James K. Dame. EVALUATION OF ECOLOGICAL NETWORK ANALYSIS FOR ECOSYSTEM-BASED MANAGEMENT (Under the direction of Dr. Robert R. Christian) Ph.D. Program in Coastal Resources Management. December 2005.

A critical assessment of ecological network analysis (ENA), a modeling technique increasingly being used to address resource management issues, was conducted. The major objectives were to evaluate the effectiveness of ENA in detecting differences in food web properties, and to validate ENA models using independent methods.

Quantitative trophic networks (n=12) representing four high marsh ponds during three times (corresponding to low stress, high stress, and post-disturbance) were constructed from an extensive field sampling program augmented by literature values. A null hypothesis was tested to determine how values of twelve indices from ENA output differed among the three stress/disturbance conditions (H_o: Low Stress = High Stress = Post-Disturbance). Statistical differences were determined using repeated measures ANOVA (with contrasts) and Friedman's Tests (with multiple comparisons). Covariance of each pair of indices was evaluated with Spearman's Rank Correlation Tests.

Hypothesis testing suggested ENA was effective in detecting differences in the food web properties examined. ANOVA results indicated mean values of 10 of 12 ENA indices were significantly different among the three stress/disturbance conditions, and results from the Friedman's Test were generally in agreement (mean rankings in 11 of 12 indices showed significant

differences). Confidence in these results was given by a relatively low amount of covariance among the indices (7 of 66 were significant).

Four separate aspects of selected models were then validated (respiration, aggregation of taxa, trophic levels, extended diet) by comparison to results derived from field measurements and stable isotope data (δ^{13}C, δ^{15}N, δ^{34}S). Validation was based on paired t-tests and graphical comparisons, and explanations were made for instances where there was not agreement. These involved assumptions associated with the models and inherent differences in the methods used for validation relative to ENA.

Implications of the validation were then examined by modifying selected models to be in agreement with the validation methods, and comparing their output to the unmodified version. Findings from this analysis support the hypothesis testing, and suggest ENA output was sensitive to changes in the amount of material available for energy flow as well as structural aspects of that flow.

EVALUATION OF ECOLOGICAL NETWORK ANALYSIS

FOR ECOSYSTEM-BASED MANAGEMENT

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CHAPTER 1. INTRODUCTION

Overview

Ecological network analysis (ENA) represents a growing area of ecology for both academics and resource managers. Researchers are using ENA to examine species interactions in a variety of ecosystems (e.g. Belgrano et al. 2005), and management entities are exploring how the quantification of these interactions can be incorporated into their programs (e.g. NCBO 2003). However in spite of increased interest and use of ENA, there has been little critical evaluation of ENA models. Moreover because of easily accessible and user-friendly software, there is a danger of ENA becoming a "black box" tool where users of the output do not fully appreciate uncertainty in the models.

This study focused on several topics seldom addressed among researchers using ENA, and even more rarely considered among those using ENA for management decisions. Few studies have addressed the level of uncertainty in their models, and even fewer validate model output. This study represents one of the first (if not the first) systematic efforts to evaluate the effectiveness of ENA. It also helps to address a long standing issue with general food web research involving data quality (e.g. Paine 1988; Cohen et al. 1993) by providing an exhaustive data set of the food webs and environmental conditions of a replicated ecosystem.

The main objectives of this dissertation are to (1) present the potential ENA has for assisting in ecosystem-based management, while at the same time,

identify some of its limitations; (2) evaluate the effectiveness of ENA in detecting differences in food web properties; (3) validate ENA models using independent methods; and (4) provide a detailed data set documenting trophic relationships in multiple ecosystems replicated in time.

Organization

A non-traditional format for the chapters is utilized. Each of the "core" chapters (2 through 5) addresses one of the main objectives given above. Each is written to stand on its own to be published separately, and is in the general format of the target journal. Chapter 2 outlines how ENA can be used for ecosystem-based fisheries management using trophic networks of the Neuse River Estuary in North Carolina as examples. Four major sources of uncertainty in ENA models are identified and discussed, and recommendations for addressing these are provided.

Chapter 3 is a "data paper" designed for electronic publication. It involves synthesis of data, and documentation of trophic models, used throughout the remainder of the study. As defined by the journal Ecology, data papers consist of two parts: data files and metadata. Chapter 3 represents the metadata component, and fully describes the content, context, quality, and structure of the data. It is presented in a standardized format described in Michener et al. (1997). The data files are not included within the body of the dissertation. The intent of this chapter is to provide a detailed description of the field and laboratory

methods, and to document sources of model input and assumptions used in model construction. The published version of this chapter will make the entire data set available on the web.

Chapters 4 and 5 involve the core issues examined in the dissertation. The ability of ENA to detect differences in food web properties is evaluated in Chapter 4. A priori hypotheses are used to statistically test ENA output from replicated networks under differing environmental conditions. Twelve networks representing four salt marsh ponds during three time periods are used in the analysis. The time periods correspond to occurrences of low stress (relatively low salinity, low temperature, and high dissolved oxygen), high stress (relatively high salinity, high temperature, and low dissolved oxygen), and post-disturbance (after recovery of water levels from drought conditions) in the ponds. Variation in twelve indices from ENA output are evaluated between stress conditions in each pond.

Chapter 5 presents results from the validation of networks examined in Chapter 4. Independent field data and stable isotope analysis are used for validation. These include (1) estimates of community and plankton respiration derived from field measurements of dissolved oxygen; (2) aggregation of taxa based on $\delta^{13}C$ vs. $\delta^{15}N$ dual isotope plots; (3) trophic level estimates from $\delta^{15}N$ data; and (4) estimates of carbon source from stable isotope mixing models (Phillips and Gregg 2001; $\delta^{13}C$ vs. $\delta^{34}S$ and $\delta^{34}S$ vs. $\delta^{15}N$). Model validation involved comparing each of these to the corresponding ENA output. For

aggregation of taxa, results using stable isotopes are compared to those from correspondence analysis.

A summary and synthesis of the findings are presented in Chapter 6. Although ENA was able to differentiate between food web properties among different environmental conditions, in many cases results from ENA were not in agreement with the validation approach used. The implications of these findings are explored by modifying several models such that they are in agreement with the results from the independent methods (i.e. validated). Then, ENA output from the unmodified and modified models are compared using the same twelve indices examined in Chapter 4.

CHAPTER 2. UNCERTAINTY AND THE USE OF NETWORK ANALYSIS FOR
ECOSYSTEM-BASED FISHERY MANAGEMENT

Abstract

There is increased emphasis by the fisheries management community to move away from single species management and towards an ecosystem-based approach. Inherent to this effort is the need for an understanding of the interactions between harvested species, their prey, predators, and competitors. One approach increasingly being used to address this understanding is ecological network analysis (ENA), a modeling technique most often used to examine food webs. The purpose of this paper is to call attention to the potential of ENA for ecosystem-based fisheries management and identify its limitations. ENA provides a method for quantifying direct and indirect trophic interactions, for comparing food web properties among different systems and/or times, and for incorporating fishery harvest into the analysis. However, four major sources of uncertainty exist: natural variability of input parameters, data collection methods, model construction, and fundamental assumptions of the algorithms. Few ENA studies address uncertainty in their models and even fewer validate model output. A priori predictions of model output and sensitivity analysis should be used to understand better the effect of variability in model input. Model construction could be improved by incorporating multivariate techniques, and concerns of how well a model depicts the real-world system should be addressed by validating model output with independent techniques.

The Problem - What Is Ecosystem-Based Fishery Management
And How Does One Do It?

Ever since the term "ecosystem management" was first used in the early

1990s (Grumbine 1994) there has been a shift in the conceptual approach we

use to manage natural resources. In 1996, the Sustainable Fisheries Act (P.L.

104-297) directed the National Marine Fisheries Service to convene a panel of

independent experts to recommend how best to integrate ecosystem principles

into future federal management and research activities (EPAP 1999). Likewise,

several recent attempts to reauthorize the Magnuson-Stevens Fishery

Conservation and Management Act have called for ecosystem-based

management approaches. For example, House Resolution 4749 from the 107th

Congress would make one of the policies of the U.S. Congress to "support and

encourage efforts to understand the interactions of species in the marine

environment and the development of ecosystem-based approaches to fisheries

conservation and management". More recently, two highly regarded

commissions recommended an ecosystem-based approach for managing our

ocean and coastal resources (U.S. Ocean Commission 2004; Pew Oceans

Commission 2003), and the National Oceanic and Atmospheric Administration's

2003-2008 Strategic Plan lists ecosystem-based management as its number one

mission goal (NOAA 2003).

In spite of these and numerous other policy efforts, resource managers

are typically uncertain about what ecosystem-based management is and are

generally at a loss of how to go about doing it. This confusion exists not only among managers, but also among policy makers and within academia. As an example, the terms "ecosystem management", "ecosystem-based management", and "ecosystem approaches to management" are often used interchangeably. The latter two terms evolved out of an effort to simplify the complexity and uncertainty involved in dealing with whole ecosystems, and are focused on using what is known about an ecosystem in the management of fisheries (Fluharty 2004).

Although the confusion remains, several concepts are consistent among these three terms and the way they are used. For example, most would agree that all three involve an integration of the various aspects of a particular fishery management issue. In practice this must involve a hierarchical approach including an integration of the biological, physical, sociological, and economic aspects, as well as a consideration of the interactions occurring within each of these disciplines.

A conceptual model of the complex, hierarchical nature of ecosystem-based fisheries management is shown in Figure 2-1. At one level it involves integrating our knowledge of wide ranging issues such as trophic relationships, physical attributes of the ecosystem, ex-vessel or wholesale prices paid to fishermen, and the human dimensions of a management action. However, each of these is affected by interactions among embedded variables. For example,

the wholesale price of a particular species is a function of the landings of that species as well as the market price of a substitute species.

Moreover, there are critical relationships that cut across traditional disciplines to bridge the two levels of the heiracrchy in Figure 2-1. For example, a holistic approach to fisheries management considers humans as the major component of an ecosystem (Ditton 2004). Humans are the top predator with respect to trophic relationships, perform engineering activities that affect geomorphology and physical attributes of a system (e.g. dredging), and have obvious affects on supply and demand of natural resources.

Ecosystem-based fisheries management clearly involves integration of various disciplines. If we consider the biological aspect only, then inherent to this integration is a multi-species approach which requires an understanding of the species being harvested as well as their prey, predators, and competitors. Up to now, the biological aspect of most fisheries management has been focused on parts of a community or a single population. At least in part, this is due to the complexity of quantifying and analyzing interactions between components of a whole ecosystem.

Hollowed et al. (2000), Whipple et al. (2000), and Latour et al. (2003) review different multi-species modeling techniques that have potential for application to ecosystem-based fisheries management. The major ones, including multi-species virtual population analysis (MSVPA), multi-species production models (MSP), and multi-species bioenergetics models (MSBE), are

restricted to fish taxa only. They do not account for direct or indirect interactions between fish and "other" taxa (e.g. primary producers, zooplankton, benthic fauna, birds, and mammals), nor do they account for these same interactions among the "other" taxa. Moreover, MSVPA does not explicitly include competitive interactions and both MSP and MSBE are limited by the number of species that can be modeled concurrently.

