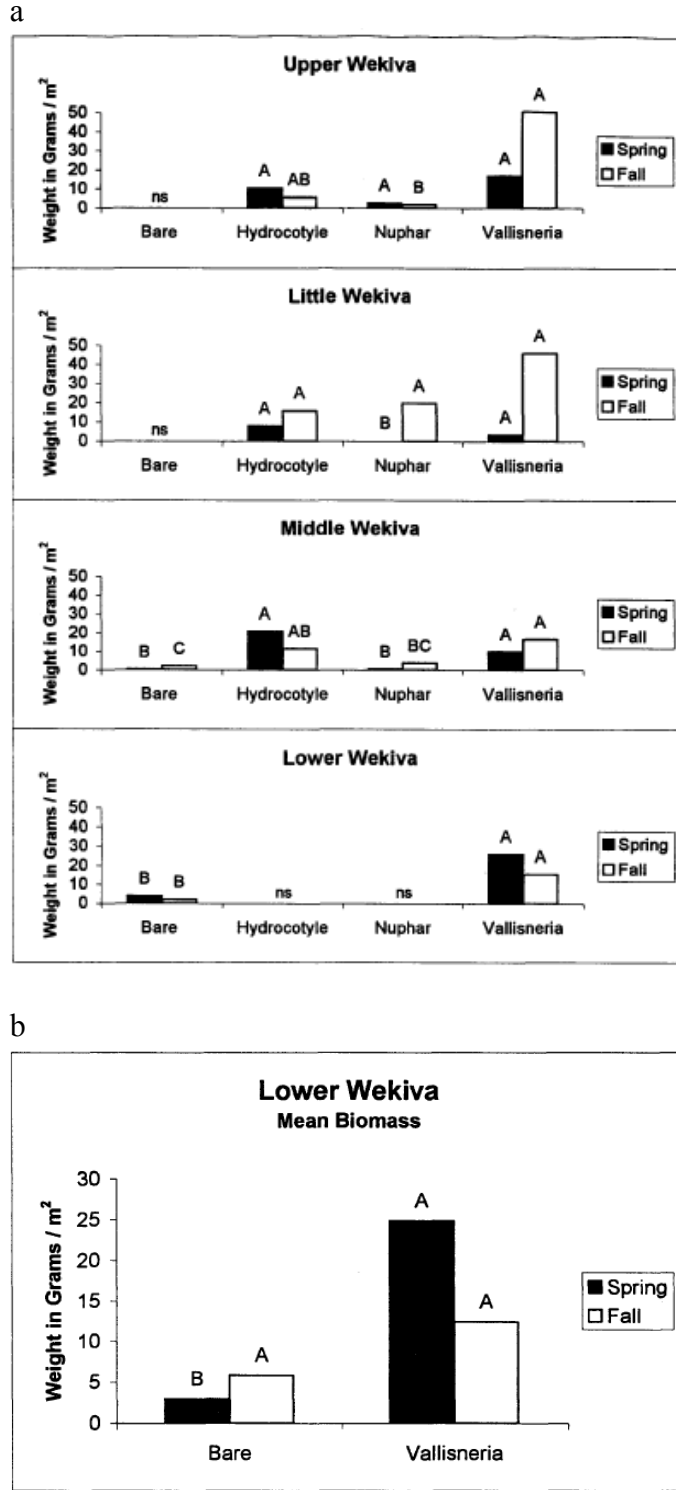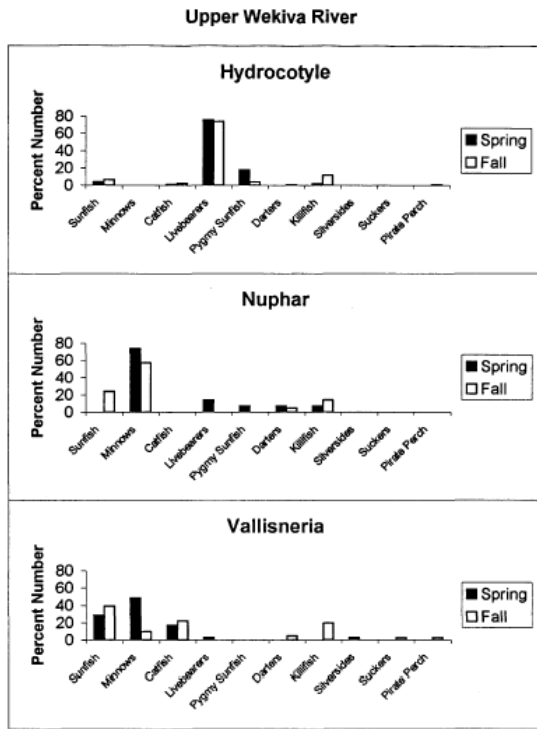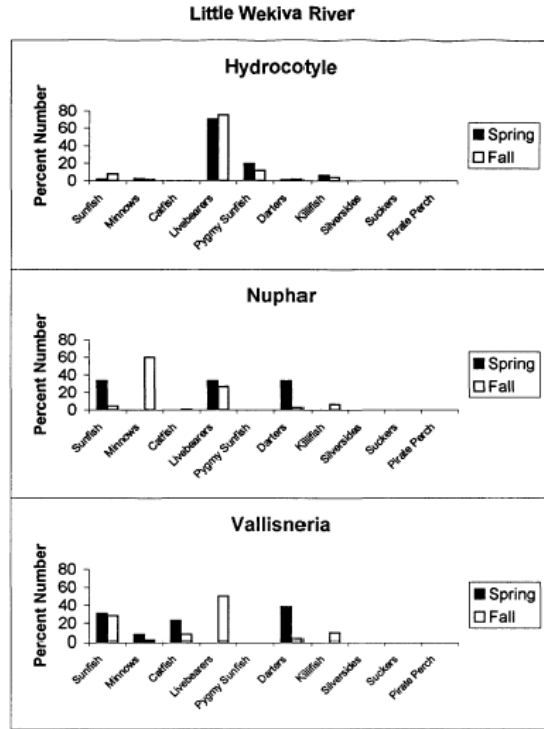
a

b

Figure 7 – *Biomass of fish in (a) throw trap and (b) blocknet samples (Warren et al. 2000).*

