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Effects of Nutrients on Spring Ecosystems

Robert L. Knight, [1,2] and Sky K. Notestein [1]
[1] Wetland Solutions, Inc.
[2] Department of Environmental Engineering Sciences, University of Florida

Summary

The relationship between nutrients and spring ecosystem structure and function primarily focuses on the state-wide increase in spring nitrate concentrations derived from anthropogenic sources and the concurrent observed visual decline of these ecosystems. However, the apparent correlation between increased nitrate loading and declining aesthetic appearance of spring ecosystems has only anecdotally provided evidence for a causative relationship. Organism-level studies, focused on single species of algae, vascular plants, aquatic macroinvertebrates, and primary and secondary consumers, have produced mixed results that do not currently present a clear or consistent link between nutrient availability and population structure in springs. Firm conclusions linking rising spring nitrogen concentrations and changes to individual components of biotic communities remain illusive, in some measure due to the immense complexity of spring ecosystems and the large number of external forcing functions (energy and matter inputs) that shape their structure and functions.

General systems theory predicts that any significant change in an important external factor (*e.g.*, nitrate nitrogen delivery in ground water feeding a spring) should result in a significant change in the target system (in this case the spring ecosystem), as long as the system of interest is highly adapted and operating at its maximum potential. If this type of cause and effect relationship holds for nutrients and springs, then measurements of whole ecosystem responses over a gradient of nitrate nitrogen concentrations should be an especially productive approach to applied research. Springs data presented and reviewed in this chapter reveal a significant correlation between rising nitrate concentrations and declining ecosystem primary production and photosynthetic efficiency. While this possible cause and effect relationship is based on detailed data from only a limited number of springs and their associated spring runs, so far this relationship has proven consistent.

While an inverse correlation between nitrate concentration and primary productivity appears counter-intuitive based on typical single plant species responses to increasing nutrients, it can be explained based on the subsidy-stress hypothesis that has been supported by a variety of ecosystem and organism-level studies. While low nitrate levels may be optimal for ecosystem productivity in pristine springs, higher nitrate levels appear to have the opposite effect of reducing overall primary productivity. It can be theorized that spring ecosystem responses to nutrient increases integrate the observed complexity of their hundreds of important plants and animals. In their natural, moderate to low nutrient state, springs may be adapted for optimal

efficiency of light utilization and maximum ecosystem metabolism (Maximum Power Theory). Rising nutrient concentrations may result in the competitive advantage of “weedy” algae and plant species that can capture light and spatial resources through higher rates of net productivity but lower gross productivity. Continuing elevated nutrient concentrations in concert with differential grazing pressure, reductions in flow, or loss of other top-down control mechanisms may be linked to observed plant community shifts from adapted submersed aquatic plants with sparse but highly productive periphyton communities to systems dominated by benthic and attached filamentous algae. Data are also presented which link elevated nitrate concentrations with alterations in the reproductive functioning of amphibians and fish. These complex interactions between increased nutrients and algae, decreased dissolved oxygen due to lowered primary productivity, and altered grazer populations illustrate the importance of employing an ecosystem perspective when examining the multiple factors potentially affecting springs.

It is the conclusion of this review that an ecosystem approach is essential for future research efforts to provide a greater understanding of the relative interactions between the myriad physical, chemical, and biological fluxes present in springs and their normal responses to rising nutrient levels. In summary these recommended applied research activities might include the following:

- Detailed baseline investigations of the “normal” or “existing” ecosystem-level structure and response of a broad sample of first and second magnitude springs to key forcing functions, including sunlight, flow, and nutrient levels. These studies need to be fairly long-term (multiple years of repeated measures) and conducted over a representative sample of springs with a range of forcing functions including discharge rates, groundwater chemistry, nutrient concentrations, and recreational intensities;
- Multivariate statistical analysis of these baseline data to look for significant and repeatable patterns between independent and dependent variables such as nutrient concentrations and photosynthetic efficiency;
- Design of *in situ* complex (multi-species) mesocosm studies to allow replication of spring plant community responses to a range of nutrient conditions under realistic and relevant spring environmental conditions;
- Whole-spring manipulation studies (if necessary) to test the effects of possible management techniques such as controlling levels of springshed nutrient loading, human recreational activities, alternative control methods for invasive species, and estimation of optimal consumer carrying capacities (*e.g.*, manatee density).

There exists a wide-spread recognition of the environmental and economic importance of artesian springs in Florida. A generous commitment of public dollars and a focused and logical research agenda will be critical to pulling these precious but threatened natural resources back from their current declining path.

ECOSYSTEM STRUCTURE

Primary Producers

The paradigm of eutrophication is that nutrient enrichment (nitrogen and or phosphorus primarily) will result in an increase in biomass in primary producers often with negative ecological and economic consequences (Cloern 2001, Nixon 1995, Rabalais 2002). Although this view assumes that nutrients are the primary limiting factor for algae and vascular plants, and not some other factor such as light or grazing, observations from a variety of aquatic systems broadly support this assertion. For marine and estuarine systems, nitrogen is typically identified as the nutrient limiting primary production. The effects of nitrogen on marine and estuarine systems, the pathways for nitrogen transport between land and aquatic habitats, and the positive correlation between nitrogen and primary production and often secondary production (*i.e.* fishery yields) have been widely reviewed (*e.g.* Hecky and Kilham 1988, Howarth 1988, Rabalais 2002). However in freshwater systems, phosphorus is the nutrient more commonly implicated as limiting for primary production and has been highly correlated with phytoplankton and fisheries biomass, especially in lakes (Bachmann *et al.* 1996, Canfield 1983, Hoyer and Canfield 1991).

Among streams and rivers, a consistent limiting nutrient for primary production does not appear to be the case (*e.g.* Tank and Dodds 2003) likely because of distinct regional differences and because the abundance and rates of primary producers represent an integration of physical, chemical, and biological conditions. Examples of the variability of stream primary producers include a cross-system analysis of temperate streams in which Dodds *et al.* (1997) established that both total nitrogen and total phosphorus in the water column were significantly related to benthic algal biomass, while Francoeur's (2001) meta-analysis of 237 nutrient enrichment studies in temperate streams found that 16% indicated a nitrogen (N) response, 18% indicated a phosphorus (P) response, 23% required N and P be added together for a response, and 43% had no response to N or P.

It is important to remember that spring runs and other lotic (flowing water) ecosystems can transform inorganic nutrients into organic material, thus preventing or reducing the downstream export of inorganic nutrients. However, these lotic ecosystems can become saturated with respect to relatively conservative inorganic nutrients such as phosphorus and recalcitrant forms of organic nitrogen and in some instances may not be able to further assimilate inorganic forms through uptake, microbial utilization, and denitrification. It is unclear at what concentration or loading rate saturation can occur, but likely is dependent on whether it is an acute or chronic nutrient load and on the magnitude of other limiting chemical constituents or forcing functions such as light availability. Once saturation occurs, nitrogen export to downstream ecosystems will increase proportionally to nitrogen loading (Bernot and Dodds 2005). Whether saturation of nitrogen or phosphorus has occurred in Florida spring ecosystems remains uncertain in most cases and is obviously a function of individual spring biogeochemistry. In at least one case within portions of the Wekiva River system, which is fed by Wekiwa and Rock Springs (WSI 2007a) there is evidence that phosphorus saturation may have occurred (as evidenced by zero or negative assimilation rates).

It is important to remember that thresholds exist for the capacity of all aquatic ecosystems to productively assimilate increased nutrient loads and that if these thresholds are exceeded, harmful ecological consequences may occur. Our use of this concept has origin in the paper by E. P. Odum *et al*. (1979), titled: *Perturbation Theory and the Subsidy-Stress Gradient*, in which the model of “too much of a good thing” is examined in the context of ecosystem response to perturbation (Figure 1). An example of a subsidy-stress gradient is the productivity of hardwood forested swamps in response to flooding, in which forested swamp productivity was observed to increase under seasonal flooding conditions but to decline under permanent flooding (Conner and Day 1992). With regard to spring ecosystems, it is likely that a variety of physical, biological, and chemical inputs may operate individually or simultaneously within subsidy-stress gradients. For instance, stream velocity can replace nutrients and remove waste products at low velocities, but is capable of scouring at high velocities, and grazing of primary producers may promote algal productivity at moderate levels but a reduction in primary production at higher levels. Knight (1980) examined and described several examples of the subsidy-stress gradient in research at Silver Springs, including the effects of herbivorous snails, carnivorous fish, and a trace metal (elemental cadmium) on primary productivity in stream mesocosms.

It is hypothesized in this chapter that nutrients also function along a subsidy-stress gradient in aquatic ecosystems. At extremely low concentrations overall system productivity of plant and animal life is low; as nutrient concentrations increase, so too does primary and secondary productivity, but at some point, productivity may decrease. Within aquatic ecosystems, nutrient subsidy-stress gradients are likely to have unique performance curves for primary producers by functional group, *i.e.* algae vs. vascular plant, or even by species within these groups. Considering this, when monitoring ecosystems for impairment or the effects of nutrient gradients, whole ecosystem measures may produce diametrically opposite results than single-species studies and will be more informative for documenting overall ecosystem impairment than measures of individual functional groups.

Algae

Pseudo-phytoplankton **–** The development of plankton in lotic ecosystems is largely prevented due to inadequate water resident times (Hynes 1970). A more accurate term is pseudo-phytoplankton, which is a mix of sloughed material from periphytic primary producers. Once entrained in the water column, this material will either be consumed, settle out or if it remains in suspension continue to grow through cellular division. The factors which affect pseudo-phytoplankton include the abundance of periphytic primary producers, light, nutrients, temperature, and velocity. In flowing water systems, stream discharge or velocity will influence their rate of delivery downstream. Given that phytoplankton populations can double in as little as one to two days, the relationship between stream flow and phytoplankton abundance is likely to be inversely correlated (Allan 1995). However, should declines in spring discharge rates occur to the point that water residence time is greater than about three days, the development of true phytoplankton communities could occur in the spring run, as was suggested by Frazer *et al.* (2002) in their examination of limiting nutrients in several spring ecosystems using traditional phytoplankton bioassay methods.