A Potential Solution - Ecological Network Analysis

Ecological network analysis (ENA) is a modeling technique used for understanding the structure and flow of material within ecosystems, and is most commonly used for evaluating food webs (Wulff et al. 1989; Christensen and Pauly 1993). Food web networks are often depicted as box and arrow diagrams where boxes represent taxa and arrows are the flow of energy between them (Figure 2-2). Values inside the boxes represent standing stock biomass, and values along flows are given by matrices of diet relationships. ENA incorporates several separate analyses, and output includes matrices and indices that quantify trophic structure, organic matter recycling, and ecosystem size and organization (Table 2-1). For each analysis, a series of mathematical algorithms is used to analyze the network model and make inferences about the corresponding ecosystem (Kay et al. 1989; Christian and Ulanowicz 2002; Ulanowicz 2005).

ENA has the potential to be a standard tool for ecosystem-based fishery management because it gives a manager the ability to evaluate an entire food

web rather than address a single component. For example, ENA allows quantification of both direct and indirect trophic interactions. This can be illustrated using a simplified portion of the 1997 early summer food web in the lower Neuse River Estuary in North Carolina (Figures 2-2 and 2-3; Christian et al. 2003). One type of direct interaction is the effect of a predator on its prey; for example, the feeding of weakfish (*Cynoscion regalis*) on menhaden (*Brevoortia tyrannus*) in Figure 2-2. Another direct interaction is the impact of bluefish (*Pomatomus saltatrix*) on weakfish because they compete for the same prey. An example of an indirect interaction is the effect of weakfish on zooplankton because weakfish feed on the major consumers of zooplankton (i.e. menhaden and spot - *Leiostomas xanthurus*).

Output from a portion of the mixed trophic impact analysis, which quantifies the overall direct and indirect interactions in the food web (Ulanowicz and Puccia 1990), is shown in Figure 2-3. Values represent the sum of beneficial and detrimental impacts of one group on another. Weakfish have relatively large detrimental impacts on menhaden (-0.333) and on themselves (-0.408). The former is due to predation and the latter to competition. The beneficial impact of weakfish on zooplankton is relatively small (+0.025) because the biomass of spot, a prey item of weakfish and predator of zooplankton, is two orders of magnitude larger than the biomass of weakfish (see Figure 2-2). The extremely small detrimental impact of weakfish on bluefish (-0.009) is also not larger because of the relatively small biomass of weakfish. In comparison, bluefish

have a greater detrimental impact on weakfish (-0.130) because their biomass is nearly twice as large (2.75 vs. 1.75 mg C/m^2) and thus their consumption is larger. Using model scenarios, predictions can be made on how these interactions change within a given set of assumptions. For example ENA makes it possible to predict how variation in harvest levels of weakfish alter their impact on menhaden and other prey items, as well as bluefish and other competitors.

ENA also provides a methodology for comparing food webs of ecosystems, or for comparing the food web of a single system at different times (Christensen and Pauly 1993; Christian et al. 2005). Various indices included in model output provide a means to compare attributes of specific compartments (e.g. prey items of predatory fish), flows (e.g. harvest), or entire food webs. Again using the lower Neuse River Estuary as an example, Table 2-2 shows ENA output from models representing early and late summer food web conditions during 1997 and 1998. Between 3 and 13 percent of primary production were required to sustain menhaden in these models. In contrast, spot required between 96 and 124 percent of primary production. Thus, during the modeled times spot not only required a larger percentage of primary production but required an import to the system to maintain their biomass. None of the indices presented in Table 2-2 show major differences with time. However their natural variability can be quantified, and scenarios of various harvest levels can then be examined to determine if the impact of harvest on a specific index is within the natural variability observed.

ENA's ability to incorporate fishery harvest into the analysis is another unique feature that demonstrates its potential for ecosystem-based fishery management. Any holistic approach to fisheries management must acknowledge man as a part of the ecosystem and include his activities in the analysis (Ditton 2004). Fishery variables such as landings and discards can be incorporated in a model, and fate of discards can be contained as separate flows (i.e. consumed by another group, incorporated into detritus, or exported from the system). Landings can be further segregated by gear type and discards by taxa. These variables, as well as several economic parameters (e.g. market and non-market prices for each group being harvested), are part of the optional input of Ecopath software (see below).

ENA should not be confused with dynamic (or simulation) modeling. Generally, one can consider systems modeling as involving construction, simulation, and analysis. Both ENA and dynamic modeling involve construction. However the emphasis of dynamic modeling is on simulation through time (and sometimes space) whereas ENA focuses on analysis of the constructed model (or network). Like the other multi-species techniques mentioned above, ENA is descriptive and gives information on ecological conditions for a snapshot in time. Dynamic modeling is intended to be predictive, and provide quantitative estimates of future conditions. Moreover, ENA generally analyzes all linkages between components of the system (as modeled). Process-based dynamic models may focus only on the dominant linkages, and cover poorly the structure

of the system as a whole. However ENA and process-based dynamic models can be used to complement each other. ENA, by providing a snapshot view, can be used to interpret how a simulation model is working, and dynamic models can provide missing information needed to construct ecosystem networks.

Examples Of Ecological Network Analysis In Use

There are numerous examples of ENA in the ecological literature, and its acceptance as an established methodology is apparently growing. Two software packages are typically employed for ENA: Ecopath (http://www.ecopath.org) and NETWRK (http://www.cbl.umces.edu/~ulan/ntwk/network.html). Ecopath is windows driven and oriented toward fisheries applications. Until recently, NETWRK was only available in DOS format. However two new windows versions are available: EcoNetwrk (http://www.glerl.noaa.gov/EcoNetwrk/) and WAND (http://www.dsa.unipr.it/~alle/ena/?Welcome_to_ESIA%21:Software). Ecopath alone has over 2500 registered users in 124 countries with more than 150 published models (Christensen and Pauly 2004). Between the early 1990s and 2004 the number of publications citing Ecopath has increased from less than 10 to over 200 (Figure 2-4). Most of these studies use ENA to characterize a single ecosystem (e.g. Baird and Ulanowicz 1989). Others use it as a tool for comparing ecosystems (e.g. Baird and Ulanowicz 1993; Christian et al. 2005), and a few use it to evaluate the magnitude of stress imposed on a system (e.g. Baird and Heymans 1996). Many authors recommend the use of ENA in

resource management decision making (e.g. Jarre-Teichmann 1998; Pauly et al. 2000), and others suggest it as a tool to quantify ecosystem health and integrity (Mageau et al. 1995; Ulanowicz, 2000).

ENA has been employed to address a number of specific management issues. Ulanowicz and Tuttle (1992) use it to evaluate the effects of over harvesting oysters on various aspects of the Chesapeake Bay food web. Pauly et al. (1998) use ENA to conclude commercial fishing is causing a global decline in the mean trophic level of marine landings and termed the phenomena "fishing down the food web". Walters et al. (1997) demonstrate the use of ENA to conduct fisheries policy analyses that explicitly accounts for multi-species interactions. Using the Exxon Valdez oil spill as an example, Okey and Pauly (1999) describe how ENA can be used as a broadly accessible tool for restoration and resource planning. Christian and Thomas (2003) use ENA to evaluate the response of nitrogen loading in the Neuse River Estuary, and Baird et al. (2004) use it to examine changes in trophic structure and energy flow due to hypoxia in the same system. Christian and Luczkovich (1999) use ENA to quantify the effects of over wintering waterfowl on the structure of a seagrass food web at a National Wildlife Refuge. These examples suggest the range of management issues that ENA can used to address.

Indeed, there are numerous examples of government entities using ENA for management of coastal and ocean resources. The Atlantic States Marine Fisheries Commission is moving toward using ENA to augment single species

management approaches (ASMFC 2003), and the South Atlantic Fishery

Management Council is incorporating ENA into its Fishery Ecosystem Plan to

examine the system level effects of fishing (SAFMC 1998; Pugliese et al. 2004).

NOAA has initiated a number of projects related to ecosystem-based

management where ENA plays an integral part. One involves development of a

food web model of the Chesapeake Bay "to support and guide ongoing multi-

species management and research, to address commitments in the Chesapeake

Bay Program's Chesapeake 2000 Agreement, and to assist in implementing the

Fishery Ecosystem Plan for the Chesapeake Bay" (NCBO 2003). A second

example involves NOAA's Great Lakes Environmental Research Laboratory

where ENA is being used to examine the impact of non-native species on the

Great Lakes food web (Mason 2004). Another federal management entity, the

Biological Resources Division of the United States Geological Survey, has used

ENA to augment simulation models that examine the response of the South

Florida Ecosystem to changes in its hydrologic regime (Ulanowicz 2003). Finally

at the international level, ENA is being used in the Large Marine Ecosystem

approach (see http://www.lme.noaa.gov) to the assessment and management of

marine resources (e.g. Pauly and Christensen 1993). Thus, ENA is widely used

by the marine resource management community.

The Devil Is In The Details - Limitations Of Ecological Network Analysis

A major source of concern with ENA is the large amount of data needed to construct a food web network. This includes the following: standing stock biomass of all producers, consumers, and detrital groups; diet distribution of all consumers; values for imports and exports of organisms and materials; process or flow rates including production, consumption, egestion, and respiration (Ecopath software calculates one of the flows, usually respiration, whereas NETWRK requires all flows as input); and data on environmental factors that affect the rate of these flows (e.g. temperature). Few studies have a field component specifically designed for ENA. For those that do (e.g. Christian and Luczkovich 1999), direct measurement of each parameter is rare. Standing stocks and diet of selected groups (e.g. fish) are often the only parameters measured. Flows are difficult to quantify and these, along with most of the input data, are often taken from the literature or derived. For example, body size estimates of consumers are typically necessary because they can be used in algorithms to calculate production (Peters 1983) which can then be used with gross food conversion efficiencies to calculate consumption (or vice versa).

Even with these large data requirements, the increased interest and use of ENA has led to few studies that validate results or recognize uncertainty in the models. Given the inherent complexities of modeling food webs, such practices should be routine. Sources of this uncertainty include: 1) natural variability of input parameters (e.g. changes in biomass and diet); 2) data collection methods

(e.g. selectivity among gear types); 3) model construction (e.g. choices in aggregating species into compartments); and 4) fundamental assumptions of ENA (e.g. steady-state conditions).

Natural variability of input parameters such as biomass, diet, and flows has the potential to introduce uncertainty in model output. The biomass of a population is a function of many things including environmental conditions, prey availability, and predator density. As a result, biomass data can be highly variable and data collected at a particular location or season may differ significantly from that collected at another point and time in the same system. Because of the considerable data requirements of ENA, a food web network is typically based on data from numerous studies collected at different times and places within the boundary conditions of the model (e.g. Baird and Ulanowicz 1989). Thus, abundance data for a consumer group is likely to have been collected from a different location and/or at a different time than that of its prey. The consequences of this on data output are generally unknown and untested.