a

Upper Wekiva River

Hydrocotyle

Nuphar

Vallisneria

b

Little Wekiva River

Hydrocotyle

Nuphar

Vallisneria

c

Middle Wekiva River

Bare

Hydrocotyle
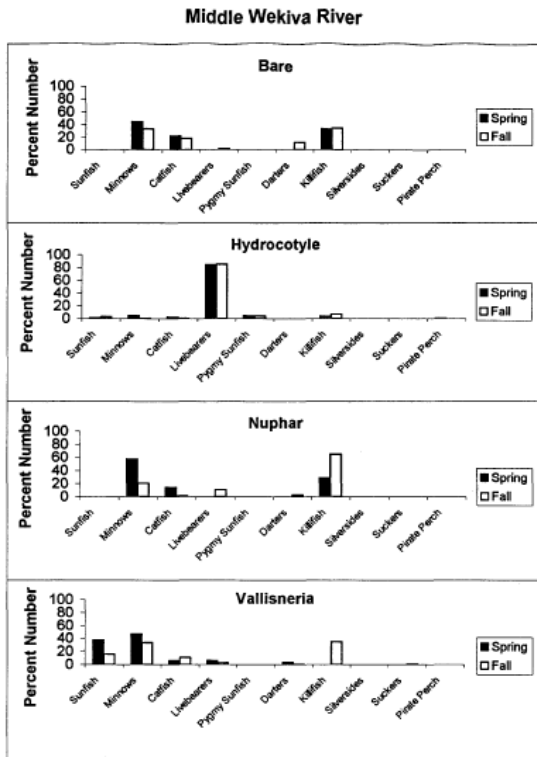
Nuphar

Vallisneria

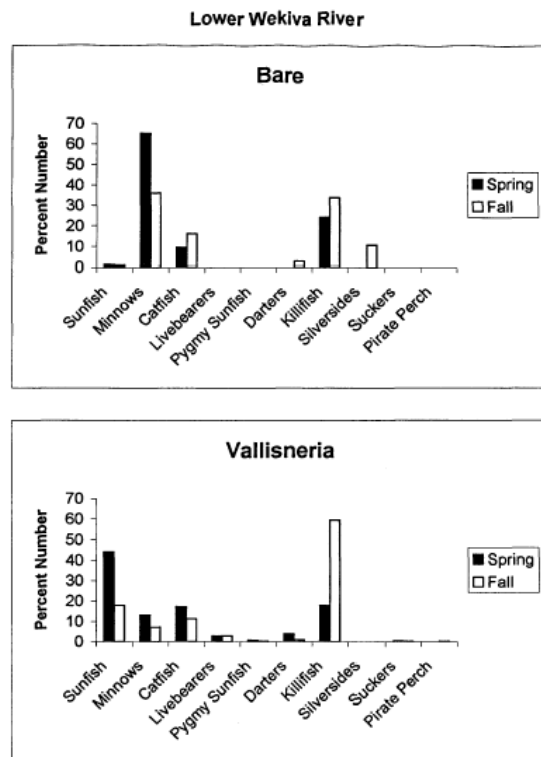d

Lower Wekiva River

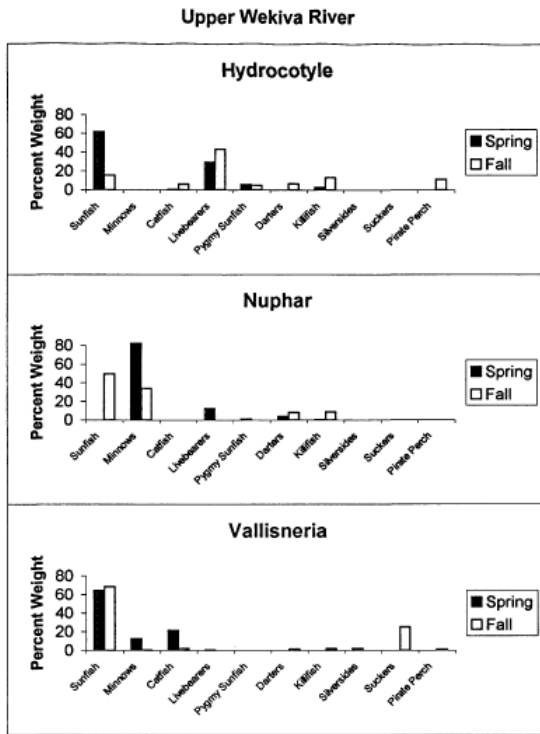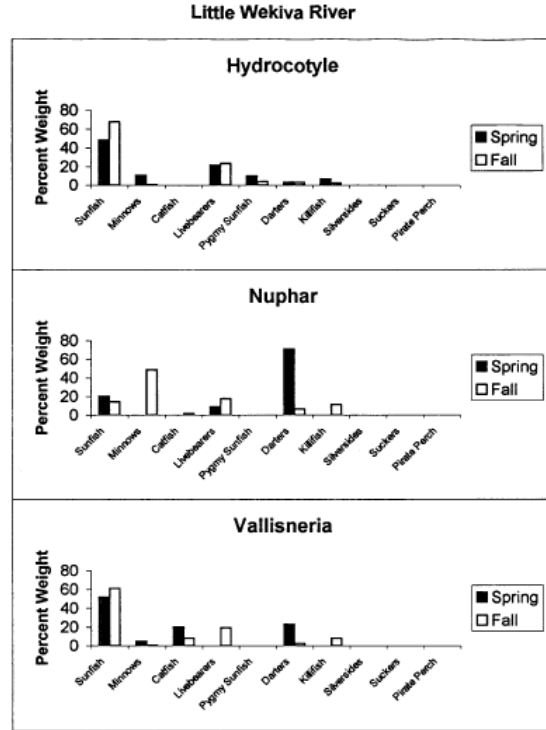**Bare**

Vallisneria

Figure 8 – *Assemblage composition by abundance in throw trap samples (Warren et al. 2000).*

a

b

c
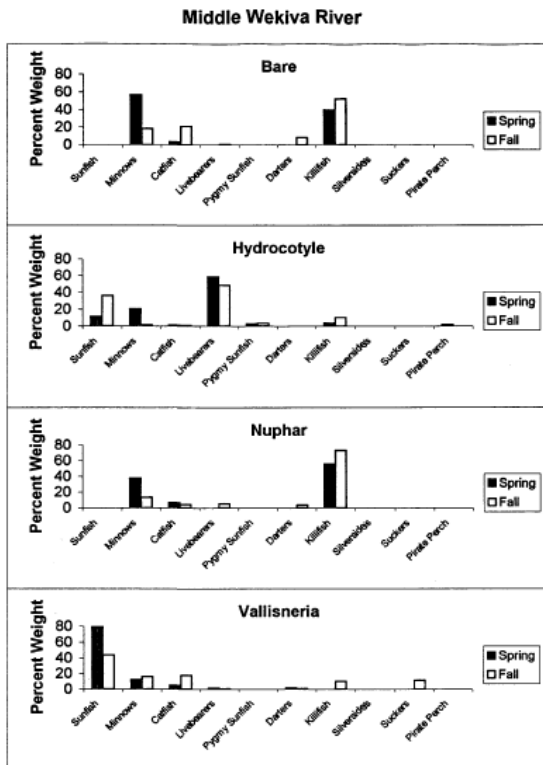
d
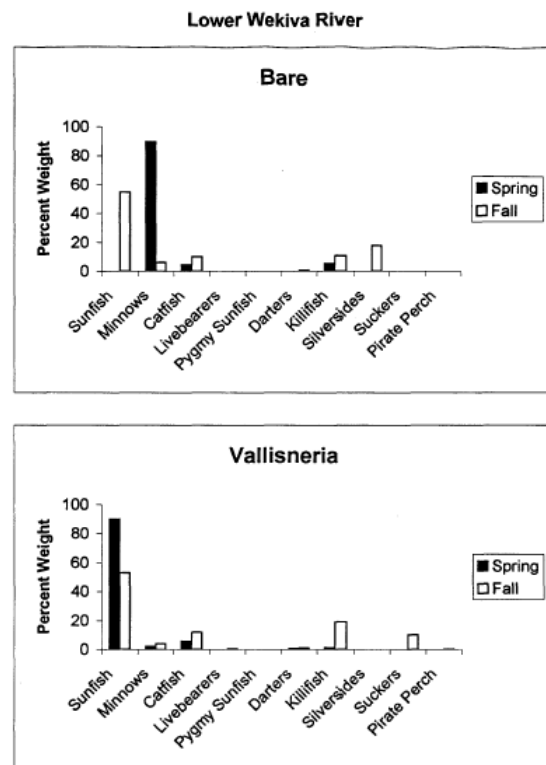
Figure 9 – Assemblage composition by biomass in throw trap samples (Warren et al. 2000).