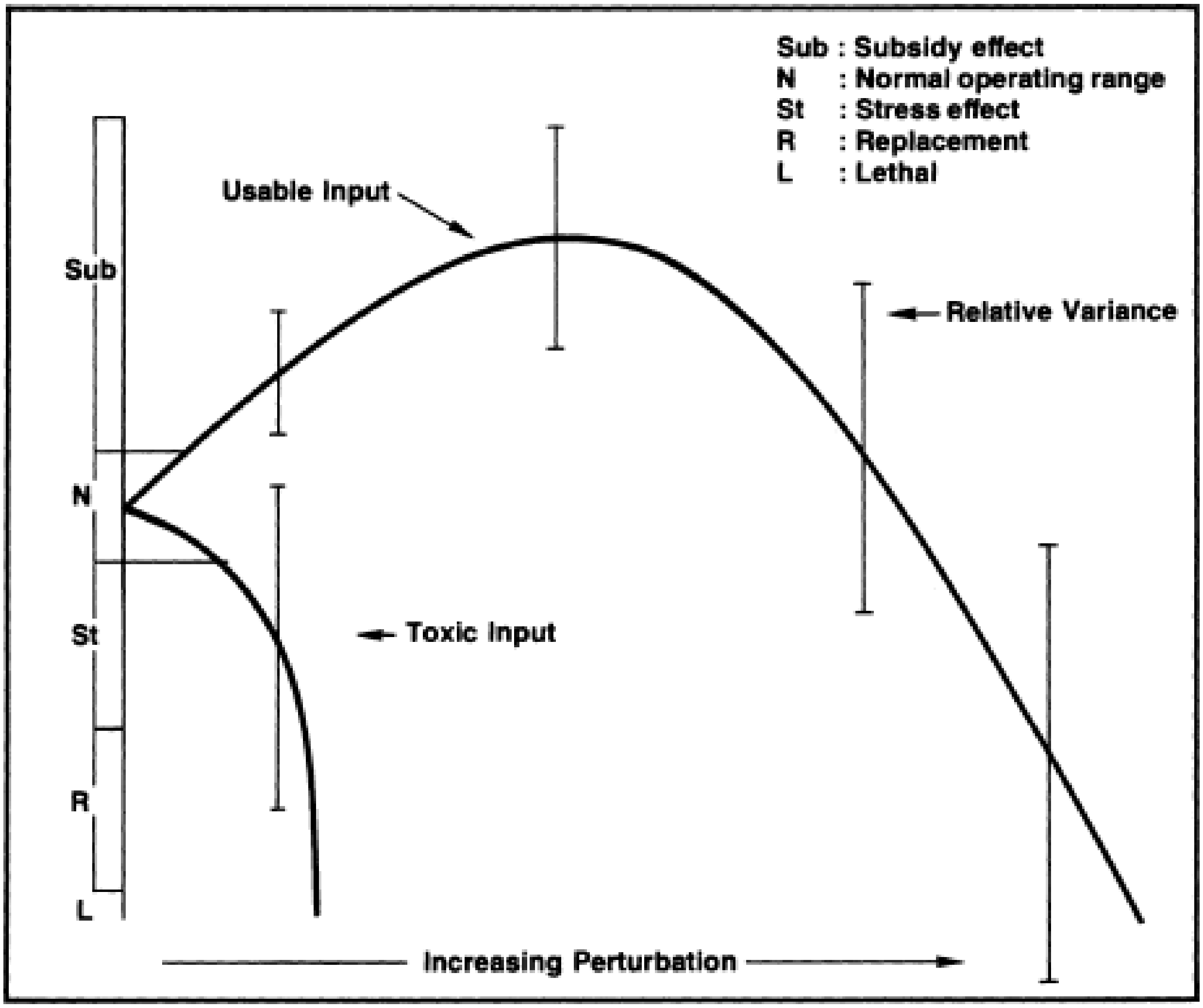

Figure 1 – *A hypothetical example of two types of inputs and their resulting ecosystem perturbations due to increasing input levels. Nutrients and nitrate in particular could be viewed as an example of a usable input (top curve), which have a subsidy effect on ecosystem productivity to a point beyond which stress is incurred (from E. P. Odum et al. 1979).*

A limited number of studies are available which have examined pseudo-phytoplankton collected from spring ecosystems and their response to manipulations of ambient nutrient levels. Frazer *et al.* (2002) conducted nutrient addition bioassay of pseudo-phytoplankton collected from several locations of the Chassahowitzka, Crystal, Homosassa, and Weeki Wachee Rivers. These samples were allowed to grow under near-optimal light and temperature conditions under a variety of nutrient treatments. These treatments included a control (without any additional nutrient additions), nitrogen (N) addition (0.4 mg NO_3-N/L), phosphorus (P) addition (0.04 mg PO_4/L), silica (Si) addition (0.4 mg Si/L), N+P addition (0.4 mgNO_3-N/L + 0.04 mgPO_4/L), and N+P+Si (0.4 mg NO_3-N/L + 0.04 mg PO_4/L + 0.4 mg Si/L). For those pseudo-phytoplankton samples collected at the upper spring run, bioassays with additional nutrients did not produce an increase in growth compared to controls with ambient nutrient levels in 61% of the experiments, suggesting that the majority of locations were not nutrient limited. In the balance of experiments, 19% were phosphorus limited, 10% were nitrogen limited and 10% were co-limited by nitrogen and phosphorus. Given that all four of these spring ecosystems are supplied by waters with elevated nitrate concentrations relative to historic values, it is not surprising that nutrients did not appear to be limiting in majority of the experiments. This observation was supported by the average (n=8) ambient concentrations of nitrate where pseudo-phytoplankton were collected: Chassahowitzka was 0.39 mg/L, Crystal was 0.03 mg/L, Homosassa was 0.42 mg/L, and Weeki Wachee was 0.53 mg NO_3-N/L. Crystal River, the spring ecosystem with the lowest ambient levels of nitrate, had a pseudo-phytoplankton community which was most responsive to nitrate additions as it exhibited nitrogen limitation in 75% of the experiments. The data from the Frazer *et al.* (2002) study suggest that when spring run nitrate concentrations exceed approximately 0.4 mg/L, nitrogen limitation is unlikely.

A second springs' study utilizing a pseudo-phytoplankton bioassay from material collected from Kings Bay, which contains multiple spring vents, was completed by Saindon (2005). This researcher compared pseudo-phytoplankton growth rates for experimentally manipulated nitrate and soluble reactive phosphorus concentrations at two sites, which had ambient nitrate concentrations of 0.083 and 0.039 mg N/L and ambient soluble reactive phosphorus concentrations of 0.011 and 0.006 mg P/L. Saindon (2005) observed significant differences between sites in the relative growth rates of pseudo-phytoplankton, and that maximum biomass was influenced by both nutrient ratios and nutrient concentrations, but that growth rates "did not determine the maximum achieved biomass, just how long it took to reach that biomass". An important conclusion from Saindon's (2005) work was that maximum pseudo-phytoplankton biomass could be maintained at very low ambient nutrient concentrations when the N:P (by weight) ratios were either very low (N:P < 3) or very high (N:P > 27) and that when the N:P (by weight) ratios were between 3 and 27, maximum pseudo-phytoplankton biomass appeared to increase with increasing nutrient availability. By way of comparison, historical N:P ratios for spring waters were reported by Odum (1957a) and Odum *et al.* (1953) and were generally below 7:1 (by weight) but not always, suggesting that historically most springs had the potential for nitrogen limitation. By way of comparison, N:P ratios can be reported by weight or by atomic (*i.e.* molar) ratio, the commonly reported Redfield ratio for N:P of 16:1 is expressed in atomic units and represents an N:P ratio of approximately 7:1 by weight (Redfield *et al.* 1963, Duarte 1992).

Filamentous and Benthic Macroalgae - These categorical terms include macroalgae commonly viewed as nuisance species and associated with declines in aesthetics of many springs such as *Lyngbya* or *Vaucheria*. It is important to note that *Lyngbya* or *Vaucheria* are indigenous genera of macroalgae and that Florida spring ecosystems have (or had) a natural diversity of macroalgae (Whitford 1956). The mere presence of these species is not an indicator of decline, but an overabundance or monoculture of macroalgae especially in conjunction with a loss of native submersed vascular species may be cause for concern.

A comprehensive synthesis report on macroalgae inhabiting Florida springs in relation to nutrients was recently completed by Stevenson *et al.* (2007). The macroalgae study included sampling from 29 different Florida springs and multiple laboratory approaches to elaborate on prior related work (Stevenson *et al.* 2004). Results for the Stevenson *et al.* (2007) report suggest that macroalgal taxa may not respond to nutrients uniformly, as the abundance of *Vaucheria* spp. in spring ecosystems was positively correlated to nitrogen and phosphorus concentrations with a clear threshold increase at 0.59 mg total nitrogen/L; however, the abundance of *Lyngbya wollei* was not correlated to either nitrogen or phosphorus water concentrations in surveyed springs, although it was related to sediment phosphorus concentrations and indices of human activities within 1,000 m of the sampling site. Several possible explanations for the lack of better correlations between ambient nutrient concentrations and macroalgae abundance are that other factors limit and influence the standing crop. Principally, instantaneous field measures are incapable of incorporating prior environmental conditions which have caused the current state. Other important forcing functions affecting macroalgae were not measured as well, for instance in spring runs with strong flow and heavy recreational use, macrophytes can easily be dislodged and swept downstream preventing the accumulation of substantial coverage or biomass. Another alternative is that some other factor, such as light, is primarily limiting, and hence nutrient supply can only be secondarily limiting. The phenomenon in which light supply appears to be the primarily limiting factor was observed by Canfield and Hoyer (1988) as well as Frazer *et al.* (2006) in their studies on the abundance of macrophytes in spring runs. The role that grazing plays on determining the standing crop of filamentous algae, particularly *Lyngbya sp.* is largely unknown as well.

Cowell and Dawes (2004) determined that increased populations of *Lyngbya* in the Rainbow River (nitrate > 1.0 mg/L) may be due to increased nitrate concentrations. *Lyngbya wollei* growth was stimulated by nitrate additions in the laboratory although no biomass increase was observed above about 1 mg/L of nitrate nitrogen. They concluded that based on their lab studies nitrate nitrogen concentrations would need to be reduced to below 0.3 mg/L in order to significantly reduce *Lyngbya* biomass. This research confirms that increased nitrate levels can increase benthic algal biomass, at least in the short term.

Stevenson *et al.* (2007) also conducted *in situ* bioassays of *Vaucheria* and *Lyngbya* in three different microcosm designs each with a matrix of nitrogen and phosphorus treatments maintained under relatively optimal growth conditions. From these experiments it appears nutrient concentrations affect the growth rate and ultimately the abundance of macroalgae as growth occurred in most nutrient treatments, even at very low nutrient concentrations, yet fastest growth rates occurred in high nutrient concentrations and were approximately two or more times greater than under low nutrient concentrations. Based on the bioassay experiments, it was preliminarily estimated that to initiate a reduction in the abundance of *Vaucheria* in Florida springs total nitrogen and total phosphorus concentrations would have to be less than 0.59 and 0.026 mg/L, respectively; while *Lyngbya* reductions would require total nitrogen and total phosphorus concentrations lower than 0.25 and 0.033 mg/L, respectively (Stevenson *et al.* 2007). These conclusions suggest that the response of macroalgae to nutrients is complex, that both nutrient ratio and concentration are important, and that growth rate (or grazing rate) can have a large impact on ultimate biomass (*i.e.* standing crop).