A similar problem exists with diet and flow data. For the most part, values for diet relationships and flows (i.e. production, consumption, egestion, and respiration) for ENA studies often come from the literature. For example, a common source of information for fishes is FishBase (http://www.fishbase.org/home.htm). Investigators must use the "best available information", but that information may be from an entirely different system, time of year, and/or collected under different environmental conditions from those

represented in the model (e.g. Haflon et al. 1996). Variability or uncertainty

introduced into the analysis of a model by using literature values for input data

has not been adequately addressed.

For the few ENA studies that do quantify feeding relationships, the diet of

larger organisms (e.g. fish) are often obtained by examining gut contents while

information for smaller organisms comes from the literature (e.g. Luczkovich et

al. 2002). Although gut content analysis is a well accepted tool for quantifying

diet relationships, there are limitations. The technique is very labor intensive

resulting in low sample sizes, differential rates of digestion can lead to false

relative abundance of each food item, and gut contents are only representative of

an organism's most recent feeding history. Most fish typically digest their food

particles on the order of hours or days (Bond 1996). However, data from gut

content analysis is used in many ENA models representing seasonal or yearly

food web conditions.

A second area of concern involves appropriate data collection methods.

These are critical for ensuring all species and size classes present in the real-

world food web are adequately represented in a model. The clearest example of

how data collection methods influence model input is to consider gear selectivity

in fisheries sampling. Practically all fish sampling methods are selective for the

species and size classes they capture (e.g. Rozas and Minello 1997). Moreover,

different species and size classes vary in how they react to different sampling

methods. Many issues such as mesh size, location of sampling in the water

column, species "catchability" or avoidance ability, and catch per unit effort will impact what is collected and therefore what is used as model input. Similar issues exist in sampling zooplankton (i.e. ctenophores, icthyoplankton, and copepods), but benthic organisms present their own set of challenges (Lewis and Stoner 1981). Species mobility is less important for the benthos; however, sampling methods need to accommodate a range of sizes (macrofauna to meiofauna), habitats (epifauna vs. infauna), types of substrate (mud, silt, sand, or gravel), and depths of organisms in that substrate. Few ENA studies, as well as food web studies in general, follow the advice of Cohen et al. (1993) to report yield-effort curves to ensure completeness of sampling.

Another source of model uncertainty involves how models are constructed. Food web scientists have long known that aggregation affects model structure (e.g. Paine 1988). In general, this means grouping similar species into compartments (aggregation) will affect the number and size of compartments as well as the number and size of interactions between compartments. Aggregation of species is necessary to simplify the real-world food web into manageable units that can be represented in a model. A common approach involves grouping taxa according to similarities in diet. However, the decision making process for determining which species are grouped together lacks methodological rigor in most ENA studies (Luczkovich et al. 2002). Species that are the focus of a study, or those considered more important to energy flow, are often placed in separate compartments (e.g. charismatic

megafauna) whereas those considered less important, or are less understood, are typically combined with ecologically similar species (e.g. microfauna). It should be intuitive that indices of trophic structure (e.g. effective trophic level), material cycling (e.g. Finn Cycling Index), and system size and development (e.g. ascendency) (see Table 2-1 for definitions) are a function of how a model is constructed. Abarca-Arenas and Ulanowicz (2002) demonstrated differences in ascendency with changes in the number of compartments, and it is likely the various analyses that make up ENA have different sensitivies to aggregation. This issue has not been adequately assessed.

A fourth source of uncertainty in ENA involves issues inherent to the analyses. There are two major assumptions of ENA: 1) a food web can be represented by a set of simultaneous linear equations; and 2) the system can be represented as being in steady-state (Whipple et al. 2000). The assumption of linearity in the algorithms may be questionable because the equations are representing biological processes (i.e. production, consumption, and respiration) that are often non-linear (i.e. have different rates, relationships to each other, and response to environmental condtions through time). In regard to the steady-state assumption, issues of natural variability in the input parameters are discussed above. Furthermore, inherent to the steady-state assumption is a mass-balance approach. In most cases, when all the information is assembled a model will not balance due to the inconsistencies in the information. When this happens the values of one or more of the terms must be changed until a balance is obtained

(Allesina and Bondavalli 2003). This results in potentially more than one "correct" model, and thus potentially more than one set of "correct" output (Morissette et al. 2003).

A relatively recent outgrowth of Ecopath software is the development of Ecosim (Walters et al. 1997) and Ecospace (Walters et al. 2000). Ecosim is designed to overcome the steady-state limitations of ENA by incorporating temporal changes in input parameters and providing a method for simulation. It was specifically developed for exploring policy scenarios. Ecospace builds on Ecosim by incorporating spatial heterogeneity into the simulation capabilities. For a given model, Ecopath input and output serves to parameterize the algorithims used in Ecosim. Ecospace then replicates Ecosim over a spatial grid where cells represent different habitats. Any uncertainty in the Ecopath base model is potentially compounded as it is used up the heirarchy in complexity from Ecosim to Ecospace.

Every decision a researcher makes during the course of an ENA study affects what the final model will look like and how well that model depicts the real-world food web. Examples of these decisions include the following: defining the geographical and temporal boundaries that a model will represent; designing a field sampling protocol and selecting sample methodology; deciding how to quantify the diet for each compartment (e.g. gut content analysis or literature values); and determining the number of compartments in a model and what species goes into each compartment. Each of these decisions will introduce

some level of uncertainty in the data that goes into a model. A major unresolved question is how does variation in the input data (whether it is due to natural variability, choices in data collection methods, or simply how a model is constructed) affect model output. A better understanding of the effects of these sources of uncertainty, coupled with validation of model output by independent techniques, is critical if ENA is to meet the rigorous challenges of both the scientific and management communities.

Conclusions And Recommendations

ENA has a solid theoretical foundation (Polovina 1984; Ulanowicz 1986, 1997; Fath and Patten 1999) and has great potential for application to both basic science and management. Its use is increasing and with the current emphasis of policy makers on ecosystem-based fishery management ENA could become a routine approach to incorporating science into fishery management decisions. It is clearly one of the few tools available that examines interactions among multiple species, and in doing so it allows a manager to evaluate an entire food web instead of a single component. However, studies to date have not adequately addressed issues of model uncertainty or performed model validation.

Four major sources of uncertainty in ENA have been identified: 1) natural variability, 2) data collection methods, 3) model construction; and 4) assumptions of algorithms. The first two sources of uncertainty result in variation in model

input, and the effect of this variation on model output needs to be more adequately assessed. One approach is to make a priori predictions (i.e. prior to the analysis) on what the resulting output and indices will look like. The predictions should be based on field observations, can be qualitative or quantitative, and should be tested using standard hypothesis testing methods. For example, predictions could involve the relative trophic level position of compartments within a model or the herbivory:detritivory ratio of one model relative to another model. These predictions will either provide support for the model output, or alert an investigator to areas of the output that may be questionable and need further review.

A second way to deal with the effect of uncertainty in model input is to utilize sensitivity analysis. A simple approach would involve altering model input, based on the range of variability observed in the input data, to determine its effect on model output (Cullen and Frey 1999). For example the biomass of a compartment would be varied such that the mean value, then +/- 1 standard deviation of the mean value, is used in separate model runs (Christian et al. 2003). The output and indices from all model runs would then be assessed graphically or statistically to determine if they differ significantly. This should be done for more than one compartment, and a similar approach could be used for other input parameters such as feeding relationships. Computationally this is very time intensive, but it gives insight into how variability in certain input parameters effect model output.

A similar deterministic type of sensitivity analysis is incorporated as a subroutine in Ecopath. Input parameters for Ecopath include biomass, production/biomass, consumption/biomass, and ecotrophic efficiency. Three of these are entered and one, typically ecotrophic efficiency, is calculated. The sensitivty analysis subroutine checks the effect of varying one input parameter, in steps between -50% to +50%, on the value of the calculated parameter (Christensen et al. 2002). Although useful, this sensitivity analysis subroutine only provides the effect of variation in the "known" input parameters on the "missing" parameter calculated by Ecoapth.

The third source of uncertainty involves model construction and how species are aggregated into compartments. Most studies have used an ad hoc approach for aggregation. Hirata and Ulanowicz (1985) proposed grouping species such that a single index, ascendency, is maximized. However a more "structural" alternative, is to employ multivariate techniques to group species based on their predator-prey relationships. In this way, compartments will be made up of species that use similar prey and are consumed by similar predators. Correspondence analysis and/or cluster analysis are examples of methods that have been used successfully (Luczkovich et al. 2002). The advantage of these multivariate techniques is that they are more objective, can be duplicated, and are based on observed feeding relationships.

A fundamental concern with ENA is how well a model depicts the real-world ecosystem. This coupled with questions concerning assumptions inherent

to the analysis (4th major source of uncertainty) can best be addressed by determining if results from ENA are similar to those obtained using a different methodology. Unfortunately, few methods are appropriate for validating ENA output because virtually none provide the same level of information. However, a few techniques provide information that correspond to specific parts of ENA output. For example, among the numerous mathematical calculations performed by Ecopath software is a value for each compartment's respiration as well as community respiration. These values can be compared to field measurements of respiration obtained with established techniques (e.g. dissolved oxygen measurements using diurnal studies - D'Avanzo et al. 1996, light/dark bottles - Valiela 1995, and/or benthic chambers - An and Joye 2001).

A second technique for validating ENA output is stable isotope analysis (SIA). Stable isotopes have been used in numerous studies over the past 30 years to gain a better understanding of aquatic food webs (e.g. Peterson and Fry 1987). In general, stable isotopes of nitrogen can be used to delineate trophic level (Yoshii et al. 1999) and those of carbon, sulfur, and nitrogen can be combined to determine the relative contribution of a primary producer to a consumer (Peterson et al. 1985). Trophic level data given by SIA can be used to validate trophic relationships (e.g. Kline and Pauly 1998) given in the Trophic Structure Analysis component of ENA (Table 2-1). Likewise, information given by SIA on the sources of carbon for consumer compartments can be used to

validate matrices that are a part of the Input-Output Analysis component of ENA (Table 2-1).

ENA has potential to play a key role in moving toward ecosystem-based fishery management because it provides a tool for quantifying direct and indirect trophic interactions, for comparing food web properties among different systems and/or times, and for incorporating fishery harvest into the analysis. However future studies should acknowledge the limitations of ENA, focus more attention on addressing the potential sources of uncertainty, and utilize methods of model validation where possible. Figure 2-5 depicts how ENA is used in most ecological studies. Observations and data are collected from a real-world system and from these a food web model is constructed. ENA is then used to obtain various output and indices that describe the trophic structure and interactions as depicted by the model. Using ecological principles, the output and indices are then interpreted to make inferences about the properties of the real-world ecosystem. Because of uncertainty inherent in the model, several components are recommended for future studies utilizing ENA (Figure 2-5). A priori predictions and sensitivity analysis will help to understand the effect of variation in model input on model output, and model output should be validated by independent techniques to ensure the model adequately represents the real-world ecosystem.