Lower Wekiva River

Bare

Vallisneria
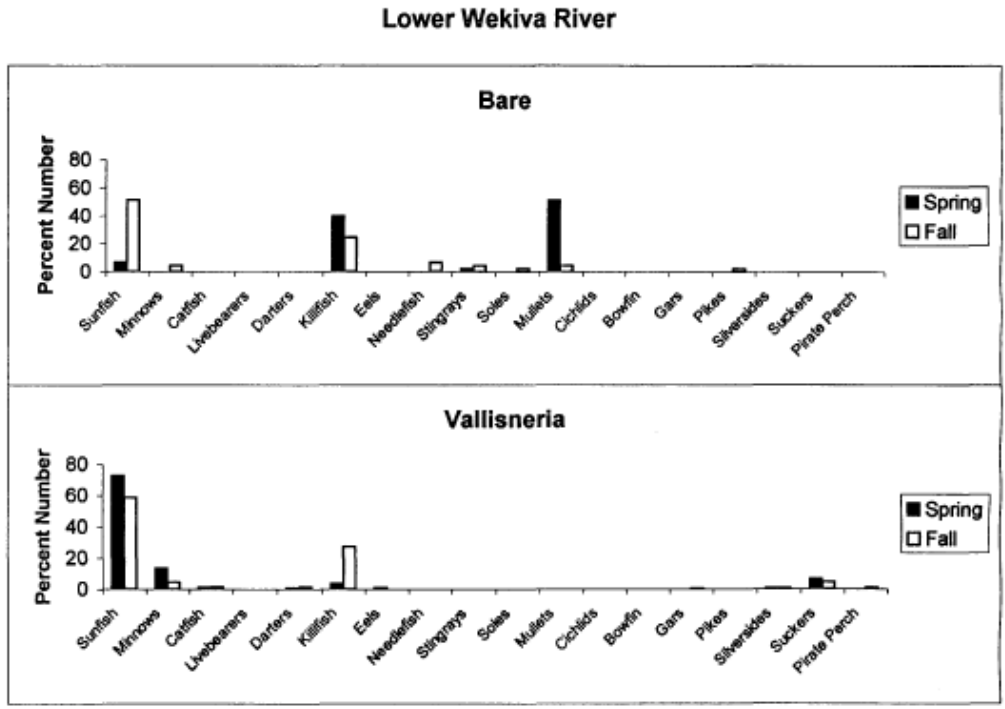
Figure 10 – *Assemblage composition by numbers for blocknet samples (Warren et al. 2000).*

Lower Wekiva River
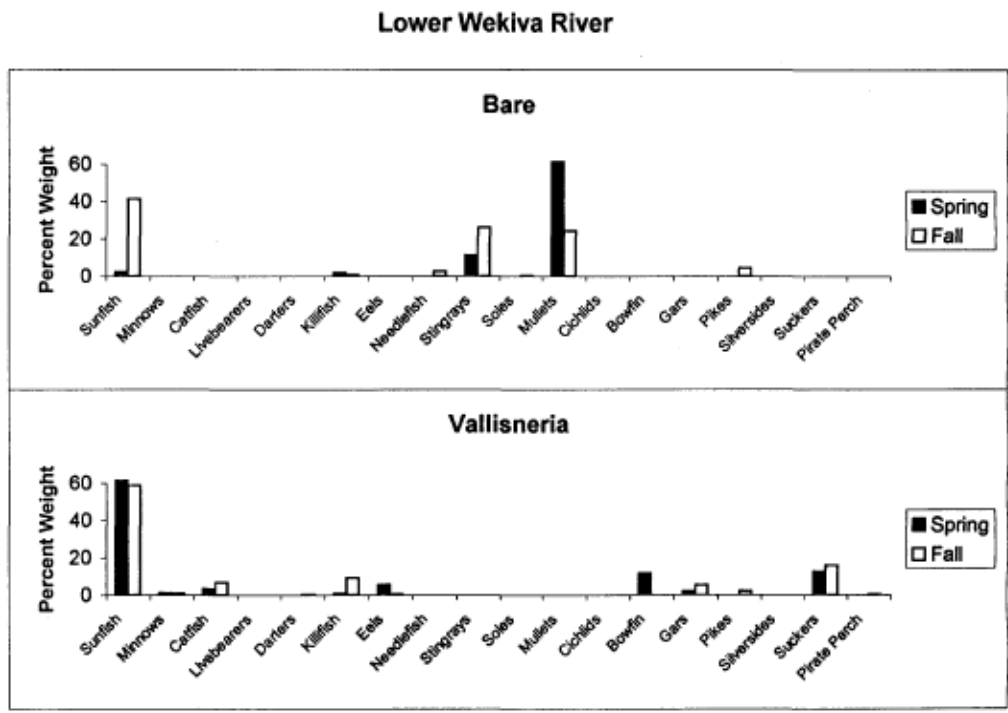
Bare

Vallisneria

Figure 11 – *Assemblage composition by biomass for blocknet samples (Warren et al. 2000).*

Summary of Relationships Between Nutrients and Fauna in Florida Springs

In summary, surveys of faunal assemblages in Florida springs have suggested the possibility of indirect influences from nutrients mediated through differences or changes in habitats. However, results of repeated surveys indicate faunal assemblages have not changed even if nitrate levels have risen. Unfortunately, these studies were not designed to identify the form and magnitude of interactions among nutrient inputs, primary producers and fauna.

Studies conducted in streams outside Florida and in lakes can provide additional insights into how nutrients affect habitat use and trophic relationships. Such insights can guide future work in Florida's spring ecosystems.