***Periphyton and Epiphytes* –** These are terms for another category of primary producers which are commonly thought to respond to nutrient enrichment of spring water through an increase in their coverage, biomass, and/or occurrence of weedy species. It is possible that these weedy species may escape top-down controls by grazers, reduce the light energy available to their vascular plant hosts, and ultimately have the potential to modify the spring ecosystem plant community. One study to directly examine nutrient effects on periphyton abundance in a spring fed system was completed by Notestein *et al.* (2003) as an *in situ* bioassay in the Chassahowitzka River, a spring run in coastal Citrus County, Florida. This study added nitrate, phosphate, and a combination of nitrate and phosphate to the upstream end of flow-through tubes containing glass microscope slides in the spring run. This experimental design allowed the amount of periphyton colonizing glass microscope slides to be compared between nutrient treatments and with a control treatment over a one month period. Increased epiphyte abundance (as measured by chlorophyll per unit area) was observed following the addition of phosphorus, as well as phosphorus in combination with nitrogen, when compared to the control (no nutrient addition treatment). The results suggested that phosphorus was the primary nutrient limiting periphyton abundance in the upper spring fed portion of this system, however nitrate additions supported additional periphyton growth over the control treatment and therefore nitrogen appeared to be secondarily limiting (Notestein *et al.* 2003). The Chassahowitzka spring system had elevated nitrate concentrations, approximately 0.45 mg/L at the time of the study, so the finding that phosphorus (approximately 0.016 mg/L) was limiting is consistent with the elevated N:P nutrient ratios (28:1 by weight) observed during the study period. The observation that additional nitrate could stimulate periphyton abundance, even though nitrogen was not the primary limiting nutrient, suggests that a nutrient need not be absolutely limiting for it to have a biological effect in a spring ecosystem.

In stream systems outside of Florida, there have been numerous publications on the relations between periphyton abundance and nutrients (*e.g.* Dodds *et al.* 1997, Francoeur 2001). Interestingly, there do not appear to be consistent trends or factors which control periphyton abundance in stream systems, which may be the case with Florida's spring systems as well. As the following interstate examples show, increased nutrient concentrations can influence periphyton abundance, but the responses to nutrient additions are frequently variable. Nitrogen alone may stimulate periphyton abundance (Crawford 1979, Mosisch *et al*. 1999) but often only when accompanied by increased light availability from removing riparian shading or adding artificial light (Busch 1978, Gregory 1980, Hill and Harvey 1990). The addition of phosphorus, both on its own and in combination with nitrogen, has been shown to increase periphyton abundance (Elwood *et al*. 1981, Peterson *et al.* 1993, Bothwell 1985). Other essential elements including carbon supplied by organic sources such as sucrose (Warren *et al*. 1964) or carbon supplied via inorganic forms such as carbon dioxide and bicarbonate (Dickman 1973 and Crawford 1979) have also been shown to increase periphyton abundance. Another factor that interacts with periphyton abundance is grazing (Steinman *et al.* 1991, Hill *et al.* 1992). In a recent study, grazers were shown to control periphyton abundance at a variety of ambient nutrient levels, while in the absence of grazers there was a significant positive correlation between periphyton and nutrients (nitrogen and phosphorus simultaneously added, Chen *et al.* 2007). These examples show that periphyton abundance in streams can be influenced by nutrients and other ecological controls originating from the bottom up (*i.e.* light availability) or top down (*i.e.* grazers controls) and suggest that the factors controlling periphyton abundance in Florida's spring systems needs further research, likely at the level of *in situ* complex mesocosms that can colonize with realistic populations and diversity of algae and invertebrates.

Vascular Plants

Studies of two Florida springs systems, the Chassahowitzka (Notestein 2001) and the Ichetucknee (Kurz *et al.* 2004), involved measuring the abundance of submersed vascular macrophytes and the concentration of water column nutrients. Chassahowitzka had ambient water column nitrate concentrations of approximately 0.45 mg/L and phosphate concentrations of 0.016 mg/L; while Ichetucknee had approximately 0.55 mg/L nitrate and 0.04 mg/L phosphate in the water column. These measurements are elevated compared to historic concentrations. Neither study showed a correlation between surface water nutrient concentrations and the growth rate and/or abundance of submersed aquatic macrophytes, which appears to be consistent with research from other non-spring fed stream systems. For example, Peltier and Welch (1969) experimented with *Potamogeton pectinatus*, and observed that nitrate concentrations above 0.44 mg/L and phosphate concentrations above 0.03 mg/L did not significantly increase growth, nor was there a correlation between the field growth of *P. pectinatu*s or *Najas sp*. when inorganic nitrogen concentrations were 0.2 to 0.3 mg/L and phosphate concentrations were 0.03 to 0.08 mg/L. Mulligan and Baranowski (1969), studied several macrophytes including *Myriophyllum spicatum* under greenhouse conditions, and found that optimum growth could be achieved with 0.1 mg/L nitrogen and 0.02 mg/L phosphorus, values which are equal to or greater than historic concentrations for many Florida springs. In a study of 17 Florida streams, many of them spring fed, Canfield and Hoyer (1988) concluded that at concentrations above 0.3 mg/L nitrogen and 0.03 mg/L phosphorus, the potential for nutrient limitation of submersed aquatic vegetation was unlikely. Therefore, in many of the spring ecosystems with current average surface water nitrate concentrations exceeding these values, particularly for nitrate, the point of nutrient limitation may have already been passed. Because rooted aquatic macrophytes are known to derive much of their nutrient supply from the interstitial pore water of the substrate (Bristowe and Whitcombe 1971, Carignan and Kalff 1980, Chambers *et al*. 1989) and that these pore-water nutrient concentrations are often much higher than the overlying waters (Stevenson *et al.* 2007), it is likely that vascular macrophyte nutrient demand can be met from sediments and helps explain the lack of correlation between water column nutrient concentrations and submersed vascular macrophytes.

Much concern over the apparent reduction from the historical abundance of submersed vascular plants in spring ecosystems exists, and is warranted due to the important habitat and the food web contributions these primary producers make (see Chapters 1 and 4). As noted in the section on periphyton above, the potential causes for declines in submersed vascular plant abundance could include resource competition from periphyton or filamentous and benthic algae, overgrazing by consumers, physical disturbance from recreation, and/or disease; it is likely that causative mechanisms responsible for declines in submersed vascular plant abundance are system specific and may not be universal. An additional explanation of reduced submersed vascular plant abundance, which could be related to elevated nitrate concentrations, was provided by Boedeltje *et al*. (2005) who reported that high water column concentrations of nitrates (> 7 mg/L, a value higher than that observed in 98% of Florida springs) can significantly reduce the growth of ammonia-preferring rooted submersed species such as *Potamogeton alpinus.* Boedeltje et al. (2005) hypothesized that nitrate dominated nitrogen assimilation may lead to strong metabolic disturbances affecting the levels of organic anions within those species adapted to ammonia uptake by the roots (such as *P. alpinus*). This may explain the observed reduction in growth under high water column nitrate concentrations, although further experiments are needed to assess potential negative effects on other species.

Primary Consumers

Invertebrates

Nitrate pollution in Florida springs has been implicated as a possible reason for the apparent decline in populations of the Florida apple snail (*Pomacea paludosa*). To explore this hypothesis, Corrao *et al.* (2006) correlated snail density with nitrate concentration measurements in six Florida springs and conducted laboratory studies to examine short-term acute impacts of nitrate on adult and juvenile apples snails. They observed no correlation between apple snail densities and ambient nitrate concentrations. LC_{50} levels (the concentration at which 50% mortality occurred) could not be determined despite nitrate concentrations >500 mg/L (Corrao *et al.* 2006). Although juvenile apple snail growth was affected in two trials with nitrate concentrations of 504 and 622 mg/L, respectively, the EC_{50} (the effective concentration of a compound where 50% of its maximal effect is observed) for these apple snails generally exhibited little to no response to these nitrate concentrations which are orders of magnitude greater than those found in Florida springs. The authors suggested that perhaps other factors, such as habitat structural changes due to non-indigenous plant invasions, might explain the observed declines in apple snail abundance (Corrao *et al.* 2006).

Studies on the effects of nitrate on invertebrates outside of Florida vary considerably, with changes in survival of caddisfly (*Hydropsyche occidentalis*) demonstrated at concentrations starting around 2.2 mg/L (Camargo and Ward 1995), while juvenile marine shrimp (*Penaeus monodon*) tolerate nitrate concentrations > 2,000 mg/L (Tsai and Chen 2002), and no mortalities were observed in juvenile Australian crayfish (*Cherax quadricarinatus*) exposed to nitrate concentrations up to 1,000 mg/L (Meade and Watts 1995) suggesting that the impact of nitrate must be assessed on a case by case basis.

Vertebrates

Studies examining the effects of nitrate on the reproduction and development of two vertebrate species found in Florida spring ecosystems, have been made for the Southern toad (*Bufo terrestris*) by Edwards *et al.* (2006) and the Eastern mosquitofish (*Gambusia holbrooki*) by Edwards and Guillette (2007). These researchers reported that nitrate concentrations in the range of 4 to 30 mg/L had the ability to alter endocrine physiology of these amphibians and fish. In laboratory experiments utilizing reverse osmosis water enriched with nitrate, Southern toad tadpoles metamorphosed earlier as nitrate concentration increased while in experiments utilizing spring water, tadpoles reared in high nitrate (30 mg/L) delayed metamorphosis, suggesting that water source was a factor besides nitrate concentration affecting *Bufo* development (Edwards *et al.* 2006). In their study of adult male mosquito fish collected from springs with varying nitrate concentrations, Edwards and Guillette (2007) observed that nitrate concentration (up to 5 mg/L) were significantly correlated with decreased total sperm counts, although other characteristics such as pH and temperature also affected physiological condition of the mosquito fish, again suggesting that nitrate alone may not have been responsible.

Secondary Consumers

Fish

Most fish utilizing spring ecosystems fall in the category of secondary and or higher level consumers. Walsh and Williams (2003) examined fish and mussel species diversity in sixteen Florida springs and spring runs. For the purposes of this review, their data were compared to ambient nitrate nitrogen concentrations reported from fifteen of those springs. Although there were trends for lower fish and mussel species numbers with higher nitrate levels, they were not significant. Fish species diversity appears to be relatively constant at Silver Springs, as evidenced by the following: Hubbs and Allen (1943) reported thirty-five species in the 1940s, Walsh and Williams (2003) collected twenty-nine species in 2002, and Munch *et al.* (2006) observed thirty-three species in 2004-05. Although the number of fish species doesn't appear to have declined in correlation with increased nitrate, the biomass of fishes in Silver Springs has declined precipitously over the past 50 years (estimated 96% reduction since the 1950's Odum study). The observed decline in fish biomass at Silver Springs (primarily channel catfish and striped mullet) may be hypothesized to be indirectly affected by elevated nitrate concentrations and their apparent contribution to plant community changes and resulting lower overall ecosystem net productivity during this 50-year period. However, at the trophic level of consumers such as herbivorous and omnivorous fish, the list of possible environmental stressors is lengthy, including limited access by riverine and marine species due to the construction of the Rodman Reservoir and dam in 1968 (Munch *et al.* 2006). This discussion illustrates the need to consider the complexity of spring ecosystems when designing experimental studies and drawing conclusions from incomplete data sources.