Table 2-1. Description of output from ecological network analysis.

Input-Output Analysis[A] - quantifies direct and indirect relationships between compartments.	Input-Output Analysis[B] - quantifies direct and indirect relationships between compartments.
● Mixed Trophic Impact Matrix - sums the positive and negative impacts of each compartment on every other compartment.	● Total Contribution Matrix - gives the percent of flow through a compartment that passes into another. ● Total Dependency Matrix - gives the percent of flow through a compartment that had once passed through another (e.g. extended diet).
Trophic Structure Analysis [C] - provides information based on the trophic concepts of Lindeman (1942). ● Effective trophic level – fractional value of a compartment's trophic level that takes into account degrees of omnivory. ● Trophic efficiency - the proportion of consumption passed up the food chain. ● Omnivory Index - variance of trophic levels in a consumer's diet.	
Pathway Analysis[A] - characterizes the pathway of flows. ● Pathway from any primary producer to a selected consumer through a specified prey. ● Primary production required to sustain the consumption of each group. ● Herbivory:Detritivory Ratio - quantifies the ratio of flow along grazing and detrital food webs.	Biogeochemical Cycle Analysis[B] - evaluates the characteristics of cycles within the system. ● Number of cycles organized by the smallest common flow. ● Length of cycles and distribution of flow along them. ● Finn Cycling Index - amount of flow involved in cycling.
Information Analysis[C] - quantifies attributes characteristic of the growth and development of the system. ● Total System Throughput - sum of all flows occurring in a system. ● Development Capacity - index of the potential of a network to develop given its particular set of connections and throughput. ● Ascendency - index of the size and developmental potential that a system has attained.	

[A] Ecopath software output.

[B] NETWRK software output.

[C] Output of both Ecopath and NETWRK.

Table 2-2. Values for a portion of the ENA output (defined in Table 2-1) from four networks representing food web conditions in the lower Neuse River Estuary, North Carolina, during early and late summers in 1997 and 1998 (Christian et al. 2003). The networks were analyzed using Ecopath with Ecosim version 5.1. See Figure 2-2 for a schematic of part of the early summer 1997 network.

Output	Early Summer 1997	Late Summer 1997	Early Summer 1998	Late Summer 1998
Trophic Structure Analysis				
Mean Effective Trophic Level	2.7	3.0	2.8	2.7
Mean Ecotrophic Efficiency	0.203	0.368	0.352	0.278
Mean Omnivory Index	0.154	0.183	0.164	0.141
Pathway Analysis				
Percent of Total Primary Production Required for Menhaden	5	3	13	7
Percent of Total Primary Production Required for Spot	124	112	96	115
Herbivory:Detritivory Ratio	0.094	0.091	0.088	0.124
Information Analysis				
Total System Throughput (mg C m^{-2} d^{-1})	18201	16274	18547	18759
Development Capacity [(mg C m^{-2} d^{-1}) (bits)]	77859	64722	76489	75221
Ascendency [(mg C m^{-2} d^{-1}) (bits)]	37281	34436	39587	38896

Figure 2-1. Conceptual model showing the hierarchical nature of ecosystem-based fishery management. Management decisions require integrating disciplines such as biology, physical sciences, sociology, and economics. Inherent to this integration is a knowledge of the interactions embedded within each discipline. For example, the salinity regime of an estuary affects the timing, abundance, and size of species present (i.e. biology). This impacts fishery harvest efforts (i.e. economics), and must be incorporated into harvest regulations (i.e. sociology). However, to understand the salinity regime one must have knowledge of the interaction between wind patterns, tides, basin geomorphology, and rainfall (stream discharge).

Biology

Trophic Relationships

| Species A | → | Species B |

Species A → Species C → Species D

Physical Sciences

Salinity Regime

Wind ↔ Geo-morphology

Tide ↔ Rainfall

Sociology

Human Dimensions

Values ↔ Politics

Regulations ↔ Policy

Economics

Ex-vessel Price

Market Price ↔ Demand

Landings ↔ Price of Substitute
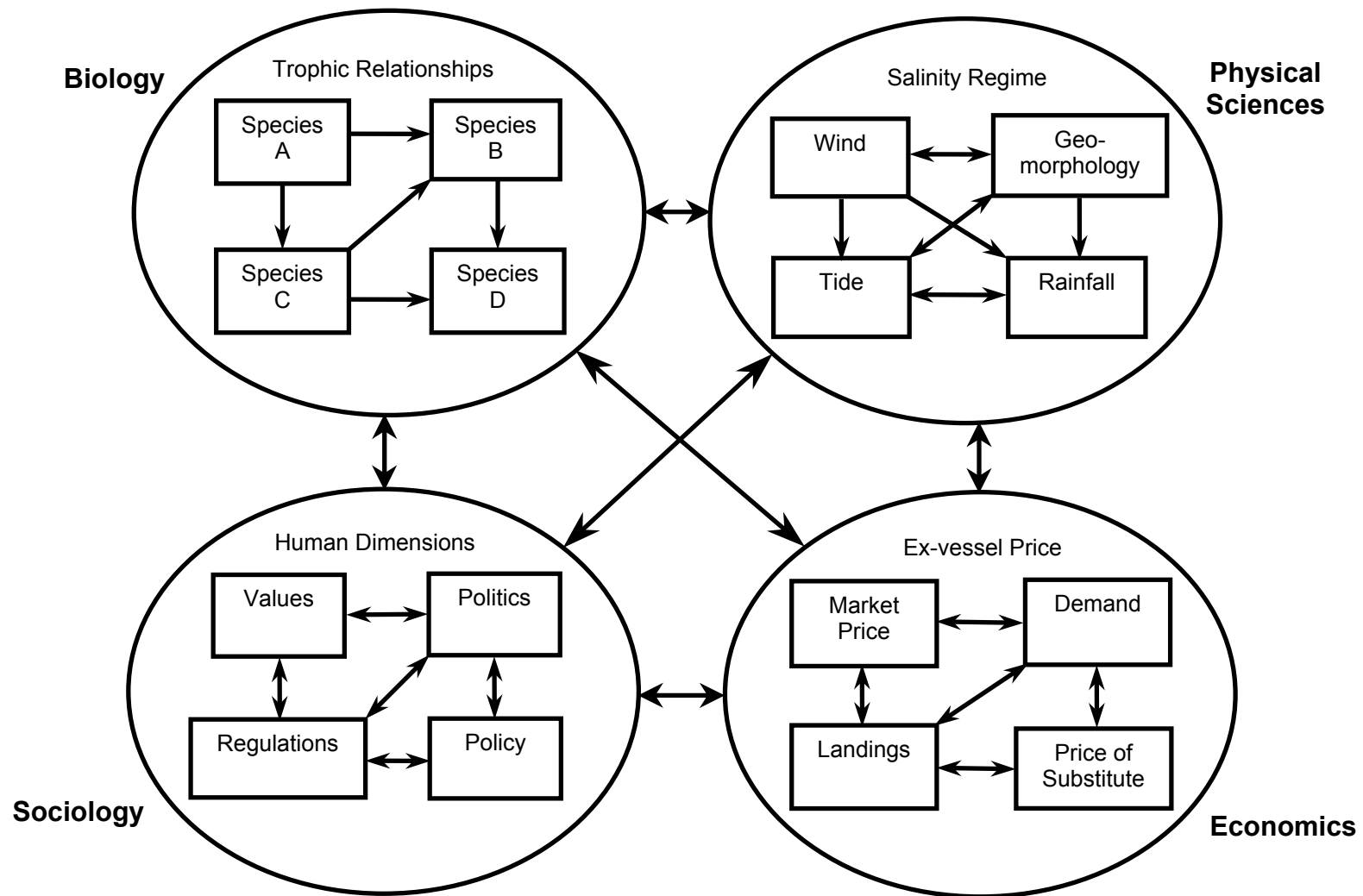
Figure 2-2. Simplified box and arrow diagram of a portion of the lower Neuse

River Estuary, North Carolina, food web during early summer 1997 (Christian et

al. 2003). Boxes, or compartments, represent single species or aggregates of

species with similar diets and predators (values are biomass in mg C m^{-2}).

Arrows represent the feeding relationships, or flow of material, between

compartments (units for flow values are mg C m^{-2} day^{-1}). This network was

analyzed using Ecopath with Ecosim version 5.1.
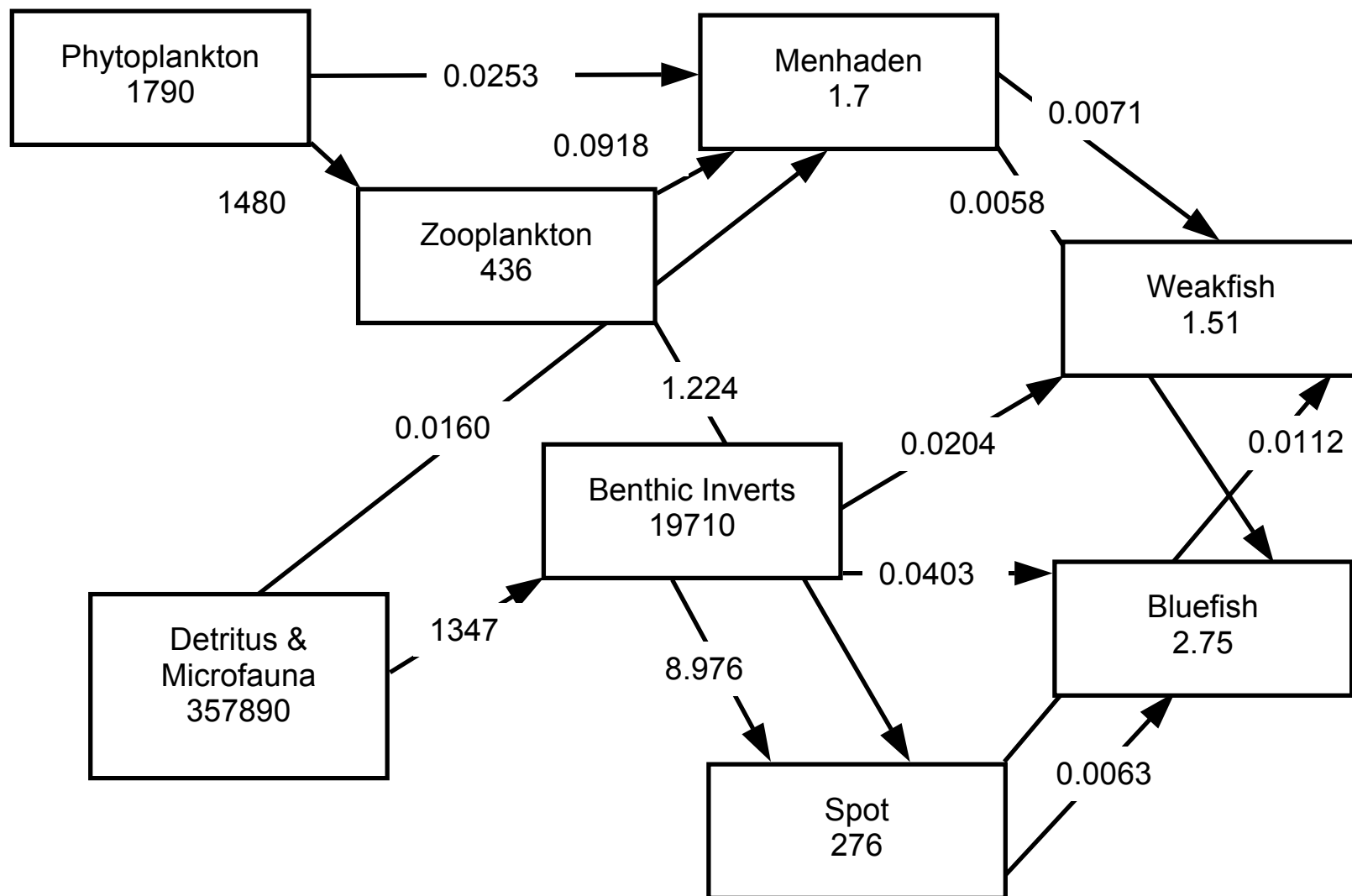
Figure 2-3. Schematic illustrating a portion of the mixed trophic impact matrix given by Ecopath analysis of the network shown in Figure 2-2. Direct and indirect impacts that a group on the left (rows) have on a group given in the columns are quantified. Net positive impacts are indicated by a bar pointing upward, while a bar pointing downwards shows a net negative impact. These, and their corresponding matrix values, should not be interpreted in an absolute sense. The impacts are relative, but comparable between groups. In this example, weakfish have a negative impact on their prey, menhaden, and an slight indirect positive impact on the prey of their prey, zooplankton. A group negatively impacting on itself indicates intra-specific competition.