Nutrient Effects Operating Through Ecosystem Processes

Interactions among animals and habitats vary not only among species but also among life history stages or ages within species. Such interactions may result in nonlinear effects on key parameters such as growth rates (Mittelbach and Osenberg 1993; Diehl and Kornijów 1998). For example, fish may rely heavily on macrophytes for refuge from predation in the early portion of their life histories even if it reduces their growth rate (Figure 12). A nonlinear change in growth rate may be associated with changing habitat use after achieving a size refuge (S) from piscivores, which emphasizes the importance of having detailed information on the form and magnitude of responses to changes in habitats (Figure 11). Such information is not available for most species of fish, including most species found in Florida springs.
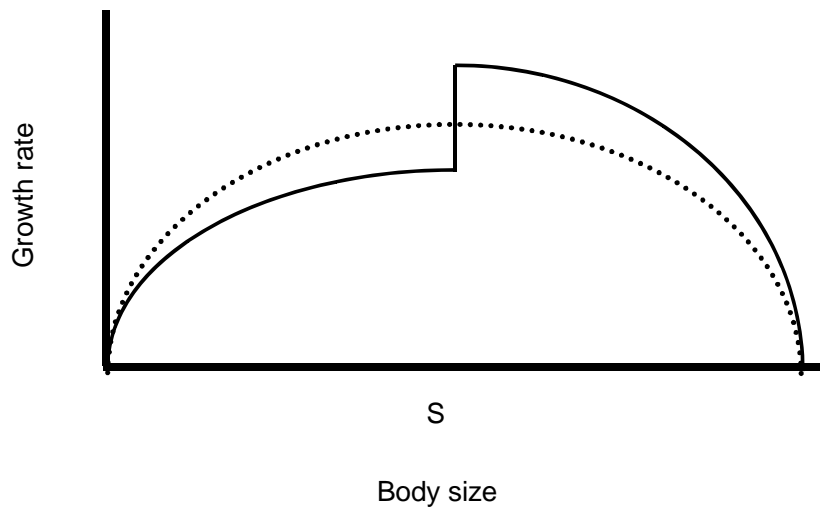
Figure 12 – *Diagram depicting a change in growth rate experienced by a fish when it switches from a suboptimal habitat used as a refuge from predators to a more optimal habitat once it has achieved a size refuge (adapted from Diehl and Kornijów 1998). Solid line represents growth rate exhibited by a fish that switches from one habitat to another once it achieves a size refuge from piscivores at body size S. Dotted line represents the growth rate of a fish able to use all available habitats and food resources due to the absence of piscivores. The difference between the lines represents the cost or benefit associated with the use of each habitat.*

Up to some level, increased nutrient inputs can increase the productivity of macrophytes, which provides more food for herbivores and may lead to increases in secondary and tertiary consumers. The role of aquatic macrophytes as a food source for the most numerous grazers remains a controversial issue (Sheldon 1987; Brönmark 1990; Sheldon 1990; Newman 1991). Living macrophytes (mosses, flowering plants and macroalgae) have been regarded as a little used food source due to high C:N ratios (e.g., 20–1,340), large quantities of cellulose and lignin, decreased digestibility of their proteins and presence of secondary chemicals that retard grazing (Brönmark 1990). Larger grazers, such as manatees, turtles and *Pomacea paludosa*, the Florida apple snail, do graze macrophytes. The effect of this grazing can be significant (Hauxwell *et al*. 2004; Jacoby *et al*. 2007). In addition, some insects, crayfish, birds and mammals have been reported to reduce macrophyte biomass through consumption or damage during grazing on periphyton (Cummins and Klug 1979; Mann 1988; Lodge 1991; Lodge *et al*. 1998; Newman 1991).

Regardless of their contribution as a source of food, macrophytes do provide a significant substratum for periphyton, which is grazed heavily by the most abundant animals. As discussed above, periphyton can outcompete macrophytes for resources, such as light and nutrients. Excessive growth of periphyton has the potential to lead to loss of macrophytes, with subsequent effects on refuge and trophic interactions.

Grazers can mediate the growth and effects of periphyton. Experiments using elevated tiles as a mean of excluding the herbivorous larvae of the caddisfly, *Helicopsyche borealis*, demonstrated effects of grazing on biomass and turnover of microalgae (Lamberti and Resh 1983). In grazed treatments, biomass, measured as chlorophyll *a* cm^{-2}, was 5–500 times lower and turnover, measured as oxygen production per unit chlorophyll *a*, was approximately 5 times higher (Lamberti and Resh 1983). Meta-analyses of experiments comparing grazers at ambient densities to treatments without grazers indicated that grazers significantly reduced periphyton biomass in 70% of experiments and measurably altered the taxonomy or physiognomic structure of assemblages in 81% of experiments (Feminella and Hawkins 1995). Typically, less than 70% of experiments showed effects on productivity, and the effects varied with the parameter measured (Feminella and Hawkins 1995). Significant effects were equally likely in laboratory and field experiments and across durations of less than and greater than 4 weeks, although factors such as the total amount of periphyton accrued, the type of grazer manipulated, and the total biomass of grazers influenced the magnitude of the effects recorded.

Grazers represent multiple levels of organismal complexity, and they interact with periphyton and each other in complex ways. For example, the extracellular polysaccharide sheath produced by *Lyngbya wollei* deterred grazing by the amphipod *Hyallela azteca* (Camacho and Thacker 2006). In streamside experimental troughs, herbivorous protozoa were shown to graze on benthic diatoms, with estimated consumption rates varying from 4,000 diatoms cm^{-2} h^{-1} in systems with few or no macrograzers to 150 diatoms cm^{-2} h^{-1} in systems with large numbers of macrograzers, supposedly due to competition or predation (McCormick 1991).

Overall, the effects of nutrients in aquatic systems appear to be mediated by a combination of bottom-up and top-down effects. For example, larvae of the chironomid *Orthocladius rivulorum* build tubes and harvest the diatoms growing on them. Addition of phosphorus and phosphorus plus nitrogen to a stream led to a greater biomass of periphyton and larger larvae (Hershey

et al. 1988). Nitrogen and phosphorus appeared to be co-limiting factors as indicated by greater accumulation of periphyton downstream of combined additions (Hershey *et al*. 1988). The system involved a positive feedback loop, with larvae in fertilized reaches growing larger, building larger tubes that supported more periphyton, and, in turn, having more food to harvest (Hershey *et al*. 1988). In addition to interactions among nutrients, periphyton may respond to other limiting factors and grazing. Periphyton in streamside, flow-through channels exhibited statistically significant increases above controls only when nitrogen and phosphorus were added, light was increased, and grazing was eliminated (Rosemond 1993). In addition, manipulating single factors led to different responses. For example, increased light elicited both a physiological response resulting in decreased chlorophyll *a* concentrations in cells and a change in the periphyton assemblage when algae adapted to low light conditions were lost (Rosemond 1993).

All the bottom-up influences of nutrients and light and top-down influences of grazing can be mediated through actual or potential trophic interactions. Manipulations of pools in a stream demonstrated that grazing minnows controlled periphyton growth unless predatory fish were present, in which case predation and avoidance of predation led to increased standing crops of periphyton in deeper sections of the stream (Power *et al*. 1985).