Higher-Level Consumers

Ecosystem level changes that have the potential to affect higher level consumers as a result of nitrate enrichment are largely hypothetical. If elevated nitrate concentrations cause qualitative or quantitative changes in primary producers, these could be reflected in quantitative changes in higher trophic levels including fish (catfish, gar, sunfish, bass, and pickerel), reptiles (alligators, softshell and snapping turtles), birds, and mammals (otters and manatees). Another potential impact to higher level consumers could be nitrate toxicity resulting in methemoglobinemia or "blue-baby" syndrome. This syndrome is known to affect mammals; hence there may be a reasonable risk of impacting manatees which are known to drink spring water. A novel hypothesis has been presented to explain the apparently high number of perinatal deaths (*i.e.* stillborn manatee calves) which account for 22% percent of all manatee deaths between 1992 and 2000 (USFWS 2001). Dr. W.T. Haller (UF professor, personal communication 2004) has suggested that if pregnant manatee feed on hydrilla which has elevated tissue concentrations of nitrate or drink nitrate rich spring water their developing *in-vitro* calves may be susceptible to methemoglobinemia. This hypothesis warrants testing given the potential of impacting a threatened species due to nitrate contamination of ground water and spring ecosystems in Florida.

Humans

A number of springs are utilized as a source for commercially bottled water (*e.g.* Gainer Springs in Bay County, Ginnie Springs in Alachua County, and Silver Springs in Marion County (Scott *et al.* 2002)). Given the widespread occurrence and generally increasing magnitude of nitrate contamination in Florida's springs, there may be the potential of spring water utilized for human consumption to approach or even exceed current USEPA drinking water standards of 10 mg/L. The USEPA (2007) has good information concerning the effects of nitrate on human health (*e.g.* http://www.ead.anl.gov/pub/doc/nitrate-ite.pdf, accessed December 2007). According to this USEPA source, nitrate is a normal component of the human diet, with the average daily intake from all sources estimated at 75 milligrams. In healthy adults, about 5% is converted to nitrite by bacteria in saliva and additional bacterial conversion of nitrate takes place inside the alimentary tract and the stomach. A pH of the gastric fluid greater than five promotes the growth of nitrate-reducing bacteria and increases the conversion of nitrate to nitrite. This is especially a concern for infants, whose gastrointestinal systems normally have a higher pH than those of adults. Nitrites and the compounds they can form with proteins (*e.g.* nitrosamines), have been implicated in causing stomach cancer, but evidence remains inconclusive. Although nitrates alone are relatively nontoxic, their conversion to nitrite in the human digestive system can in turn react with hemoglobin in the blood and creating methemoglobin, a form of the protein hemoglobin that is unable to transport oxygen. This results in a decreased capacity of the blood to transport oxygen causing a condition known as methemoglobinemia. Although some amount of methemoglobin in blood is common, levels of 10% can cause the skin and lips to take on a bluish tinge (cyanosis), levels above 25% can cause weakness and a rapid pulse, and levels above 50 to 60% can cause loss of consciousness and death. It appears that infants are much more sensitive than adults to nitrates/nitrites, as essentially all deaths from nitrate/nitrite poisoning have occurred in infants; however adults are susceptible to long-term exposure to lower levels of nitrates and nitrites which can cause diuresis (an increase in the amount of urine, and starchy deposits and hemorrhaging of the spleen). Given the potential for human health impacts, the USEPA has designated a drinking water standard of 10 mg/L for nitrate and 1 mg/L for nitrite. At this time, only a minority (< 2%) of Florida springs currently have nitrate concentrations greater than 10 mg/L.

ECOSYSTEM FUNCTION

Community Metabolism

We define the term community metabolism to include the characterization of the primary producers (autotrophs) and consumers (primary, secondary, etc. heterotrophs and decomposers) which collectively comprise the living component of spring ecosystems (see Chapter 1). Because primary producers generate oxygen through photosynthesis and both producers and consumers use oxygen through respiration, the measurement of oxygen (or carbon dioxide) dissolved in the water can serve as a proxy for community metabolism. In addition, the ability to characterize changes in oxygen per unit area and per unit time allow rates of change in metabolism to be estimated further strengthening this techniques' ability to document ecosystem responses. An example of the daily cycle in dissolved oxygen (DO) is shown in Figure 2, which shows average daily DO change at Silver Springs, Florida over three different time intervals separated by about 50 years.

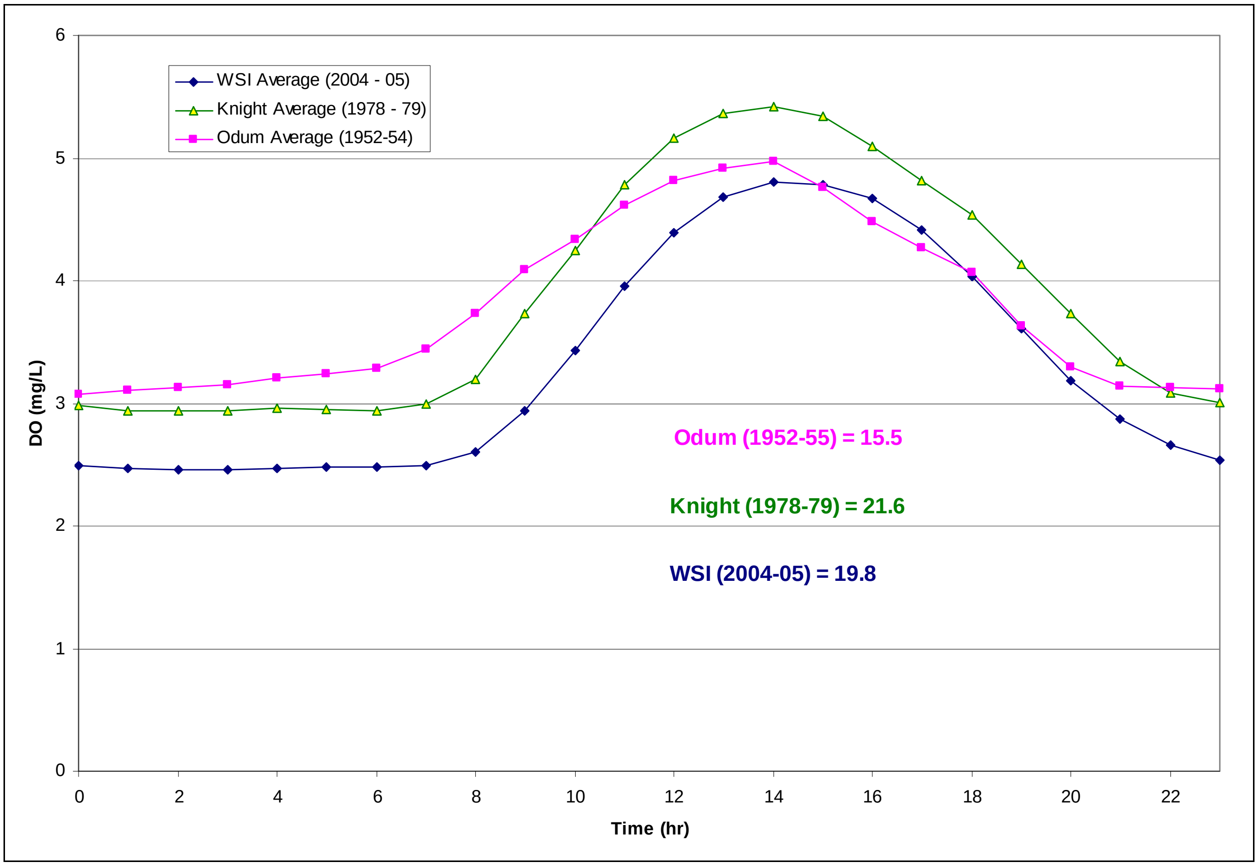

Figure 2 – *Comparison of Silver Springs average diurnal oxygen curves at the 1,200-m downstream station with the resultant estimated gross primary productivity in units of ppm-hrs. Higher total GPP estimates for Knight (1978-79) and WSI (2004-05) are a result of increased wetted area in Silver Springs Run above the 1,200-m station due to the addition of the Back Channel Extension in the late 1970s. Estimated GPP rates corrected for the total aquatic area declined from about 15.7 to 15.6 g $O_2/m^2/d$ in the 1950s and the late 1970s, to about 11.2 g $O_2/m^2/d$ during 2004/2005 (from Munch et al. 2006).*

Gross and Net Productivity

Odum (1957a) measured nitrate and gross primary productivity (GPP) in eleven Florida springs in 1955, which reveal a weak but negative relationship between nitrate concentration and GPP (R^2 = 0.14). The recent findings of lower GPP in Silver Springs at higher nitrate nitrogen concentrations are consistent with the patterns revealed by Odum's earlier research (Table 1). A more recent study of community metabolism has been completed as part of the Wekiva River Pollutant Load Reduction Goal evaluation conducted by the St. Johns River Water Management District, in which spring runs from the Wekiva River System with high nitrate levels were compared to spring runs with minimal nitrate levels (WSI 2007a). The Wekiva River and Rock Springs Run were found to have higher nitrate nitrogen concentrations (averaging 0.69 and 0.84

mg/L respectively) compared to the reference streams located in the Ocala National Forest (Juniper Creek – 0.05 mg/L and Alexander Springs Creek – 0.02 mg/L). Within the spring runs, the upstream nitrate concentrations in the Wekiva River and in Rock Springs were significantly higher than downstream concentrations (Wekiva – 1.1 vs. 0.36 mg/L; Rock Springs Run – 1.1 vs. 0.55 mg/L, respectively). Gross primary productivity and PAR efficiency were found to be significantly lower in all of these spring-fed streams with higher nitrate nitrogen concentrations (Figure 3 and Table 2, WSI 2007a). This trend is consistent with the observations made at Silver Springs during the fifty-year retrospective study described by Munch *et al.* (2006).

Table 1. A comparison of gross primary productivity (GPP) over a fifty-year time period for Silver Springs (from Munch et al. 2006).