IMPACTED GROUP

IMPACTING GROUP

	Zooplankton	Menhaden	Weakfish	Bluefish
Zooplankton	-0.483	+0.342	+0.010	+0.026
Menhaden	-0.034	-0.078	+0.022	0.074
Weakfish	+0.025	-0.333	-0.408	-0.009
Bluefish	+0.020	-0.276	-0.130	-0.028
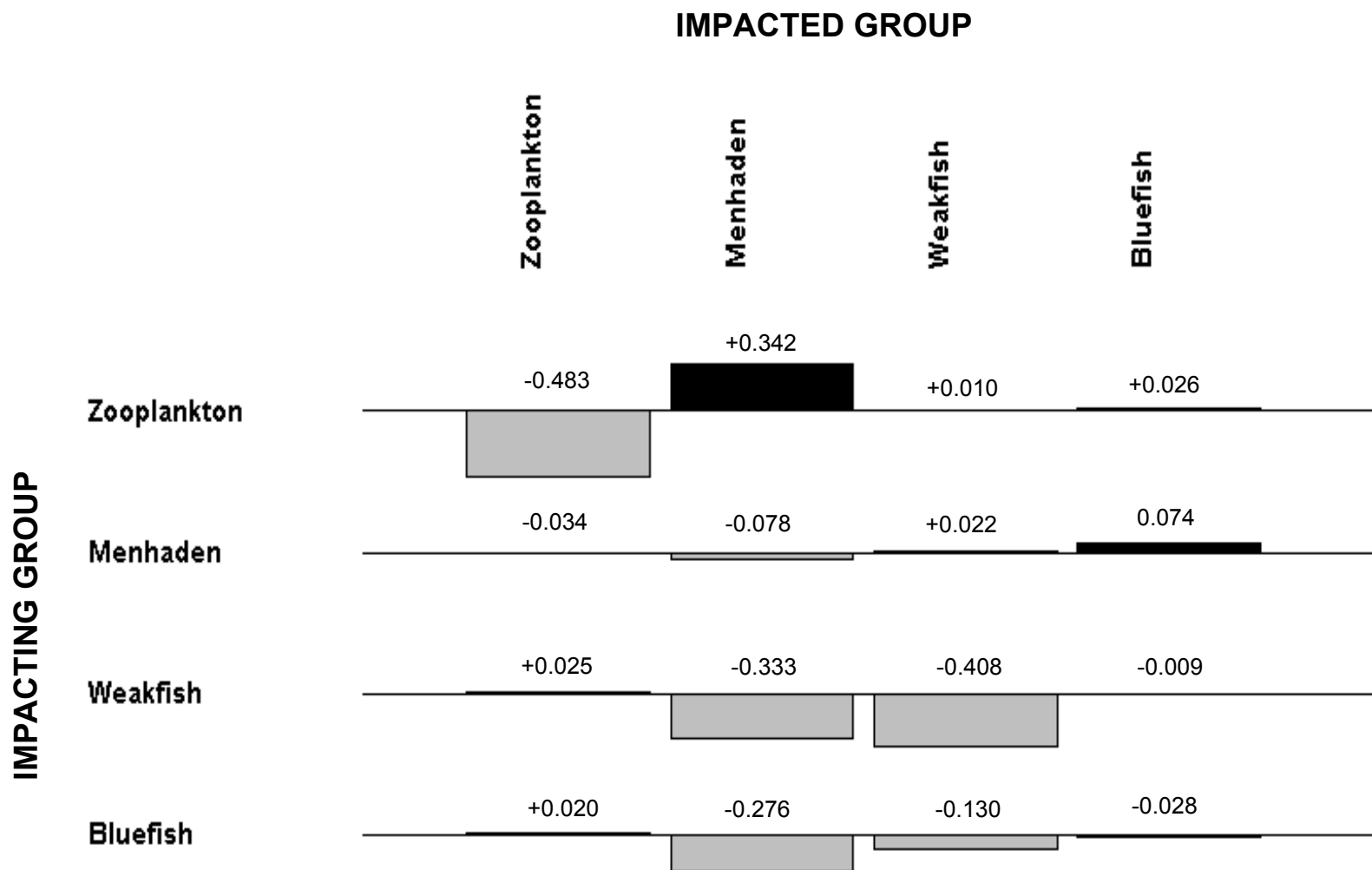
Figure 2-4. Trends in the number of publications citing Ecopath in their abstract.

Data is from searching the following databases: Biological Abstracts, BioOne,

Cambridge Scientific Abstracts, and Web of Science.
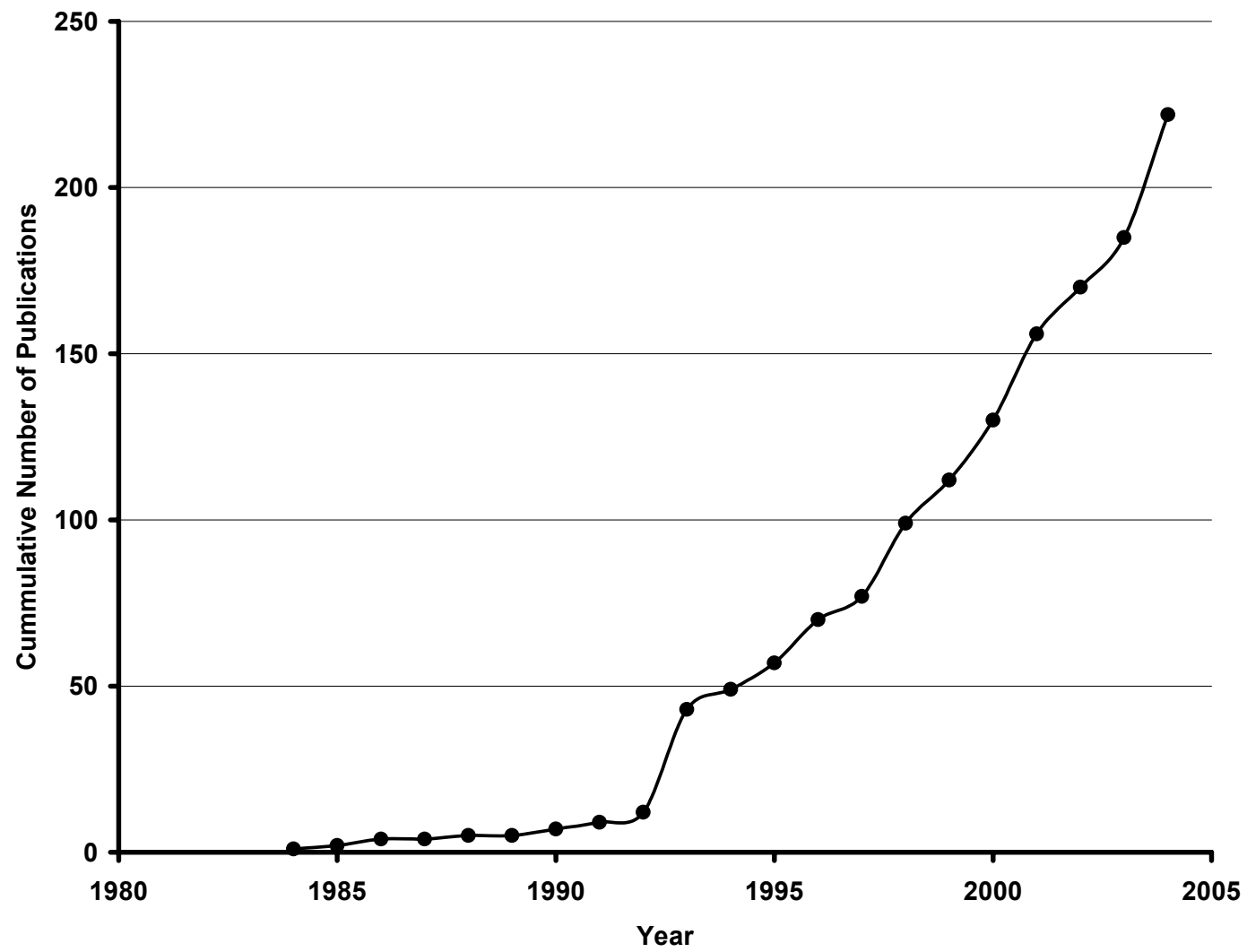
Figure 2-5. Conceptual model of how ENA is used in most studies (solid lines).
Dashed/italicized components represent recommendations to incorporate into
future studies. Predictions made prior to performing ENA (a priori) and sensitivity
analysis will help to understand the effect of variation in input data on model
output. Model validation is necessary to provide confidence in the output.

| Real World System | | Network Model | | Output & Indices | | Ecosystem Properties |