In some cases, spring systems may exhibit very complex trophic cascades, which involve reciprocal interactions among predators and prey that result in ecological changes across trophic links. Manipulations of lakes demonstrated that such effects remain stable across a range of conditions (Carpenter *et al*. 1987; Carpenter *et al*. 2001). For example, a reference lake was characterized by variable productivity of phytoplankton related to the effects of climatic factors and the natural dynamics of food webs. Manipulating numbers of zooplanktivores and piscivores in other lakes yielded consistent changes in zooplankton assemblages and their grazing, which led to altered phytoplankton assemblages and productivity. For example, gelatinous, colonial green algae became more abundant in a lake with reduced feeding on zooplankton. Additions of significant quantities of nitrogen and phosphorus did not disrupt the trophic cascade. Piscivorous fishes reduced numbers of zooplanktivorous fishes to an extent that allowed zooplankton numbers to increase. The resulting increased grazing by zooplankton controlled the phytoplankton assemblage.

Over time, the interactions among bottom-up and top-down effects can change. For example, nutrient additions to a river led to increased primary production in years 1 and 2, with increased production of insects and fish lagging until years 3 and 4 (Peterson *et al*. 1993). Competition among insects moderated the flow of increased primary production through the trophic web (Peterson *et al*. 1993).

Given the complexity of potential interactions, it is not surprising that an attempt to correlate standing crop of fish with phosphorus levels in streams led to the conclusion that increases in fish biomass with increased phosphorus were greater at lower initial phosphorus concentrations (Hoyer and Canfield 1991). Other responses of lotic systems to increased nutrients also are likely to be nonlinear, with the potential for thresholds and feedback loops.

In fact, nonlinear responses, such as thresholds and feedback loops, characterize one of the most cogent descriptions of eutrophication (Duarte 1995). In the eutrophication progression, interactions among nutrients, plants and algae, and grazers combine to produce changes that are not linearly correlated with nutrient concentrations. With large and chronic increases in nutrients, an aquatic system is highly likely to follow a qualitative progression from long-lived, rooted, vascular plants that provide a key structural habitat to systems dominated by fast-growing macroalgae and microalgae and potentially to systems dominated by phytoplankton if residence times are long enough. Nonlinearities make the quantitative responses of any given system difficult to predict, without detailed information.

In addition to effects on trophic interactions among primary producers, grazers and consumers, nutrient enrichment also may alter interactions between parasites and hosts. Additions of nutrients to mesocosms led to increased algal production, higher densities of snails, greater production of trematode cercariae, and ultimately, increased infection of frogs yielding deformities that decreased their survival (Johnson *et al*. 2007). The prevalence of such interactions and their consequences in aquatic systems remains unknown.

Nutrient Effects Operating Through Toxicity

Ammonia, nitrate and other chemicals can have toxic effects at a variety of levels from sublethal effects on the physiology, development, reproduction, behavior or morphology of individual organisms through to lethal effects that operate at the level of populations, assemblages and ecosystems (Mattson *et al*. 2006; Mattson *et al*. 2007). In general, toxicological experiments address endpoints related to individuals. For example, acute toxicity often is addressed by determining a concentration that kills 50% of the test organisms over a defined time interval, or an LC_{50}. Florida law contains a definition of a 96-hour LC_{50}, which is a concentration that kills 50% of the test organisms over a 4-day period (Florida Administrative Code Chapter 62–302.200). Sublethal endpoints can also be used to yield concentrations that cause a defined effect in a defined time interval. Terms for such endpoints include least observable effect level (LOEL), which is the lowest concentration at which a change of interest is observed, or no observable effect level (NOEL), which is the concentration at which a change of interest ceases to be observed. In addition, experiments can examine chronic toxicity or the effect of longer exposures to lower concentrations of toxicant; however, the time needed to conduct such experiments has led to various approaches to extrapolate results of acute tests. The results of laboratory experiments documenting effects on individuals typically are extrapolated to predict ecotoxicological effects, or effects at the population, assemblage or ecosystem levels. Effects at higher levels of organization may arise from density-mediated changes resulting from the death of organisms or trait-mediated changes resulting from sublethal influences, with extrapolations from laboratory results often relying on untested effects on reproductive success or trophic links. Trait-mediated effects represent a primary focus because they typically occur at lower levels of contamination.

Predictions of ecotoxicological effects may employ uncertainty factors to account for limitations imposed by testing relatively few individuals of relatively few species for relatively short periods at concentrations that cause an observable effect (Mattson *et al* 2006). For example, an intraspecies uncertainty factor can be used to account for variation in sensitivity among individuals within a species. The USEPA recommended an intraspecies uncertainty factor

of 10 applied to any NOEL especially for endangered species (Mattson *et al.* 2006). An interspecies uncertainty factor ranging from 1 to 1,000 may be applied to account for uncertainties associated with extrapolating toxicokinetics (processes determining uptake rates, distribution among tissues, detoxification and elimination) and toxicodynamics (processes determining effects such as enzyme inhibition, enzyme facilitation and binding to DNA) from one species to another (Mattson *et al.* 2006). The magnitude of the uncertainty factor can be derived from allometric scaling of body sizes, known variation in acute and chronic sensitivity, and best professional judgment. Another uncertainty factor is used to translate the results of short-term studies to protection during long-term exposures. This adjustment accounts for the facts that contaminants often reach equilibrium levels only after long-term exposure; physiological compensations to toxins may fail after longer periods of exposure; latent, adverse effects may appear after exposure ceases; and longer exposures may affect life history stages that are more sensitive than those tested. Larger adjustments are needed when available data come from studies much shorter than the life expectancy of the target organism or much shorter than the time needed to achieve equilibrium concentrations in the tissues of the target organism. An uncertainty factor also can be used to predict NOELs from observed LOELs, with severe effects calling for a larger factor and unbounded LOELs from steep dose-response curves requiring smaller uncertainty factors than unbounded LOELs from curves with shallow slopes. Whenever they are applied, uncertainty factors lower the concentration considered acceptable in the environment.

The toxicity of aqueous solutions of ammonia, nitrite and nitrate has received attention primarily due to harmful effects in closed circulating systems, such as aquaria or fish hatcheries, and through drinking water for livestock that has been contaminated by urine, manure or fertilizer (Armstrong 1979; Shimura *et al.* 2002; Mattson *et al.* 2006). Ammonia tends to be more toxic than nitrite, which is typically more toxic than nitrate (Armstrong 1979).