Odum (1957b)		**Knight (1980)**		**Munch *et al.* (2006)**	
Date	**GPP ($gO_2/m^2/d$)**	**Date**	**GPP ($gO_2/m^2/d$)**	**Date**	**GPP ($gO_2/m^2/d$)**
2/19/1953	12.4	8/31/1978	19.3	Feb-04	8.2
3/7/1953	14.0	10/5/1978	13.6	Mar-04	11.4
3/25/1953	17.5	12/13/1978	7.8	Apr-04	13.2
1/7/1954	10.1	3/7/1979	10.7	May-04	13.9
5/23/1954	24.4	4/15/1979	16.8	Jun-04	12.7
7/12/1955	12.1	5/16/1979	23.4	Jul-04	13.6
8/11/1955	19.7	6/19/1979	20.7	Aug-04	12.3
		7/17/1979	11.2	Sep-04	10.9
		8/15/1979	17.1	Oct-04	11.7
				Nov-04	9.8
				Dec-04	8.5
				Jan-05	8.6
				Feb-05	11.1
				Mar-05	10.8
Average	15.7		15.6		11.2

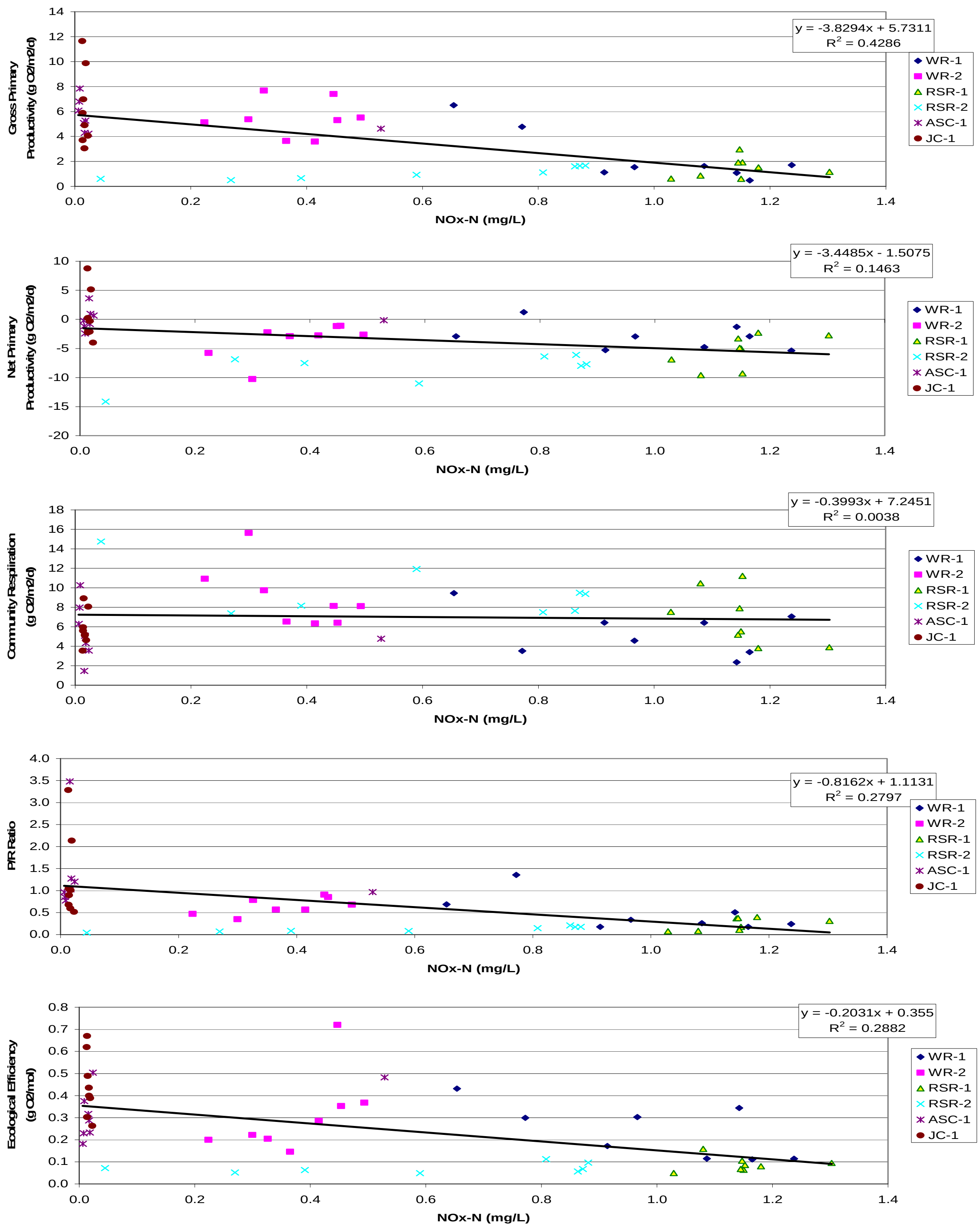

***Figure 3** – The relationship between nitrate and community metabolism parameters from six spring run segments, ASC is Alexander Springs Creek, JC is Juniper Creek, RSR is Rock Springs Run (1 is upstream, 2 is downstream), WR is Wekiva River (1 is upstream, 2 is downstream). Eight sampling events for each spring segment (from WSI 2007a).*

Table 2. Summary comparison of ecological indices between the spring run segments from Wekiwa Springs and Rock Springs and the reference spring runs from, Alexander Springs and Juniper Springs (from WSI 2007a).

Parameter	**Units**	**Wekiwa Springs**			**Rock Springs**			**Reference Springs**		
		SEG 1	**SEG 2**	**Average**	**SEG 1**	**SEG 2**	**Average**	**Alexander Springs**	**Juniper Springs**	**Average**
Dissolved Oxygen	mg/L	1.93	6.81	4.37	5.67	6.40	6.03	5.54	6.81	6.17
pH	s.u.	7.47	7.71	7.59	7.84	7.67	7.76	7.97	7.82	7.90
Specific Conductance	µS/cm	316	426	371	242	257	250	898	1,645	1,272
Total Nitrogen	mg/L	1.20	1.36	1.28	1.31	1.15	1.23	0.25	0.17	0.21
Nitrate + Nitrite N	mg/L	1.01	0.364	0.686	1.13	0.554	0.841	0.051	0.024	0.038
Total Phosphorus	mg/L	0.130	0.118	0.124	0.095	0.101	0.098	0.049	0.024	0.037
Soluble Reactive Phosphorus	mg/L	0.120	0.105	0.112	0.084	0.084	0.084	0.041	0.016	0.028
Community GPP	g $O_2/m^2/d$	2.12	5.39	3.76	1.41	1.00	1.20	5.71	5.67	5.69
Community NPP	g $O_2/m^2/d$	-3.50	-3.78	-3.64	-5.85	-8.59	-7.22	-0.39	-0.52	-0.46
Community Respiration	g $O_2/m^2/d$	5.62	9.18	7.40	7.26	9.58	8.42	6.10	6.19	6.15
P/R ratio	unit less	0.44	0.63	0.54	0.23	0.11	0.17	1.16	0.96	1.06
Community GPP Efficiency	g O_2/mol	0.22	0.29	0.26	0.09	0.09	0.09	0.31	0.45	0.38
Community GPP Efficiency	%	6.06	3.57	4.82	1.87	3.63	2.75	1.82	3.05	2.44

Factors influencing stream metabolism have been estimated in eight streams from several biomes in North America by Mulholland *et al.* (2001). These streams were not spring runs, but were relatively free from human disturbance and had dissolved inorganic nitrogen concentrations less than 0.15 mg/L and soluble reactive phosphorus concentrations of 0.014 mg/L or less. These streams had GPP estimates ranging from less than 0.1 to 15 g $O_2/m^2/d$, community respiration (CR) estimates ranging from about 2.0 to 11.1 g $O_2/m^2/d$, and photosynthetically active radiation (PAR) efficiencies ranging from 0.042 to 0.45 g O_2/mol. Mullholland *et al.* (2001) reported that GPP and NPP were not correlated with either dissolved inorganic nitrogen or soluble reactive phosphorus concentrations but were significantly correlated with PAR, although CR was correlated with soluble reactive phosphorus concentrations.

Other estimates of primary production from spring ecosystems include those made by Duarte and Canfield (1990a), who published plant community and productivity data for thirty-one Florida springs. Productivity data were not directly comparable to data collected by Odum (1957a, b), Knight (1980), WSI (2006), or Munch *et al.* (2006) as they were based on a short term, rapid assessment technique rather than on a full diurnal cycle. Still Duarte and Canfield (1990a) observed no correlation between total nitrogen (which was mostly in the form of nitrate) or total phosphorus with the biomass or productivity of submersed aquatic vegetation (SAV) in the spring runs they examined. SAV standing crop and maximum daily productivity were correlated with degree of shading by shoreline vegetation. These results suggest that light may have been the primary limiting factor during their study.

Respiration

Changes in community respiration are likely to be influenced by nutrients if nutrients are influencing the abundance or productivity of primary producers. Changes in primary producers would in turn affect the consumer or decomposer community and hence the respiration of this part of the ecosystem. The practical differentiation of autotrophic versus heterotrophic respiration is not feasible in field studies. From Florida spring studies that have compared community respiration rates to water column nutrient concentrations, it appears that a negative correlation does exist (*i.e.* Munch *et al.* 2006; Figure 3, WSI 2007a), but the correlation is weak. Community respiration was also observed to increase downstream in the Wekiva River and in Rock Springs Run as nitrate concentrations decreased. Community respiration also declined at Silver Springs at the same time nitrate nitrogen concentrations were increasing (Munch *et al.* 2006).

Productivity/Respiration Ratio

The productivity/respiration (P/R) ratio is commonly used to classify ecosystems as either autotrophic (P/R > 1) or heterotrophic (P/R < 1) depending on whether they are net producers or net consumers of organic matter (Odum 1956). Florida spring ecosystems were noted by Odum (1956) to have P/R ratios greater than one, indicating net production of organic matter. Estimates from Silver Springs illustrate that during the winter of 1952/1953 and March 1954, this ecosystem had P/R ratios of 2.9 and 7.0 respectively. The effects of elevated nitrate concentrations on the P/R ratio are revealed by the results of Munch *et al.* (2006), in that period-of-record (February 2004 to March 2005) average P/R ratio at Silver Springs was estimated as 1.06, with the monthly maximum P/R ratio being 1.26 in June 2004 and a minimum monthly average of 0.73 in February 2004. In a comparison of spring run segments with nitrate concentrations ranging from natural ambient levels (< 0.05 mg/L) to elevated levels (> 1.0 mg/L), P/R ratios were found to decline significantly in relation to increasing nitrate concentrations (Figure 3, WSI 2007a). This negative correlation suggests that the P/R ratio can be used to evaluate nutrient impairment of spring ecosystems.