• *A Priori Predictions*
• *Sensitivity Analysis*

Observations   Inference from Theory   Network Analysis

Validation
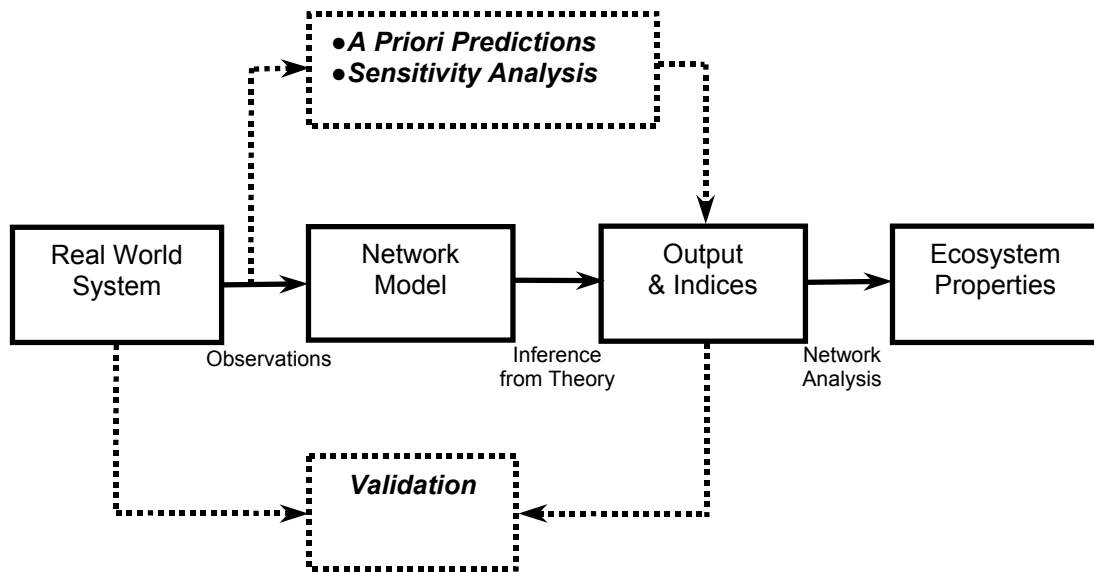
CHAPTER 3. FOOD WEB DATA FOR A REPLICATED ECOSYSTEM

Abstract

Detailed data and metadata are provided for defining food web

relationships in four salt marsh ponds during three times. The ponds are located

in the Virginia Coast Reserve Long Term Ecological Research site (37.48^o North

Latitude, 75.66^o West Longitude), and times correspond to periods of low stress

(spring 2002), high stress (late summer 2001), and post-disturbance (period

immediately following recovery of water levels from drought in summer 2002).

Stress/disturbance levels are characterized by changes in dissolved oxygen,

salinity, temperature, and water depth. Containing a total of 4,640 records, this

data set represents an intense sampling and comprehensive documentation of

both physical and biologic variables. Physical data includes area, depth,

dissolved oxygen, salinity, and temperature. Biologic data includes abundance

and body size of 60 taxa utilizing the ponds. The original purpose for collecting

the data was to construct trophic models for evaluating the effectiveness of

network analysis in detecting change in trophic conditions. Input parameters and

quantitative diet relationships for use in Ecopath software are included from this

effort. These involve biomass, production/biomass, consumption/biomass,

egestion/consumption, and quantitative diet matrices for aggregated taxa.

Additionally, stomach content data are provided that include the percent of each

prey item for different size classes of five fish taxa representing the three times.

Stable isotope data including δ^{13}C, δ^{15}N, and δ^{34}S values for both primary

producer and consumer taxa are also provided. All physical data, abundance, body size, biomass, stomach content data, and stable isotope data are based on field sampling. Other information was either calculated from accepted algorithms (e.g. production from body size) or obtained from the literature (e.g. diet of aquatic insects). This data set should prove useful for augmenting other efforts in food web research and network ecology given the detail and replication involved.

Introduction

Food webs have been suggested as a unifying concept for ecology (e.g. Cohen 1991), and they are the basis of various advances in our understanding of community structure and dynamics including keystone predation and herbivory (e.g. Paine 1966), intermediate disturbance hypothesis (Connell 1978), trophic cascades (Pace et al. 1999), and the importance of indirect effects (Abrams et al. 1996) and feedbacks (Ulanowicz 2005). However, the "white elephant" with respect to food web studies is the quality and completeness of the data. Paine (1988) raised this question, and Cohen et al. (1993) made recommendations to address the issue. These include: 1) be as explicit as possible in documenting sampling design, methods, and effort; 2) be as exhaustive as possible in field collections and laboratory analyses; and 3) make detailed data available for secondary analysis or incorporation into larger studies of ecological patterns. Although some projects have provided extensive information of this type (e.g.

Martinez 1991), additional efforts are needed to make whole data sets available for further advancement of food web research and the corresponding development of network ecology (see Belgrano et al. 2005).

The objective of this paper is to carry out the recommendations of Cohen et al. (1993), and apply them to an extensive study of replicate salt marsh pond food webs (n = 12). The ponds provide ideal systems for analyzing trophic relations in a natural setting because they have relatively well defined boundaries, provide an opportunity for thorough documentation of all taxa, and allow for replicate systems to be documented. Few food web data sets of natural systems have the level of detail or replication provided here. Intense field sampling involved documenting changes in water quality parameters (dissolved oxygen, salinity, temperature, and depth) and quantifying the density, size, and where possible feeding habits of all taxa (bacteria to birds) utilizing four ponds during three sampling events. To insure completeness of sampling, separate yield-effort curves (Cohen et al. 1993) were performed for different methods involving fish, macro-invertebrates, meiofauna, zooplankton, and stomach content analysis. Sampling occurred over approximately one month periods during late summer 2001 and 2002, and spring of 2002. Based on water quality data, these times roughly corresponded to periods of low stress (spring 2002 = relatively low salinity, low temperature, high dissolved oxygen), high stress (late summer 2001 = relatively high salinity, high temperature, low dissolved oxygen),

and post-disturbance (late summer 2002 = immediately after recovery of water levels from drought).

The data were originally collected to construct trophic models for evaluating the effectiveness of network analysis in detecting change in trophic conditions (see Chapter 4). Taxa were aggregated into compartments based on common prey and predators using correspondence analysis (Luczkovich et al. 2002). In addition to data on density and body size, biologic variables in this data set include input parameters to analyze the pond food webs using Ecopath software (Christensen and Pauly 1992). These involve biomass, production/biomass (P/B), consumption/biomass (Q/B), egestion/consumption (E/Q), and quantitative diet matrices for aggregated taxa. Biomass was based on field sampling while P/B, Q/B, and E/Q were calculated from published algorithms or obtained from the literature (e.g. P/B from Banse and Mosher 1980; Q/B using similar methods as Christian and Luczkovich 1999; E/Q from Ulanowicz et al. 2003). Diet was either obtained from the literature (e.g. aquatic insects from Merritt and Cummins 1984), or from stomach content analysis (for all fish taxa excluding *Anguilla rostrata*) using the sieve fractionation technique (Carr and Adams 1972, 1973; Luczkovich and Stellwag 1993). An additional component of this data set is information on stable isotopes including δ^{13}C, δ^{15}N, and δ^{34}S values for primary producers and consumers representing pond food webs in spring 2002 and late summer 2002.

Issues of data quality and completeness are well documented in the food web literature (e.g. Hall and Raffaelli 1993), and similar concerns exist with network analysis (see Chapter 2). Making data available on well documented food webs will aid in addressing these issues and increase our understanding of trophic interactions.

Metadata Class I. Data Set Descriptors

Data Set Identity

Title: Food web data for a replicated ecosystem.

Data Set Identification Code

Suggested Data Set Identity Codes:

Area-Depth.txt

Salinity-Temp.txt

Diurnal_Curve_DO.txt

Light-Dark_Bottle_DO.txt

Abundance-Size.txt

Stomach_Contents.txt

Ecopath_Input_Parameters.txt

Ecopath_Stacked_Diet_Matrix.txt

Stable_Isotope_Data.txt

Data Set Description

Principal Investigators:

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North Carolina 27858 USA.

Key Words

dissolved oxygen; disturbance; Ecopath; food webs; salinity; salt marsh ponds;

stomach contents; stable isotopes; stress; temperature; trophic networks.

Metadata Class II. Research Origin Descriptors

Overall Project Description

Identity

      Detailed food web data for replicate salt marsh ponds

Originator

      James K. Dame

Period Of Study

      Late Summer 2001 (03 September - 10 October), Spring 2002 (20 May -

25 June), and Summer 2002 (25 July - 17 August).

Objectives

To construct relatively thorough trophic networks (n = 12) of four salt marsh ponds under three different stress/disturbance conditions.

Abstract

This project endeavors to make detailed food web data available for analysis of trophic interactions or incorporation into larger studies of ecological patterns. Few examples of replicated trophic networks exist in the literature, and even data sets of single food webs are rarely based on comprehensive field sampling designed specifically for quantifying trophic interactions. It is anticipated that this data set will help to fill that void and be easily accessible to the public.

Specific Subproject Description

Site Description

The four study ponds are located in the upper elevations of a mainland salt marsh at the headwaters of Upper Phillips Creek within the Virginia Coast Reserve Long Term Ecological Research site on Virginia's Eastern Shore (37.48°

North Latitude, 75.66^o West Longitude). Pond dimensions were roughly 30 m x

3-5 m x 0.5 m. Pond walls were nearly vertical, and their bottoms contained a 5

to 15-cm layer of fluid organic muck (or fluff layer) underlain by marsh peat.

North and South Ponds lie in a hummock and hollow zone dominated by *Spartina*

patens and *Distichilis spicata*. East and West Ponds are located further up-

gradient in a forest transition zone (mixture of *Spartina patens*, *Distichilis spicata*,

Iva sp., and *Juniperus virginiana*).

Experimental Or Sampling Design

Separate sampling events were performed during late summer 2001 (03

September - 10 October), spring 2002 (20 May - 25 June), and summer 2002 (25

July - 17 August) to document a range of trophic conditions in each pond. For

each event, sampling occurred over approximately a one month period. The

times correspond to different levels of stress and disturbance. Late summers are

characterized by high stress conditions because of relatively high salinity, high

temperature, and low dissolved oxygen. The opposite is found in early spring

when low stress conditions prevail. The summer 2002 sampling event was

performed immediately after recovery from a drop in water level due to drought.

Sampling began approximately 5 days after water levels were restored after

rainfall. The food web in each pond at this time represents a post-disturbance

condition. For the purposes of sampling and enumerating organisms the ponds

were conceptually separated into 3 communities: benthic, epiphytic, and pelagic.

Spatial boundaries were defined by the sides of each pond and the base of the "fluff" layer at the bottom of each pond.

Research Methods

Field And Laboratory Methods

Given below are details of the field and laboratory methods used in data collection and analysis. They are separated into the following sections: 1) density, size, and biomass estimates; 2) diet analysis; 3) aggregation of taxa for construction of Ecopath models; and 4) physical parameters. Numbers correspond to compartments in the models. Next, documentation of the Ecopath models is given. This includes two sections: 1) sources and references of all compartment inputs; and 2) rules used in balancing the models.