Toxicity of Ammonia

The USEPA has invested significant effort in identifying water quality criteria for ammonia (e.g., USEPA 1999). Their results provide important guidance for establishing criteria related to concentrations of ammonia considered safe in aquatic environments. Some key findings from their analysis of data related to ammonia toxicity are:

- toxicity of ammonia can depend on the ionic composition, pH and temperature of water due to poorly understood mechanisms, with pH dependence strongly suggesting joint toxicity of unionized ammonia and ammonium ions

- ionic composition generates small effects on ammonia speciation in freshwater, and its other effects on toxicity are poorly established; therefore, the ionic composition of water was not considered in the derivations of criteria

- temperature can strongly affect the relative amounts of unionized ammonia and ammonium ion, but its effect on the toxicity of ammonia is not strongly indicative of joint toxicity so it was addressed strictly by an empirical approach

- pH effects were described by equations that include basic features of joint toxicity due to unionized ammonia and ammonium ion, along with an empirical component that addressed the incomplete knowledge of these effects

Detailed and updated analysis of acute toxicity data yielded criteria expected to protect aquatic life, except for unusually sensitive species. Criterion maximum concentrations (CMCs) drawn from acute toxicity tests address short-term exposure, and analysis of chronic toxicity data yields criterion continuous concentrations (CCCs) that address long-term exposure. The ultimate efficacy of the proposed criteria will be known only after monitoring. For example, chronic exposures to ammonia showed that a mayfly species in New Zealand would be protected minimally by the proposed criteria (Hickey *et al*. 1999). In Florida springs, the effects on obligate residents in springs and stygobiota represent special concerns. For example, a survey of stygobitic crayfish linked their presence or absence to ammonia concentrations in source water (Spanjer and Cipollini 2006). Regardless of the associated uncertainties, the proposed criteria are based on detailed interpretation of the best available information. Given temperature (T) and pH of a waterbody, the proposed criteria are:

1. The one-hour average concentration of total ammonia nitrogen (in mg–N L^{-1}) does not exceed, more than once every three years on the average, the CMC (acute criterion) calculated as:

$$\text{CMC} = \frac{0.275}{1 + 10^{7.204 - pH}} + \frac{39.0}{1 + 10^{pH - 7.204}} \quad \text{where salmonid fish are present}$$

$$\text{CMC} = \frac{0.411}{1 + 10^{7.204 - pH}} + \frac{58.4}{1 + 10^{pH - 7.204}} \quad \text{where salmonid fish are not present}$$

2A. The thirty-day average concentration of total ammonia nitrogen (in mg–N L^{-1}) does not exceed, more than once every three years on the average, the CCC (chronic criterion) calculated as:

$$\text{CCC} = \left(\frac{0.577}{1 + 10^{7.688 - pH}} + \frac{2.487}{1 + 10^{pH - 7.688}} \right) \cdot \text{Minimum of } (2.85, 1.45 \cdot 10^{0.028 \cdot (25 - T)})$$

where early life history stages of fish are present

$$\text{CCC} = \left(\frac{0.577}{1 + 10^{7.688 - pH}} + \frac{2.487}{1 + 10^{pH - 7.688}} \right) \cdot 1.45 \cdot 10^{0.028 \cdot (25 - \text{Maximum of } (T, 7))}$$

where early life history stages of fish are not present

2B. In addition, the highest, four-day average concentration of total ammonia nitrogen (in mg–N L^{-1}) within the 30-day period should not exceed 2.5 times the CCC.

Toxicity of Nitrate

Nitrate (NO_3^-) and nitrite (NO_2^-) coexist as the two major forms of oxidized nitrogen in aquatic systems. Nitrifying bacteria produce nitrite from organic forms of nitrogen and from ammonia as part of the nitrogen cycle (Lewis and Morris 1986). In aerobic environments, nitrite concentrations tend to be low because nitrite is converted rapidly to nitrate, a more stable form (Guillette and Edwards 2005). Analyses of nitrate in aquatic systems typically measure both nitrate and nitrite (Edwards *et al*. 2004). The solubility of nitrate means that any nitrate not taken up by plants or converted to nitrogen gas by denitrification is transported in surface water and groundwater.

Like all chemicals, the toxicity of nitrate and nitrite is mediated by its uptake and metabolism. Nitrate and nitrite enter animals either through permeable membranes, such as the gills of fish or tadpoles and the skin of adult amphibians, or through the gut wall after ingestion, which is the primary means for birds and mammals. It appears that the chloride transport mechanism of gill membranes actively transports nitrate (Jensen 2003; Lewis and Morris 1986). Nitrate in body fluids can be converted to nitrite or it can accumulate because of hepatic detoxification of nitrite (Edwards *et al*. 2004).

Several detrimental effects have been ascribed to nitrate. Concerns related to drinking water, including groundwater, have centered on nitrate interference with hemoglobin in the bloodstream resulting in a reduced ability to carry oxygen that leads to tissue hypoxia and cyanosis. In vertebrate animals, nitrate oxidizes the iron in hemoglobin molecules disrupting the reversible binding of oxygen and causing methemoglobinemia (Lewis and Morris 1986). In human infants, the resulting cyanosis is one cause of blue baby syndrome. Nitrate also can act as an endocrine disruptor, interfere with steroid hormone synthesis, affect sperm motility and viability, affect fecundity, or be toxic to embryos (Edwards *et al*. 2004; Edwards 2005; Guillette and Edwards 2005; Edwards *et al*. 2006a; Edwards and Guillette 2007). In fact, nitrate levels in some freshwater systems may reach and be sustained at levels that lead to declines in amphibians (Rouse *et al*. 1999).

Nitrate toxicity represents a concern in Florida springs because nitrate levels have risen during the past decades (Mattson *et al*. 2006; Mattson *et al*. 2007). Estimates of nitrate toxicity are available for a variety of organisms (Table 3; Camargo *et al*. 2005; Corrao *et al*. 2006; Mattson *et al*. 2007 and references therein). The bulk of the tests have been conducted on fishes, amphibians and aquatic invertebrates, including crustaceans, insects, and mollusks.

Table 3. Nitrate concentrations yielding 50% mortality in 96-hour tests (96-hour LC$_{50}$) or other changes deemed relevant, e.g., altered growth, time to metamorphosis or reproductive characteristics (Camargo *et al.* 2005; Corrao *et al.* 2006; Mattson *et al.* 2007).

Taxonomy	Life stage	96-hour LC$_{50}$ (mg L^{-1} NO$_3$–N)	Other threshold (mg L^{-1} NO$_3$–N)
Order: Ephemeroptera			
Baetis vernus	Nymph		0.73
Potamanthus luteus	Nymph		0.70
Baetis fuscatus	Nymph		0.74
Cloeon bifidum	Nymph		0.72
Ephemerella lenoki	Nymph		0.74
Heptagenia sulphurea	Nymph		0.70
Order: Trichoptera			
Cheumatopsyche pettiti	Early instar larvae	113.5	2.4
	Last instar larvae	165.5	3.5
Hydropsyche exocellata	Last instar larvae	269.5	11.9
Hydropsyche occidentalis	Early instar larvae	97.3	1.4
	Last instar larvae	109	2.2
Order: Cladocera			
Ceriodaphnia dubia	Neonates		7.1–56.5
Daphnia magna	Neonates		358
Daphnia sp.	Adults		34.8–87.1
Order: Amphipoda			
Eulimnogammarus toletanus	Adults	85.0	4.4
Eulimnogammarus echinosetosus	Adults	62.5	2.8
Order: Decapoda			
Cherax quadricarinatus	Juveniles		1,000
Astacus astacus	Juveniles		14
Penaeus monodon (marine)	Protozoea		0.23
Macrobrachium rosenbergii	Juveniles		160
	Juveniles		175
Class: Gastropoda			
Pomacea paludosa	Adults & juveniles	> 500	> 500
Potamopyrgus antipodarum	Adults	1,042	195
Family: Salmonidae			
Oncorhynchus mykiss	Eggs		1.1
	Fry		1.1 - 4.5
	Fry		2.3
	Fingerlings	1,355	14
Oncorhynchus kisutch	Eggs		4.5
	Fry		4.5
Oncorhynchus tshawytscha	Eggs		4.5
	Fry		2.3 & 4.5
	Fingerlings	1,310	
Oncorhynchus clarki	Eggs		2.3 & 4.5
	Fry		4.5 & 7.6
Salvelinus namaycush	Swim-up fry	1,121.4	
	Embryo		100
	Alevin	2,342.5	400
	Embryo to swim-up fry		1.6
Coregonus clupeaformis	Swim-up fry	1,902.7	
	Embryo		25
	Alevin	2,185.7	25
	Embryo to swim-up fry		6.25