Photosynthetic Efficiency

The efficiencies of photosynthetic primary producers have been measured most comprehensively at Silver Springs, where Odum (1957b) reported an average value of 1.09 g O_2/mol of PAR (ranging from 0.79 to 1.53), while Knight (1980) reported an average value of 1.06 g O_2/mol of PAR (ranging from 0.61 to 1.50), and Munch *et al.* (2006) reported an average value of 0.95 g O_2/mol of PAR (ranging from 0.07 to 2.71). The Munch *et al.* (2006) period-of-record average photosynthetic efficiency of 0.95 g O_2/mol, is equivalent to an estimated PAR efficiency of 7.6% using estimated conversion factors and PAR efficiency was observed to vary seasonally with the highest monthly average value of 10.5% (1.30 g O_2/mol) measured in December 2004 and the lowest value of 5.5% (0.68 g O_2/mol) measured in June 2004. These estimates of photosynthetic efficiency at Silver Springs indicate a slight reduction in the magnitude of this parameter over the past 50 years although differences in the number of estimates make it difficult to determine if this change is significant and if it has any relation to increasing nitrate concentrations. Photosynthetic efficiency estimates for other spring ecosystems have recently been made for Alexander, Juniper, Rock, and Wekiwa Springs with average values of 0.32, 0.45, 0.09, and 0.26 g O_2/mol of PAR respectively (Figure 3, WSI 2007a). These estimates were derived from a similar number of daily measures collected during the same time periods and illustrate a reduction in photosynthetic efficiency correlated with increasing nitrate concentrations.

Community Metabolism Conclusions

As the previous examples illustrate, traditional measures of describing community structure which use biomass metrics allow few generalizations about the response of primary producers to elevated nutrient conditions in stream systems. This dilemma supports the use of ecosystem-level metabolism functional measures, such as GPP, NPP, P/R ratio and photosynthetic efficiency, which integrate the overall functional response of the ecosystem components and may reduce variance caused by site-specific conditions. The observations summarized in this chapter of reduced primary productivity and ecological efficiency in correlation with increasing nutrients may have multiple explanations.

First, Florida's spring run ecosystems were likely adapted to naturally lower ambient nutrient conditions and higher nutrient levels could represent an imbalance or stress; this explanation follows the subsidy-stress hypothesis of Odum *et al.* (1979). Support for this concept was provided in an ecosystem-level metabolism study of Silver Springs, where ecosystem production and efficiency were also reduced in inverse proportion to rising nitrate nitrogen concentrations (Munch *et al.* 2006).

A second possible mechanism that might help to explain a decline in ecosystem primary production could be reduced macrophyte photosynthesis resulting from increased epiphytic algae growth and shading as a result of elevated water column nutrients. While epiphytic growths can reduce the light energy available to host plants, some research found these effects were primarily observed at low light levels and did not affect host photosynthetic rates at typical midday intensities As spring waters are generally clear and light transmission is excellent (Duarte and Canfield 1990b), epiphytic reductions in light availability to vascular plants may not reach the level of causing host plant death. In an experimental manipulation of epiphytes and light levels, Asaeda *et al*. (2004) noted that both epiphytic algae and low light reduce growth and production; plants tended to optimize low light-created shade by changing their physiology and morphology, but were unable to do the same when epiphytic algae-created shade occurred on the leaf surface.

A third alternative explanation to reduced primary productivity in macrophytes in relation to elevated nitrate concentrations was provided by Boedeltje *et al.* (2005) who reported high water column nitrate nitrogen concentrations can significantly reduce the growth of ammonia-preferring species such as *Potamogeton alpinus*, because the uptake and reduction of nitrate has a much higher energy and carbon requirement than ammonia uptake and assimilation (Runge

1986, Marschner 1995). Boedeltje *et al.* (2005) hypothesized that nitrate-dominated nitrogen assimilation may lead to strong metabolic disturbances in species adapted to ammonia uptake by the roots (such as *P. alpinus*) resulting in the observed reduction in growth under high water column nitrate concentrations, although further experiments are needed to assess potential negative effects on species found in the springs of Florida.

Although the causative mechanisms which result in reduced primary productivity and ecological efficiency in correlation with increasing water column nutrient concentrations (especially nitrate and perhaps phosphorus) remain unknown; the averages, extremes, and seasonal patterns for these ecological measures are becoming more apparent as additional ecosystem metabolism data are being collected in Florida's springs. Year-to-year measures of ecosystem metabolism are remarkably consistent compared to structural measures such as plant or fish populations and provide a valuable database for comparison to other springs, spring runs, and streams in Florida.

HUMAN AND AESTHETIC USES

Visitor Satisfaction

There are little or no published data that relate recreational satisfaction from Florida springs to their trophic status. It is readily apparent that people prefer to swim in clear, clean water rather than turbid, colored, or phytoplankton-rich waters. It is likely that highly abundant macrophytes discourage water recreation as well. This presents a dilemma, in that, native benthic vascular plants represent a natural and ecologically important component of spring ecosystems. Species such as *Vallisneria americana* or *Sagittaria kurziana* are typically not viewed as nuisance solely due to their presence.

Economic Impacts

As detailed in Chapter 1, the number of visitors attending the springs in Florida and their cumulative economic impact is substantial enough to impact local economies. If recreational attendance were to decline due to real or perceived diminished appeal of a spring system, there would be associated declines in the economic impacts generated. Preservation of spring ecosystems should not be based solely on an economic basis, unless considerations for environmental services are factored in as well, but they provide support towards these systems protection. At this time insufficient quantitative public use data exist from most springs to develop correlations with increasing nutrient concentrations.

Effects of Eutrophication on Human Recreation

The type of recreation will obviously influence tolerance for the presence of SAV, with water dependent recreation perhaps having lower tolerance levels than non water dependent contact activities. SAV can attract waterfowl, wading birds, and other wildlife which would affect perceptions of persons engaged in observation of wildlife as well. Limited data on recreational satisfaction in relation to primary producers exists, but Holland and Cichra (1994) reported that what respondents liked best about their trip to Rainbow River was: clear, clean water (35%), scenery (19%), quiet, relaxing (17%), and seeing wildlife and fish (11%) and that what respondents liked least about their trip: rainy weather (13%), motorboat noise (12%), vegetation and algae in water (10%), and river float too long (10%). These results suggest that while water clarity is very important, there is tolerance for submersed aquatic vegetation among the surveyed recreationalists, possibly because this vegetation may have been viewed as a natural component of a healthy ecosystem. Declines in water clarity as a result of reductions in stream flow with corresponding increases in water residence time and phytoplankton abundance would likely be associated with decreased recreational satisfaction. Similarly, increases in the abundance of vascular plants or macroalgae are likely to be viewed negatively by recreational users of spring ecosystems, given that people do not like to swim through or stand in vegetation. If the abundance of filamentous algae increases as a result of eutrophication in a spring ecosystem, there would likely be a decline in recreational user satisfaction. Real or perceived declines in the aesthetics of a spring ecosystem as a result of eutrophication would likely result in less human recreational use and visitation, with resulting declines in the economic impact. It is important to note that human uses of the terms, such as *natural* or *healthy*, are subjectively defined, and that an educational component aimed to identify these biases would serve both the managers and users of springs and other ecosystems.

DISCUSSION

In the past century, many of Florida's spring ecosystems have experienced changes which include declines in discharge, increases in conductivity, increased recreation, and colonization by non-indigenous species. However, the widespread increase in nitrate concentrations during this time period continues to be one of the key elements that spring ecosystems have had to adjust to, and it has been hypothesized that the increasing concentration of that form of nitrogen is a principal causative factor for observed changes in ecosystem metabolism. Strong (2004) examined temporal trends in water quality characteristics in 109 Florida springs. Parameters that were generally found to be increasing were specific conductance, alkalinity, hardness, and nitrate. Only pH concentrations were observed to be declining. There were no apparent trends in phosphorus concentrations. Mean nitrate concentrations in spring samples generally increased from about 0.43 mg/L before 1977 to about 1.13 mg/L since 1990. Strong's (2004) study suggests that state-wide trends for declining water quality of springs are occurring. An illustration showing the range of nitrate plus nitrite concentrations (NO_x-N mg/L, although nitrite is generally a minimal component of these aggregate values) for 130 Florida springs compiled from 2006 Florida Geological Survey data is shown in Figure 4. This figure presents the wide range of NO_X-N concentrations that Florida springs exhibit and suggests that 83% of the surveyed springs have NO_X-N concentrations greater than 0.1 mg/L, which is a commonly assumed background level for groundwater nitrate levels in Florida.

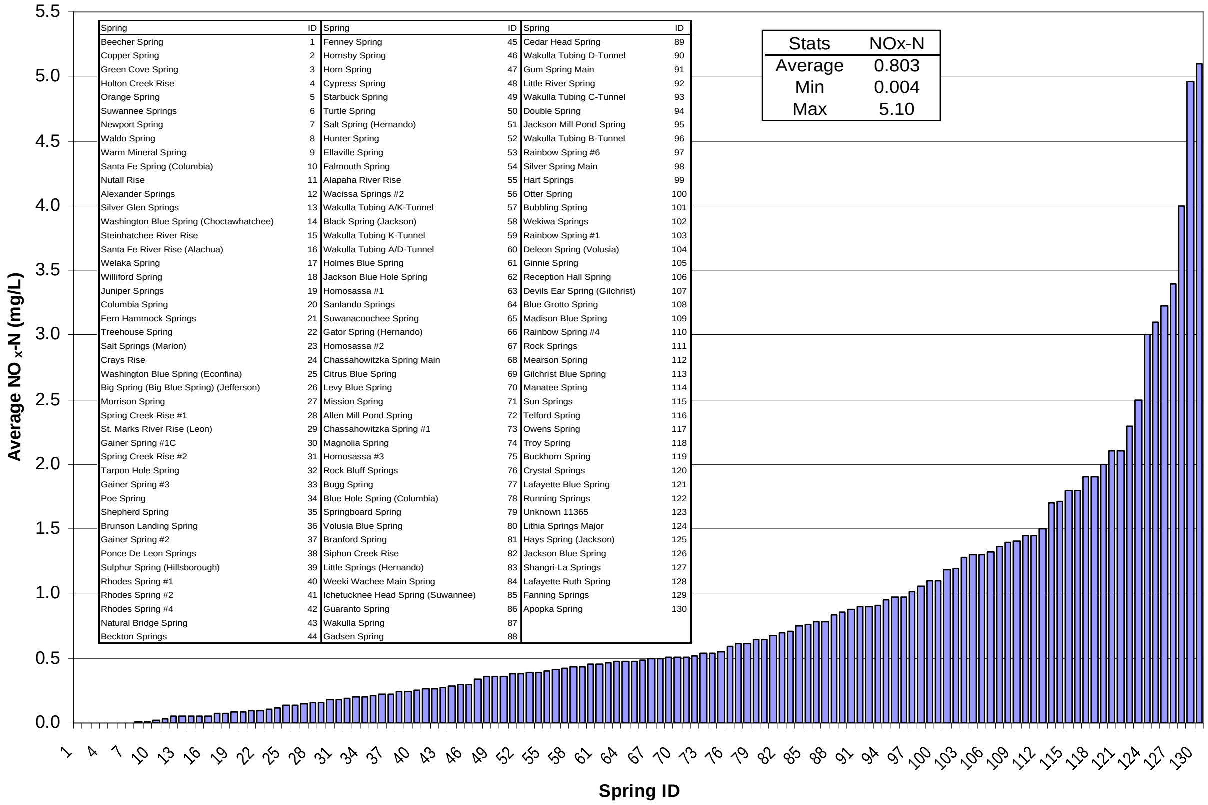

***Figure 4 –** Illustrates the average NO_X-N concentrations (mg/L) for 130 Florida springs (data from Florida Geological Survey, Scott* et al. *2004).*

A similar illustration showing the range of total phosphorus concentrations (TP mg/L), for 130 Florida springs compiled from 2006 Florida Geological Survey data is shown in Figure 5. Although total phosphorus (the aggregate of all forms of phosphorus) data are presented, in the case of groundwater, this total phosphorus is almost entirely composed of dissolved inorganic forms of phosphorus such as orthophosphate or soluble reactive phosphorus. This figure suggests that the total phosphorus concentrations of spring water are less variable than the wide range of NO_x-N concentrations that Florida springs exhibit. Groundwater phosphorus concentrations are largely a function of the associated geology and many of the higher phosphorus values are observed from springs which overlie natural phosphatic deposits.