Density, Size, And Biomass Estimates

Primary producers (compartments 1-5). Phytoplankton (4) and benthic microalgae (1) biomass were estimated from fluorometric measurements of chlorophyll a as outlined by Strickland and Parsons (1972: pages 201-203) and modified by L. Clough and T. West (East Carolina University, personal communication). Phytoplankton biomass was estimated from four 100-ml filtered water samples (Whatman 934-AH filter) collected at mid-water depth, and benthic microalgae from four 5-ml samples collected from the fluff layer with a sterile syringe. In both cases, samples were taken from randomly selected

locations in each pond during each sampling event. Laboratory analysis involved extraction of pigments using 10-ml of an acetone mixture (45% acetone, 45% ethanol, 10% deionized water), and reading on a Turner fluorometer (Model TD 700). Extractions occurred over a 12-24 hour period at a temperature below 32 ^{o}C. Following initial readings, the samples were acidified with 10% HCL and read a second time to provide a correction for pheophytin pigments. Chlorophyll values were then converted to µg C m^{-2} (see Table 3-1).

Biomass estimates of submerged aquatic vegetation (5), overhanging vegetation (3), and associated epiphytic algae (2) were made from random samples collected from each pond. During spring, percent cover of Ruppia maritima in each pond was visually estimated, and then it was harvested from 4 to 6 0.5-m^2 quadrats along a pond's edge and 4 0.25-m^2 quadrats away from the edge (see Macroinvertebrates section for detailed description of quadrats). For all sampling events, submerged overhanging leaves of Spartina patens and Distichilis spicata were harvested from 4 to 6 of the "edge" quadrats. All harvested material was bagged and refrigerated. Within 24 hours of collection, macroinvertebrates were removed from all samples (see below) and the vegetation was weighed and frozen. Epiphytic algae and small invertebrates were removed from sub-samples of the frozen vegetation by scraping (Dauby and Poulicek 1995), and then they were sorted, weighed, and dried. Loss on ignition (Allen 1989) was then used to calculate biomass in µg C m^{-2} from ash free dry weight (Table 3-1).

Microbes (compartments 6-9). Microbal biomass was calculated from total direct counts and biovolume estimates using epifluorescence microscopy. Samples were collected from four random locations in each pond during each sampling event. Separate samples were collected for H_2O column bacteria (6), H_2O column microprotozoans (7), sediment bacteria (8), and sediment microprotozoans (9). H_2O column samples were taken from mid depth and consisted of 19-ml of pond water with 1-ml of 37% formaldehyde. Sediment samples were taken from the fluff layer and contained 1-ml fluff and 19-ml of 2% bacteria free formaldehyde. Acridine orange stain was used for H_2O column bacteria (Hobbie et al. 1977), fluoroscein isothiocyanate (FITC) for microprotozoans (Sherr and Sherr 1983), and 4'6-diamino-2-phenlindole (DAPI) for sediment bacteria (Porter and Feig 1980; Schallenberg et al. 1989).

Laboratory techniques followed those of Heath (1989) when using acridine orange and FITC stains. The DAPI protocol involved a 0.1 mg/ml stock solution that was refrigerated for 24 hours prior to use. Sediment bacteria field samples were diluted with 0.1 M tetrasodium pyrophosphate (0.25-ml field sample + 9.25-ml TSP), and sonicated (FS14H Fischer Scientific) for 10 minutes to separate bacteria from sediment particles. Sonicated samples were then stained with 0.5-ml of DAPI stock solution and incubated for 20 minutes prior to being vacuum filtered through hydrolan soaked 0.2-μm Nuclepore filters. Each filter was mounted on a microscope slide, and density counts and biovolume estimates

were then performed as described in Heath (1989). Microscope work was accomplished with a Nikon epifluorescent microscope (Model XF-EF) equipped with a Mercury lamp (HBO-50) and a 100x oil immersion objective (1250x magnification). Density and biovolume were converted into biomass in μg C m^{-2} (Table 3-1).

Zooplankton and meiofauna (compartments 10-11). Both zooplankton (10) and meiofauna (11) were collected from 4-6 random locations in each pond during each sampling event. Zooplankton samples were collected by filtering 75 L of pond water (Figure 3-2) through an 80-μm mesh plankton net. Concentrated pond water (100 ml) from the cod end of the net was then fixed in 10% formalin with rose bengal. Meiofauna samples consisted of 6.5-cm diameter cores from the fluff layer (Figure 3-3) and were also preserved in 10% formalin with rose bengal.

In the laboratory, zooplankton density and biovolume/individual were estimated from 1-ml sub-samples of each concentrated field sample using an Olympus stereo-zoom trinocular microscope and a Sedgewick-Rafter cell. Four to six sub-samples were used to estimate zooplankton density in each field sample. Biovolume of 4-6 individuals of each taxa were estimated from each sub-sample and converted to biomass in μg C/individual (page 182, Higgins and Thiel 1988). The same general approach was used for meiofauna. However, meiofauna field samples were first washed through a series of seives (250-μm,

125-µm, and 63-µm screens) to separate detrital material. Densities for each field sample were calculated from a 1-ml sub-sample from each screen, and estimates of biomass/individual were obtained in the same manner as for zooplankton. Densities and biomass per individual were then used to calculate biomass in µg C m^{-2}.

   Macroinvertebrates (compartments 12-20). Macroinvertebrates were associated with microhabitat located along pond edges and when present within leaves of Ruppia maritima. Quadrats used in sampling several of the primary producers (i.e. overhanging vegetation, R. maritima, and associated epiphytic algae) were also used for sampling macroinvertebrates. "Edge" quadrats consisted of a 3 sided (0.5-m per side) enclosure made of 1-mm nylon mesh with wooden stakes for support. With the pond bank acting as the fourth side, a 0.5-m^2 quadrat was formed when the ends of the mesh were placed snugly against the pond's bank. "Ruppia" quadrats were essentially a 0.25-m^2 enclosure made of 1 mm nylon mesh and PVC pipe. For both types of quadrats, the mesh extended from above the water's surface to the pond's bottom.

   Between 4-6 "edge" quadrats were sampled in each pond per sampling event (Figure 3-4), and 4 "Ruppia" quadrats were sampled in each pond when *R. maritima* was present (low stress conditions). Sampling consisted of removing the vegetation for primary producer biomass estimates, and then using a dip net (1-mm mesh) to collect macroinvertebrates from the area within each quadrat.

The pond's edge and sides of the quadrat were repeatedly agitated, and sweeps made until four consecutive sweeps result in no additional individuals. Specimens were preserved in 70% isopropyl alcohol, later identified to the lowest possible taxon (McCafferty 1981; Merritt and Cummings 1984), sorted into size classes (0.5-cm increments), and enumerated. Loss on ignition (Allen 1989) was performed on representatives from each taxa and size class, and biomass (μg C m^{-2}) of each taxa was calculated (Table 3-1).

Nekton (compartments 21-25). *Anguilla rostrata* (21) was collected in quadrats, and its biomass estimated, as described for macroinvertebrates above. Other fishes (22-25) were sampled with a seine net (3.2-mm mesh, 1.2-m high). Sampling was performed by positioning the seine tight against both banks at one end of a pond, and then pulling the seine along the length of the pond while maintaining contact with the banks. Both ends of the seine were then brought together using the bank as a barrier, and the contents were placed into a graduated container. To limit mortality and inhibit disturbance to the pond community, contents of the seine were mixed and sub-sampled (Figure 3-5) with the remainder returned to the pond. Specimens were placed on ice and returned to the laboratory where they were identified to genus or species (Hildebrand and Schroeder 1927), sorted into size classes (1-cm increments), and enumerated. Wet weights were obtained for each size class and used to convert to biomass in μg C m^{-2} (Table 3-1). Data were corrected to account for differences in volume

of sub-sample vs. volume of catch, and for seine efficiency (Allen et al. 1992; Rozas and Minello 1997). Seining was performed 1-2 times in each pond for each sampling event after all other data had been collected. Seining was disruptive to water quality and pond morphology, and performing multiple seines could potentially affect subsequent sampling events. As a result, it was necessary to obtain estimates of seine efficiency visually from observations of the ponds during and after seining.

Amphibians (compartments 26-27). Two techniques were used to quantify use of the ponds by amphibians. Tadpoles (26) were collected in quadrats, and their biomass estimated, as described for macroinvertebrates above. Adult frogs (27) were counted during night surveys (eye shining) of the area within and immediately surrounding the ponds (Heyer 1994) at four times during each sampling event. Several adults from each set of ponds were collected to obtain size and wet weight information. This was used with density estimates to calculate biomass in µg C m^{-2} (Table 3-1).

Snakes and birds (compartments 28-29). Snakes (28) were prevalent in the ponds during spring (low stress conditions). Densities were estimated from funnel trap collections and observations of their swimming habits. On a minimum of 4 instances per sampling event, two funnel traps were placed in each pond for between 4-8 hours. For a portion of this period the pond was monitored for

snake activity. Density estimates involved keeping track of the number of free-swimming individuals and adding the number of trapped individuals. Trapped individuals were then returned to the pond. Biomass in µg C m^{-2} was estimated for each pond (Table 3-1) using an average wet weight of 291-g (Ulanowicz et al. 2003).

Wading bird (29) use of the ponds was highly variable. Large numbers were present when water levels in adjacent areas of the marsh were too low to permit their use for feeding (Gawlick 1996). Other variables which appeared to affect their presence was tide level and activity of fish in the ponds. Density and feeding activity of wading birds were estimated from 3-5 surveys of each pond during each sampling event. Surveys were conducted from before sunrise until mid-morning, and involved discreetly observing the ponds from a distance that allowed documenting individuals and their successful feeding attempts. Because of proximity, North and South Ponds were surveyed simultaneously and likewise for East and West Ponds. Average wet weights from Dunning (1993) were used to calculate biomass in µg C m^{-2} (Table 3-1).

Detritus (compartments 30-31). Biomass of dissolved organic carbon (DOC - 30) was estimated from four 20-ml surface water samples. A set of samples was collected from each pond during spring (low stress) and post-disturbance sampling events. Analysis was performed at the University of North Carolina's Institute for Marine Science. Samples for estimating particulate

Table 3-1. Conversion factors used in biomass calculations. AFDW = ash free dry weight from loss on ignition.