Taxonomy	Life stage	96-hour LC$_{50}$ (mg L^{-1} NO$_3$–N)	Other threshold (mg L^{-1} NO$_3$–N)
Family: Moronidae			
Morone sp. hybrid	Unknown (juveniles?)		200
Family: Centrarchidae			
Lepomis macrochirus	Fingerlings	1,975	761
Micropterus salmoides	Fingerlings?	140	
Micropterus treculi	Fingerlings	1,261	
Family: Ictaluridae			
Ictalurus punctatus		1,355–1,423	90
Family: Cyprinidae			
Pimephales promelas	Larvae	1,010–1,607	358
Catla catla	Fry		1,484
Family: Poeciliidae			
Gambusia affinis	Embryos		4
	Adults		4
Poecilia reticulatus	Fry	191	
Family: Adrianichthyidae			
Oryzias latipes	Spawning		30
	Juveniles		50
Family: Bufonidae			
Bufo bufo	Tadpoles	384.8	22.6
Bufo americanus	Tadpoles	13.6–39.3	
	Fertilized eggs		9.0
Bufo terrestris	Tadpoles		30.0
	Tadpoles		4.9
Xenopus laevis	Embryos		24.8
	Tadpoles	1,655.8	65.6
Family: Hylidae			
Hyla versicolor			20
Litoria caerulea	Tadpoles		22.7
Pseudacris triseriata	Tadpoles	17	10.0
Pseudacris regilla	Embryos	643	56.7
	Tadpoles	1,749.8	30.1
Family: Ranidae			
Rana pipiens	Tadpoles	22.96	10.0 & 30.0
Rana clamitans	Tadpoles	32.4	
Rana sylvatica	Fertilized eggs		9.0
Rana pretiosa	Newly-hatched larvae		16.5
Rana temporaria	Larvae		5
Rana aurora	Embryos		29.0
Family: Ambystomatidae			
Ambystoma maculatum	Fertilized eggs		9.0
Ambystoma jeffersonianum	Fertilized eggs		9.0
Ambystoma gracile	Newly-hatched larvae		23.4
Family: Salamandridae			
Triturus vulgaris	Larvae		17.4
Order: Crocodilia			
Alligator mississippiensis	Juveniles		0.01–0.04

Nitrate concentrations from 0.23 mg L^{-1} NO_3–N to over 1,000 mg L^{-1} NO_3–N produced effects on invertebrates. Lethal concentrations in 96-hour tests ranged from 62.5 mg L^{-1} for an adult amphipod to over 1,000 mg L^{-1} for an adult snail. The lowest concentration causing an effect was 0.23 mg L^{-1}, which was reported for a larval stage of a marine shrimp. The lowest levels recorded or calculated to be of import for freshwater species were 0.70 mg L^{-1} NO_3–N for nymphs of mayfly species when they were subjected to a mixture of nitrate, nitrite and ammonia. A value of 2.2 mg L^{-1} was calculated as a safe concentration for long-term exposure of caddisfly larvae to nitrate alone. In general, early life history stages appear to be most sensitive, and freshwater invertebrates appear to be more sensitive than marine invertebrates. Thus, nitrate toxicity may have its most severe ecotoxicological effect on invertebrate growth and development rates, due to greater effects on larval phases.

Toxicity values for the effects of nitrate on fishes range from 1.1 mg L^{-1} NO_3–N for least observable and no observable effects on eggs to over 1,000 mg L^{-1} NO_3–N for effects on early life history stages. Channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), and mosquitofish (*Gambusia holbrooki*) all exhibited responses to nitrate in laboratory experiments or abnormalities correlated with nitrate concentrations in freshwater habitats. Impacts on eggs, embryos or fry may represent special concerns. Responses may vary with environmental conditions and exposure. For example, increased salinity may buffer the toxic effects of nitrate and nitrite, and longer-term exposures may produce increased tolerance (Knepp and Arkin 1973; Russo *et al*. 1981).

Amphibians have been promoted as sentinel species or species that will provide an early warning of the ecological effects of toxicants. They suit this role because their skin increases uptake rates for contaminants as a byproduct of being adapted for respiration and hydration and because they have complex life histories spanning aquatic and terrestrial habitats. Negative effects of nitrate on amphibians include decreased activity, reduced feeding, disequilibrium, deformities, mortality, and altered timing of metamorphosis. Evidence points to nitrate acting as an endocrine disruptor. Overall, sensitivity to nitrate varies among amphibian species, with 13.6 mg L^{-1} to over 1,000 mg L^{-1} being reported to cause mortality in embryos over 96 hours and 4.9 mg L^{-1} to 65 mg L^{-1} reported to cause other effects in tadpoles. Factors such as water chemistry and the type of nitrogen compound introducing the nitrate may influence toxicity. For example, tadpoles of *Bufo terrestris*, the southern toad, raised at nitrate levels of 30 mg L^{-1} took less time than controls to reach metamorphosis in water prepared by reverse osmosis and about 6 days longer than controls in unfiltered spring water (Edwards *et al*. 2006b). Furthermore, tests using ammonium nitrate may yield results that confound ammonia and nitrate toxicities. In addition, chronic, sublethal effects may not appear within the 96-hour time limit used in many assays. The role of nitrate toxicity in the decline of amphibian populations is confounded by habitat destruction and the impacts of introduced species; however, nitrate toxicity should factor into amphibian conservation.

Toxicity tests using birds and most mammals are lacking, and one study addressed nitrate effects on the reptile, *Alligator mississippiensis*. Preliminary findings indicate that nitrate concentrations between 0.01–0.04 mg L^{-1} NO_3–N appear to affect alligators (Guillette and Edwards 2005). Plasma testosterone levels in both male and female juvenile alligators were negatively correlated with nitrate levels, and estradiol levels in juvenile females were positively correlated with nitrate levels. Toxicity to birds and mammals probably occurs through ingestion of contaminated water.