Of these 130 springs, 80% have total phosphorus concentrations greater than 0.025 mg/L, a value which has been defined as a break point separating mesotrophic and eutrophic lakes (Table 3, Forsberg and Ryding 1980). Although other indices of trophic status exist (*e.g.* the Florida Trophic State Index created by the Florida Department of Environmental Protection), their application to lotic systems has been problematic. This is principally due to the index's inability to incorporate macrophytes and benthic algae that are not accounted for by standard methods which measure water column chlorophyll content in non-flowing systems.

Because phosphorus levels have not been increasing in Florida's springs, even though the groundwater is regionally rich in phosphorus, (Strong 2004), it can be suggested that the majority of Florida springs would have been historically nitrogen limited, based on known ratios of nitrogen to phosphorus demand by plants (*e.g.* Redfield ratio, Redfield *et al.* 1963). However, in the last several decades, a great majority of springs appear to have been impacted by high nitrate concentration (83% exceed 0.1 mg/L NO_x) and the resulting nitrogen to phosphorus ratios have become skewed to favor phosphorus limitation. This view should not be misinterpreted by the reader to suggest that nitrate is not important because it may no longer be limiting on a ratio basis. Instead it should be viewed that there is such an over abundance of nitrogen (principally in the form of nitrate) relative to historic ambient conditions, that an un-natural phosphorus limited state has evolved. While efforts to limit the input of phosphorus to aquatic ecosystems should be continued, the supply of this element to Florida's springs appears to be linked to geological conditions which are largely beyond human control. Nitrate loading from anthropogenic activities can, however, be moderated and hence represents our best ability to effect a nutrient reduction strategy to benefit Florida spring ecosystems.

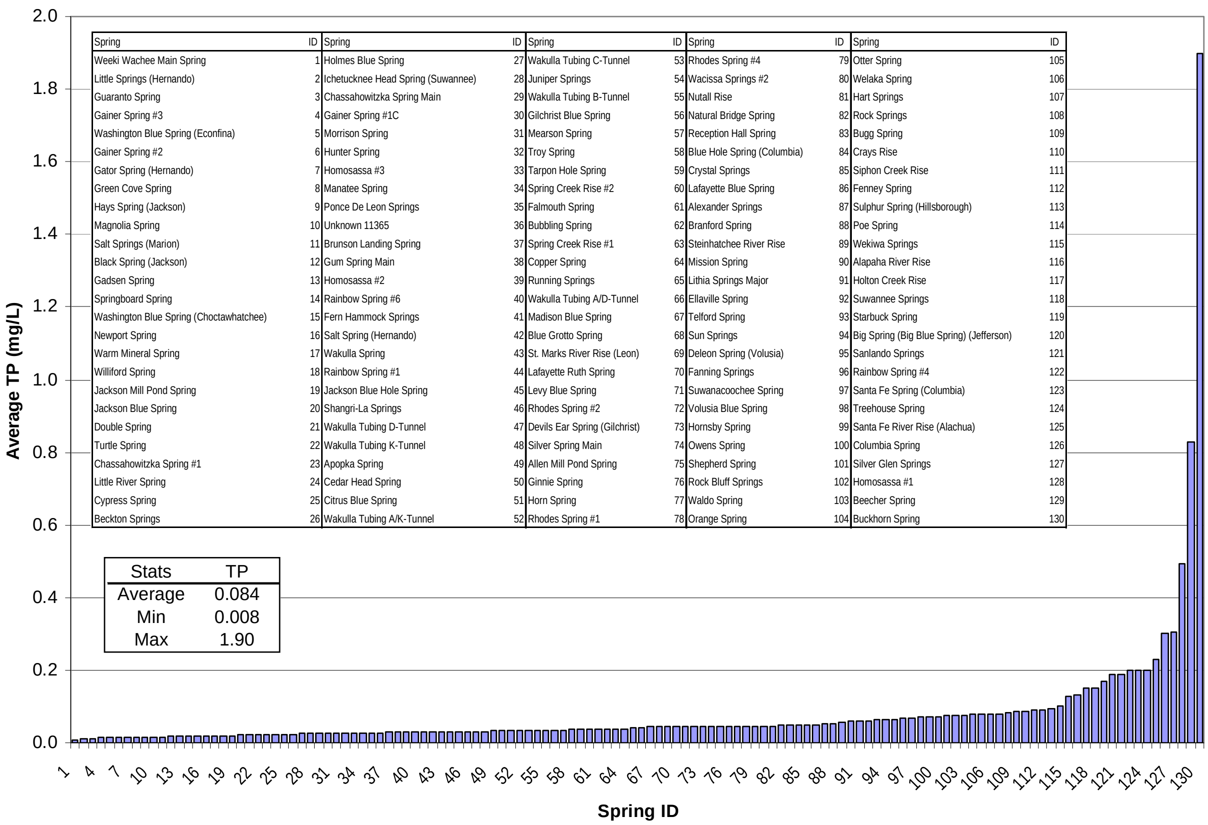

Figure 5 - *Illustrates the average total phosphorus concentrations (mg TP/L) for 130 Florida springs (data from Florida Geological Survey, Scott et al. 2004).*

Table 3. Lake trophic state classification system based on Forsberg an Ryding (1980).

Trophic State	Chlorophyll (mg/L)	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	Water Clarity (ft)
Oligotrophic	< 0.003	< 0.015	< 0.40	> 13.1
Mesotrophic	0.003 – 0.007	0.015 – 0.025	0.40 – 0.60	8.2 - 13.1
Eutrophic	0.007 – 0.04	0.025 – 0.10	0.60 – 1.50	3.3 – 8.2
Hypereutrophic	> 0.04	> 0.10	> 1.50	< 3.3

The best evidence suggesting a decline in the health of Florida's spring ecosystems comes from studies that have been conducted over a half-century time period in Silver Springs. Both the direct measurements and the estimated system metabolism analyses indicate that the Silver Springs ecosystem may be considerably less productive than it was fifty years ago (Munch *et al.* 2006). This appears to be a consequence of lowered gross primary productivity and efficiency of light utilization. This result appears to be counter-intuitive to the observation that nutrient levels (nitrate nitrogen) which support plant growth have increased in Silver Springs. It is generally thought that an increase in nutrients will stimulate both gross and net primary productivity in aquatic ecosystems (Wetzel 2001). However, spring ecosystems are characterized by flowing waters which continually re-supply nutrient demands of primary producers. H.T. Odum (1957a) theorized that prior to modern anthropogenic impacts, Florida's springs existed as a balanced aquatic ecosystem which had evolved to maximize community metabolism, and the relatively recent increase of nutrients in the form of nitrate may have resulted in a stress rather than a subsidy (E.P. Odum *et al.* 1979). The subsidy-stress hypothesis may be the best model to describe the response of spring ecosystems to increasing nitrate concentrations; however, controlled field experiments need to be made in order to test this hypothesis.

One of the most comprehensive examinations of the effects of nitrate on spring ecosystem structure and function is the 50-year retrospective study of Silver Springs by Munch *et al.* (2006). The key findings from that study are reproduced here:

- *Sagittaria kurziana* remains the dominant submersed aquatic plant species in Silver Springs and represents one of the main physical features of the ecosystem.
- Biomass estimates for submersed aquatic plants in the summer season were not significantly different from estimates made by Odum in the early 1950s. However, estimates for winter biomass were 31% lower than Odum's, who reported no seasonal difference in submersed aquatic plant biomass.
- Biomass estimates for the epiphyte community in the summer were approximately three-fold higher than those reported by Odum, while winter values were not significantly different between the two studies.
- The largest disparity between the Munch *et al.* (2006) estimates of primary producer community biomass and those of Odum from the 1950s was the substantial increase in biomass for the benthic algal mat community. While Odum discounted the importance of algal mats, in terms of biomass, the Munch *et al.* (2006) study indicated that this primary producer category had biomass estimates similar to those observed for epiphytes and

submersed aquatic plants. However, it is important to note that these estimates may not be an accurate indicator of primary productivity attributable to benthic mats, since mat biomass includes large proportions of bacteria, fauna, dead algae, and other detrital material.

- Total species richness for birds, fish, and reptiles in the Munch *et al.* (2006) study were similar to historical records at Silver River.
- Estimated annual average fish live-weight biomass in the Munch *et al.* (2006) study has declined in Silver Springs since Odum's study in the early 1950s by about 96%; and by 61% since Knight's 1978-79 study (Knight 1980). These declines in biomass were primarily due to large reductions in a few species (channel catfish, mullet, and gizzard shad), while the remaining fish species were found in similar abundance across the fifty-year span. It is likely that declines in channel catfish, mullet, and gizzard shad abundance are the result of diminished access to the St. Johns River and the Atlantic Ocean due to the Kirkpatrick (AKA Rodman) Dam
- Annual average gross primary productivity (GPP) declined from about 15.6 grams oxygen per square meter per day (g $O_2/m^2/d$) in the 1950s and late 1970s to about 11.2 g $O_2/m^2/d$ during the current study, a decline of about 27%.
- Community respiration also declined from about 14.8 g $O_2/m^2/d$ during the earlier studies to about 10.9 g $O_2/m^2/d$ during the Munch *et al.* (2006) study, a 26% reduction.
- The resulting net community primary productivity declined from about 1.0 g $O_2/m^2/d$ in the 1950s, to 0.80 g $O_2/m^2/d$ in the late 1970s, to about 0.42 g $O_2/m^2/d$ during the Munch *et al.* (2006) study, a decline of about 59% over the past fifty years.
- The production to respiration (P/R) ratio remained relatively consistent between the three studies, ranging from about 1.11 during Odum's study in the 1950s to about 1.06 during Knight's study in the late 1970s, and 1.06 in the Munch *et al.* (2006) study.
- Ecological efficiency declined from about 1.09 gram of oxygen per mol (g O_2/mol) of Photosynthetically Active Radiation (PAR) during Odum's study to about 0.94 g O_2/mol of PAR during the Munch *et al.* (2006) study, a decline of about 13%.
- Average particulate export rates were found to be 72% lower during the Munch *et al.* (2006) study compared to data published by Odum (1957). These particulates are a representation of the net system productivity, and their decline strengthens the evidence that overall productivity has declined in Silver Springs.

The Munch *et al.* (2006) study observed that the percentage of developed lands within the springshed (5 mile radius) was positively correlated with actual nitrate concentrations and spring discharge was found to be inversely correlated with nitrate. The resulting correlation models were used in turn to predict future nitrogen loading to the Silver Springs ecosystem if the future land use development estimates for 2055 are realized (Table 4).

Table 4. Modeled changes in the nitrogen concentration of Silver Springs water based on projected changes in land use and corresponding nitrogen loading rates (Munch et al. 2006).

Year	Observed Spring Flow	MODFLOW Simulated Spring Flow	Observed Nitrogen Load (lbs/yr)	Land Use/Land Cover Model Est. N Load (lbs/yr)	Observed Spring N Concentration (mg/L)	Land Use/Land Cover Model Est. N Concentration (mg/L)	N Concentration Change (Model Predictions, %)
1957	640.0	716.5	94,416.0	399,054.10	0.10	0.38	N/A
1979	778.0	710.8	814,898.6	802,633.06	0.71	0.76	102.75
1995	720.0	708.3	955,962.0	1,036,198.93	0.90	0.99	29.56
2005	680.0	687.6	1,057,606.7	1,120,813.63	1.07	1.10	11.42
2055	N/A	687.6	N/A	1,760,000.00	N/A	2.02	84.00

The apparent decline in ecosystem metabolism and efficiency observed in Silver Springs over the past fifty years may be the result of a several ecosystem-level factors working in concert or at odds. Four possible hypotheses were offered to explain these observations (Munch *et al.* 2006):

- Decreased usable solar radiation is reaching the level of submersed aquatic plants in Silver Springs due to increased shading by a growing tree canopy (natural wetland forest succession) along the river and this reduced input of solar energy may have lowered GPP and PAR efficiency.
- GPP may have declined as areas of optimal submersed aquatic vegetation growth (*Sagittaria kurziana* beds with adapted periphytic algae) have been replaced with benthic algal mats, possibly due to increasing nitrate nitrogen concentrations, flow decreases, or physical factors related to human uses.
- Decreases in flow rate and water velocities due to natural climatic conditions or consumptive groundwater uses may have reduced the previous subsidy needed for maximum plant/periphyton growth and higher GPP and PAR efficiency.
- Decreased top-down consumer control of the primary producers and lowered GPP is resulting from lower fish/consumer populations below optimal grazing densities, possibly due to obstruction of fish migration by the Kirkpatrick Dam, or lower minimum daily dissolved oxygen concentrations, or due to indirect effects of nitrate on consumers. The role of striped mullet (*Mugil cephalus*) in the top-down control of the periphytic growths on submersed vascular plants in Silver Springs was reported by Allen (1946, p. 32), who wrote: "*These attractive fish are very prominent, always swimming about, forever feeding on algae off the blades of underwater grasses* [*Sagittaria kurziana*]. *They start at one end of the blade of grass, sucking up food as they slowly swim up toward the other end*."

Overall, the Munch *et al.* (2006) study indicates that multiple factors may have resulted in the observed ecosystem changes at Silver Springs over the past 50 years. These factors include reductions in the volume of spring discharge, increases in riparian shading, reductions in the abundance of herbivorous fish, and increases in nitrate concentration and load. The authors of the Munch *et al.* (2006) study suggest that the corresponding increases in nitrate concentration over this time period cannot exclusively be responsible for this degradation, but that nitrate pollution has likely contributed to this decline. Given the anticipated future increases in nitrate pollution, additional concern due to this factor remains warranted. Support for the concept that nitrate can act as both a subsidy at low concentration and a stressor at high concentrations has been provided by the WSI (2007a) study in the Wekiva River and Rock Springs Run which observed negative correlations between measures of ecosystem productivity and nitrate concentrations. Based on these lines of available evidence, nitrate alone may not be the sole factor negatively impacting Florida spring ecosystems, but it is the single most important water quality parameter that has changed during the period of observed declines in spring ecosystem structure and function.

RECOMMENDATIONS FOR FURTHER RESEARCH

Despite a wide variety of springs' research, there remains a significant knowledge gap between the real and perceived threats that nitrate pollution plays on the ecology of spring ecosystems. This situation is not unique to Florida, as Bernot and Dodds (2005, p. 442) observed that for North American streams: "*There is a great need for long-term studies of nitrogen additions in lotic ecosystems and clear distinctions need to be made between ecosystem responses to short-term or periodic increases in nitrogen loading and alterations in ecosystem functions due to chronic nitrogen loading.*" In addition to the general research questions to be answered, Florida's springs represent important cultural and economic resources which also provide real-time windows into the condition of Florida's most essential natural resource – high-quality groundwater.

With these caveats in mind, we suggest that nutrients are not the only stressors of concern in spring ecosystems. Recent work in the headwater areas of Wekiwa and Rock springs indicates that ecosystem metabolism can be negatively impacted by high levels of human recreation and by flow reductions (WSI 2007a, 2007b). Many, if not most, of Florida's springs are threatened by these additional stressors as well as by invasions from non-indigenous plant and animal species, aquatic weed management activity, erosion and deposition of sediments, and increasing stormwater inflows from surrounding urbanized areas. Future research to be conducted should attempt to effectively understand the separate and synergistic effects of these multiple forcing functions and the associated responses of our spring ecosystems.

With the multiple stressors affecting spring ecosystems, any metric used to monitor their health should to the fullest extent possible be integrative. The single best way to characterize these systems would be through estimates of community metabolism and its constituent parameters: gross primary productivity, net primary productivity, community respiration, productivity to respiration ratios, and photosynthetic efficiency. These metrics can be relatively easily estimated using upstream-downstream changes in dissolved oxygen concentrations and have the best ability to describe ecosystem level functions, and their value is enhanced when combined with measures of fauna and flora abundance and water chemistry, other typical single-component ecosystem metrics.

Proposed Research Priorities

The following springs' research priorities are suggested:

- Recognize the importance of the collection of water quality samples, particularly for dissolved oxygen (diel changes) and nitrates, on a periodic schedule, from major springs. Continue the collection of discharge estimates from major springs (all first and second magnitude and a representative sample of springs with lower discharge rates), as declining discharge represents a major threat to springs. These types of data are regularly collected by local, state, and federal agencies, but improvements in the spatial and temporal sampling could be realized as well as improved assembly, analysis, and reporting of the resulting data.
- Continue to expand and refine the development of Land Use and Land Cover (LULC) datasets, which in conjunction with improved infrared and color imagery, will greatly increase the relative accuracy in the LULC classifications for springsheds. In conjunction with accurate estimates of nitrogen loading per LULC category, assessment of springshed land use changes on the nutrient concentration of associated spring discharge could be made. Thus, rather than solely reacting to spring pollution after the source is in place, the ability to predict and as a result to limit or prevent springshed pollution would be enhanced.
- Better utilize current technology of *in situ* data loggers in a representative set of major springs ecosystems so as to provide regular or continuous records of changing water quality conditions, including temperature, dissolved oxygen, pH, and specific conductance (and other parameters including nutrients as instruments and technologies become available). These measures, particularly if made in conjunction with levels of ambient solar radiation and spring discharge would allow the direct estimation of ecosystem metabolism and photosynthetic efficiency. Given the inter-specific range of physical and chemical conditions that Florida springs currently exhibit, simultaneous estimates of ecosystem metabolism would allow development of a response surface for comparison to changing environmental conditions (forcing functions). For example, multivariate analysis could be applied to determining the relative importance of a number of forcing functions on ecosystem metabolism, including identification of the concentration or intensity of each variable (*e.g.*, nitrate) as it transitions from a subsidy to a stressor.
- Quantify invertebrate, vertebrate, and floral diversity and abundance on a three-to-five year schedule at 1st and 2nd magnitude springs so that trends in these ecosystem components can be observed. This sampling should be conducted on springs that are also being monitored for ecosystem metabolism and should be scheduled on a monthly basis for at least one year during each three-to-five -year period so as to elucidate seasonal trends.
- Conduct *in situ* mesocosm experiments which quantify the role that bottom-up processes (*i.e.* nutrient limitation) versus top-down processes (*i.e.* herbivore grazing) have on the growth and abundance of primary producers, particularly epiphytes and filamentous algae. The primary question to be answered is whether future increases or decreases in nutrient concentrations will result in responses by the primary producer community.

- Better monitor and inventory human use activities in a representative group of important springs on a routine basis so that optimal (as opposed to high) levels of human recreation can be maintained without degradation of the springs' resource. Human recreation levels or uses which are identified to be impairing spring health or degrading spring aesthetics should be modified through adaptive management strategies. The most successful example of this balance may be Ichetucknee Springs State Park which annually accommodates hundreds of thousands of in water recreationalists while maintaining sustainable populations of submersed macrophytes.

Recommendations

Based on the synthesis of information provided concerning the effects of nutrients on springs in Florida, the following recommendations are provided:

- It is recommended that the Florida Department of Environmental Protection (DEP) Springs Initiative continue funding applied research under field conditions in order to develop a consistent base of scientific knowledge for spring ecosystems. A critical need exists to establish a comprehensive baseline database for a large number of artesian spring ecosystems, including their ecosystem metabolism, trophic structure, and key forcing functions. A substantial increase in the annual funding for this research is justified by the ecological and economic importance of artesian springs in Florida.
- It is recommended that counties with significant springs' resources utilize existing governmental agencies to develop springs protection plans that address such components as land development regulations and land acquisition, along with other groundwater and spring protection measures in order to prevent further and, if possible, reverse the observed increases in nitrate-nitrogen concentrations. Regulatory programs and tax incentives should be utilized to reduce nitrogen loads to the artesian groundwater in karst areas of the state. These actions have the ability to reduce other ground water pollutants introduced through anthropogenic activities and would contribute to high quality ground water recharge which would be widely beneficial.
- It is recommended that the DEP with support from the Florida Legislature develop applicable standards that define nutrient impairment in artesian springs. Springs that meet these impairment metrics should be moved as quickly as possible into the state's Total Maximum Daily Load (TMDL) program. Nitrate concentrations significantly above historical spring levels could be used as a criterion for listing under the Impaired Waters Rule. For springs that are above recommended thresholds for nitrate nitrogen concentrations, these TMDLs could provide a basis for enhanced nitrogen-removal standards for municipal and on-site wastewater facilities as well as for stormwater management systems and the active and pro-active enforcement of nitrogen mass loading limits by existing and future point and non-point sources in the affected springsheds.

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