Herds of cattle were unaffected by exposure to water contaminated with 0–44 mg L^{-1} NO_3–N; were at risk if continually exposed to 133–220 mg L^{-1}; and exhibited losses at 221–660 mg L^{-1}, more losses at 661–880 mg L^{-1}, and heavy losses above 880 mg L^{-1} (http://www.vetmed.ucdavis.edu/vetext/INF-BE_cca/INF-BE_cca01/INF-BE_cca0111.html). In addition, the human health standard for nitrate in drinking water is 10 mg L^{-1} (http://www.dep.state.fl.us/water/drinkingwater/standard.htm).

Average nitrate levels in 92% of 130 Florida springs were reported to be below 2 mg L^{-1}; therefore, they were typically lower than concentrations reported to cause effects on animals (Munch *et al.* 2006). However, nitrate concentrations exceed levels of concern in some springs, and they may reach levels of concern for short periods in other springs, especially after the application of safety factors. Given the uncertainty surrounding estimates of toxicity, nitrate concentrations in Florida's springs do represent a cause for concern.

Summary of Nutrient Effects on Fauna in Springs

In summary, a synthesis of information indicates that nutrients can affect faunal assemblages in aquatic systems. Effects are generated in three primary ways: 1) toxicity, 2) changes to trophic webs, and 3) changes in habitats.

Toxicity from elevated ammonia concentrations and changes to respiratory functioning from elevated nitrate concentrations are well-established facts. In addition, evidence of other detrimental effects from elevated nitrate concentrations is accumulating. Increases in concentrations of nitrate may initially lead to changes in traits of individual organisms. These sublethal effects on individuals could translate to detrimental effects on populations, assemblages or ecosystems, although we lack definitive data on these outcomes. Concentrations of ammonia and nitrate in most Florida springs have not reached toxic levels, but concentrations are of concern in some springs. The ecotoxicological effects of nutrients should factor into management of nutrients in Florida's springs.

Increases in concentrations of nutrients also can change trophic webs and habitats, with outcomes determined by complex interactions among bottom-up and top-down processes. At this time, we lack the data needed to rigorously evaluate the role of nutrients in creating perceived and observed changes in the habitats or trophic webs of Florida springs.

Overall, management of nutrients in spring ecosystems would benefit from diagnostic studies of bottom-up and top-down interactions. These interactions, with their associated complexity and nonlinearity, ultimately determine the level of management needed to protect springs ecosystems. Furthermore, the complex and nonlinear nature of key interactions make it highly likely that management applied at real world, spatiotemporal scales will generate unforeseen and unforeseeable responses or emergent properties, which create a need to manage adaptively.

RESEARCH TO IMPROVE MANAGEMENT OF NUTRIENTS IN SPRINGS

Florida's springs and spring runs are enriched in nitrate due to broad-scale contamination of groundwater supplies. As a result, the potential for eutrophication becomes a major concern. The eutrophication progression scheme suggests that increased nutrient delivery to aquatic systems favors production of fast-growing algae that ultimately outcompete and displace native vascular plants (Duarte 1995).

In some of Florida's spring systems, both the apparent proliferation of nuisance algae and the apparent decline of native macrophytes are consistent with such a progression. However, we have few quantitative data that definitively link these changes to an increase in nitrate delivery, and the eutrophication progression scheme predicts that nonlinear responses in systems may have "decoupled" them from contemporary concentrations of nutrients in the water column. Currently, patterns in the distribution and abundance of plants and algae do correlate with other physical and chemical parameters. However, we know little about their individual or combined effects or the form and strength of potential feedback mechanisms, including those linked to nutrient concentrations. Overall, numerous processes and their interactions probably dictate the structure and function of submersed aquatic vegetation, and changes in these vegetated communities generate effects on faunal assemblages.

In general, nutrients can affect the faunal assemblages in aquatic systems in three primary ways: 1) toxicity, 2) changes to trophic webs, and 3) changes in habitats. Toxicity from elevated concentrations of ammonia and nitrate is not a major concern in most Florida springs at this time. In addition, sublethal effects of nitrate on individuals may not impact populations, assemblages or ecosystems. Regardless, the ecotoxicological effects of nutrients should factor into management of nutrients. Changes in trophic webs and habitats, along with flow-on effects on faunal assemblages, are determined by complex interactions among bottom-up and top-down processes. At this time, we lack data to support rigorous evaluation of the role of nutrients in such changes.

Overall, management of nutrients in spring ecosystems would benefit from compilation of inventories, collection of baseline data, and completion of diagnostic studies focused on bottom-up and top-down interactions. The interactions, complexities and nonlinearities uncovered by these studies should influence the management of spring systems. Furthermore, the likelihood that unpredictable, emergent properties will arise when management is implemented at real world, spatiotemporal scales creates a need to manage adaptively.

Designing, implementing, evaluating and applying research that improves management of Florida's springs and spring-fed systems requires input from multiple stakeholders, including policy makers, managers, the public, and scientists from many disciplines. Initial targets for discussion include:

- inventories and baselines designed to:
  - measure nutrients, oxygen, carbon, light, flow, substrates, grazing, algae, plants, invertebrates, fish, and other key drivers and valued components of spring systems chosen with the best available information and, eventually, with guidance from the results of diagnostic studies

- target systems where effectiveness of management can be assessed (e.g., systems with total maximum daily loads, minimum flows and levels, pollution load reduction goals, surface water improvement plans or basin management action plans)
- sample synoptically or at spatial and temporal scales that support rigorous analyses of interactions (note that these inventories and baselines form the platform for adaptive management)
- balance breadth with depth (e.g., optimize taxonomic detail and replication)

- diagnostic studies that determine assimilation of nutrients by flora as key elements in nutrient budgets for spring systems, including contributions by epiphytes, macroalgae, vascular plants and microphytobenthos or microalgae found in sediments

- diagnostic studies that determine how nutrients and other drivers affect overgrowth, shading and other relationships among periphyton, macroalgae and vascular plants in an effort to determine the need for management actions, including aquatic plant management and restoration of native species

- diagnostic studies designed to:
  - focus on species of algae and plants that are hypothesized or known to play major roles in nutrient assimilation
  - extend beyond nitrogen and phosphorus to include other potentially limiting factors and interactions among limiting factors that can significantly influence the outcomes of management (e.g., micronutrients, iron, oxygen, carbon, light, flow and substrate type)
  - explore relationships and consequences across multiple levels of biological and ecological complexity, including uptake and other physiological responses, growth and reproduction of individual organisms, and ecological changes in populations or assemblages to promote the success of management actions at the system level (note that some of the key, large-scale relationships form the core of an adaptive management approach)
  - elucidate the direction, magnitude and form of limitations (e.g., nonlinear and nonreversible) to identify suitable targets and goals for management actions

- diagnostic studies to identify and elucidate changes in composition and function of faunal assemblages related to changes in habitats in an effort to identify habitats to be protected or restored by management actions

- diagnostic studies to determine palatability of flora to grazers, rates of grazing, and the form and magnitude of predation by primary consumers in an effort to identify flora, grazers and trophic links to be protected or restored by management actions

- diagnostic studies to identify and elucidate the toxic effects of ammonia and nitrate beyond physiological and individual levels of organization (e.g., population and assemblage effects) in an effort to determine threshold levels, safety factors and the need for management actions

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