Compartment	Parameter	Value Used	Source
Benthic Microaglae (1)	Carbon from Chlorophyll a	42 x Chlorophyll a	Foreman 1985; Taguchi 1976; Valiela 1995
Epiphytic Algae (2)	Carbon from AFDW	45% AFDW	USACE 1997; Jorgensen et al. 1991
Herbacious Vegetation (3)	Carbon from AFDW	45% AFDW	Bowen 1966; Jorgensen et al. 1991
Phytoplankton (4)	Carbon from Chlorophyll a	62 x Chlorophyll a	Enryquez et al. 1996; Valiela 1995
Ruppia maritima (5)	Carbon from AFDW	32.7% AFDW	Twilley et al. 1986
Bacteria (6 & 8)	Carbon from Biovolume	425 fg C/μm^3	Loferer-Krößbacher et al. 1997
Microprotozoa (7 & 9)	Carbon from Biovolume	170 fg C/μm^3	Putt and Stoecker 1989
Hydrobiidae (12)	Carbon from AFDW	39.9% AFDW	Bowen 1966
Amphipod (13)	Carbon from AFDW	49% AFDW	Salonen et al. 1976
Crabs and Shrimp (13)	Carbon from AFDW	44.6% AFDW	Bowen 1966
Aquatic Insects (14-20)	Carbon from AFDW	47.5-53.5% AFDW	Salonen et al. 1976
Fish (21 - 25); Frogs (27); Snakes (28); Birds (29)	Dry Weight from Wet Weight	20% Wet Weight	Waters 1997; Jorgensen et al. 1991
	Carbon from AFDW	45% Dry Weight	USACE 1997; Jorgensen et al. 1991
Fish (21-25)	Seine Efficiency	30-50%	Visual estimates; Allen et al. 1992; Rozas and Minello 1997

organic carbon biomass (POC - 31) were collected at the same time as those for

DOC, and during the high stress sampling event (late summer 2001). POC was

estimated from filters (Whatman 934-AH) obtained from vacuum filtering 100-ml

of pond water (n = 4). The filters were analyzed on Exeter Analytical's Model 440

Elemental Analyzer. Ratios of DOC to POC were calculated for each pond with

the samples that were collected to estimate DOC for the high stress time period.

Diet Analysis

Diet information for fish compartments 22 through 25 was obtained from

stomach content analysis. Literature values were used for all other consumer

compartments (see Model Documentation). Stomach content analysis was

performed using the sieve fractionation methodology of Carr and Adams (1972,

1973) as modified by Luczkovich and Stellwag (1993). Stomach contents of

each taxa within 1-cm size classes from each pond were pooled and filtered

through a series of sieves ranging from 75 to 200 μm mesh. The contents of

each sieve were then grouped into food categories and enumerated. Each sieve

fraction was then dried at 60°C for 24-48 hours and weighed. The proportional

contribution of each food source was estimated using the numerical counts and

mass of each sieve fraction. A maximum of 15 individuals were combined when

pooling stomach contents (Figure 3-6).

Aggregation Of Taxa For Ecopath Models

Taxa, and their different size classes, were aggregated into compartments using correspondence analysis as outlined by Luczkovich et al. (2002). First, a stacked rectangular binary diet matrix was constructed from literature values and stomach content analysis. Columns of the matrix were made up of prey items, and rows represented consumers broken down into taxa, size class, pond, and time. Rectangular refers to the number of rows not being equal to the number of columns, and stacked refers to the matrix including data from all four ponds and all three sampling events. Next, factor scores were computed for both consumers (rows) and prey items (columns) using UCINET 6 software (Borgatti et al. 2002). These scores were then plotted in the same multivariate space and taxa were grouped into compartments using Mage 3D visualization software (http://kinemage.biochem.duke.edu). Delineation of compartment boundaries was somewhat arbitrary and based on relative spatial distance between taxa. However, this approach was more objective than grouping species based on intutition and experience because it utilized similarities of both prey and predators and followed the trophospecies concept (Yodzis and Winemiller 1999).

Physical Parameters

On a daily basis at each pond (for duration of sampling events) the air and water temperature was recorded, salinity was read with a refractometer, and water level documented with a permanent staff gauge. Also in each pond during

each sampling event, the surface and bottom water dissolved oxygen was measured every 3 hours over one randomly selected 24 hr period. A combination of Winkler titrations (pages 21-26: Strickland and Parsons 1972) and automated instruments (Model 55 YSI DO Meter) were used. To obtain area and volume estimates, the ponds were surveyed using modified plane table and alidade techniques. For each set of ponds, a laser level was positioned at a central location. From there a series of data points were obtained which included distance and azimuth from the instrument and water depth.

Ecopath Model Documentation

General Considerations

- Units for model parameters were $\mu g\ C\ m^{-2}$ for biomass and $\mu g\ C\ m^{-2}\ d^{-1}$ for flows.

- When sampling indicated an entire compartment was not present, the compartment's biomass was set at 1×10^{-5} and diet values were taken from a model of an adjacent pond where the compartment was present.

- Final input values for P/B and Q/B were corrected for temperature differences between seasons using Q_{10}. Assuming a Q_{10} of 2.5 (Pandian and Vernberg 1987a, b; Withers 1992) and temperature difference of $6.3^{o}C$, a correction factor of 1.6 was used for High Stress and Post-Disturbance time periods.

- Diet percentages for each compartment often were altered to balance the model. However, binary relationships were retained.

- Christian and Luczkovich (1999) and Ulanowicz et al. (2003) represent indirect references for values of several parameters (e.g. P/B of Birds - compartment 29, GE - gross food conversion efficiency, egestion, and binary diets of some compartments). Where these references are cited, values are based on food web models within the reference.

Compartment Inputs

1 Benthic Microalgae

Biomass: based on chlorophyll a; 4 5-ml samples from surface of fluff layer.

Production/Biomass Ratio: from Ulanowicz et al. (2003).

2 Epiphytic Algae

Biomass: based on Edge Samples (0.5-m lengths) and Ruppia Quadrats (0.0625-m^2), and loss on ignition.

Production/Biomass Ratio: from Frankovich and Zieman (1994).

3 Overhanging Vegetation

Biomass: based on Edge Samples (0.5-m lengths), and loss on ignition.

Production/Biomass Ratio: from Roberts (2000).

4 Phytoplankton

Biomass: based on chlorophyll a; 4 300-ml surface samples.

Production/Biomass Ratio: from Christian and Luczkovich (1999).

5 *Ruppia maritima*

Biomass: based on Ruppia Quadrats (0.0625-m^2), and loss on ignition.

Production/Biomass Ratio: from Kinney and Roman (1998).

6 H$_2$O Column Bacteria

Biomass: based on epifuorescence microscopy; direct counts using acridine orange (Hobbie et al. 1977; Sherr et al. 1993); 4 surface samples.

Production/Biomass Ratio: From WU (1995).

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Christian and Luczkovich 1999), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of Post-Disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

7 H$_2$O Column Microprotozoans

Biomass: based on epifuorescence microscopy; direct counts using FITC (Sherr and Sherr 1983; Sherr et al. 1993); 4 surface samples.

Production/Biomass Ratio: From WU (1995).

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Christian and Luczkovich 1999), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

8 Sediment Bacteria

Biomass: based on epifuorescence microscopy; Direct counts using DAPI (Porter and Feig 1980; Schallenberg et al. 1989; Sherr et al. 1993); 4 samples of "fluff" layer.

Production/Biomass Ratio: from Aiosa (1996).

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Christian and Luczkovich 1999), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

9 Sediment Microprotozoans

Biomass: based on epifuorescence microscopy; Direct counts using FITC (Sherr and Sherr 1983; Sherr et al. 1993); 4 surface samples.

Production/Biomass Ratio: based on similar size, assumed to be 80% of sediment bacteria value from Aiosa (1996).

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Christian and Luczkovich 1999), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

10 Zooplankton

Biomass: based on density and biovolume estimates; 75 L collected from surface, poured through plankton net, subsampled and analyzed with dissecting microscope & Sedgewick Rafter counting cell (Higgins and Thiel 1988).

Production/Biomass Ratio: P/B = (0.000069) $W^{-0.37}$ (Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Wet weight per individual in kg from biovolume calculations - average of all taxa present (Higgins and Thiel 1988).

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Christian and Luczkovich 1999), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

11 Meiofauna

Biomass: based on density and biovolume estimates; 6.5-cm diameter cores of "fluff" layer subsampled and analyzed with dissecting microscope & Sedgewick Rafter counting cell (Higgins and Thiel 1988).

Production/Biomass Ratio: P/B = (0.000069) $W^{-0.37}$ (Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Wet weight per individual in kg from biovolume calculations - average of all taxa present (Higgins and Thiel 1988).

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Christian and Luczkovich 1999), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

12 Hydrobiidae

Biomass: based on "edge" quadrats (0.5-m^2) and "Ruppia" quadrats (0.0625-m^2), and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) W$^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Wet weight per individual in kg from Mandracchia and Ruber (1990).

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Mandracchia and Ruber 1990), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

13 Amphipods and Decapods

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) W$^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Dry wet weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (LaFrance and Ruber. 1985; Sanders et al 1962; Anderson 1985; Welsh 1975; Hill et al. 1989), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

14 Detritivorous Insects

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) W$^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Dry wet weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (McCafferty 1981; Merritt and Cummings 1984), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

15 Small Herbacious Insects

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) W$^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Dry wet weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (McCafferty 1981; Merritt and Cummings 1984), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

16 Large Herbacious Insects

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) W$^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Dry wet weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (McCafferty 1981; Merritt and Cummings 1984), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

17 Small Predacious Insects

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) $W^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Dry wet weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (McCafferty 1981; Merritt and Cummings 1984), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

18 Large Predacious Insects

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) W$^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Dry wet weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (McCafferty 1981; Merritt and Cummings 1984), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

19 Piscivorous Insects & Spiders

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) W$^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Dry wet weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (McCafferty 1981; Merritt and Cummings 1984; Zimmermann and Spence 1989), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

20 Omnivorous Insects

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000069) W$^{-0.37}$(Banse and Mosher 1980; Peters 1983; for inverts at 5-20°C); Dry wet weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (McCafferty 1981; Merritt and Cummings 1984), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

21 *Anguilla rostrata*

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.002368) W$^{-0.26}$ (Banse and Mosher 1980; Peters 1983; for fish); Wet weight per individual in kg measured directly.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Van Den Avyle 1984), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

22 Fish - Pelagic Feeders

Biomass: based on density (barrier seine) and wet weight (measured directly).

Production/Biomass Ratio: $P/B = (0.002368) W^{-0.26}$ (Banse and Mosher 1980; Peters 1983; for fish); Wet weight per individual in kg measured directly.

Consumption/Biomass Ratio: $Q/B = (P/B)/(GE)$ where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from $E=C-(P+R)$.

Diet Composition: based on gut content analysis (GCA; Carr and Adams 1972, 1973; Luczkovich and Stellwag 1993). Various taxa and size classes make up a compartment, and binary GCA data are used for aggregation. Representative taxa/size are selected to provide quantitative values of diet for a compartment. Selection is based on largest biomass of compartment and largest number of guts analyzed. When no

representation occurs, data are used from: 1) adjacent pond at same time; 2) nearest pond at same time; 3) nearest pond at different time.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

23 Fish - Herbivores

Biomass: based on density (barrier seine) and wet weight (measured directly).

Production/Biomass Ratio: P/B = (0.002368) $W^{-0.26}$ (Banse and Mosher 1980; Peters 1983; for fish); Wet weight per individual in kg measured directly.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: based on gut content analysis (GCA; Carr and Adams 1972, 1973; Luczkovich and Stellwag 1993). Various taxa and size

classes make up a compartment, and binary GCA data are used for aggregation. Representative taxa/size are selected to provide quantitative values of diet for a compartment. Selection is based on largest biomass of compartment and largest number of guts analyzed. When no representation occurs, data are used from: 1) adjacent pond at same time; 2) nearest pond at same time; 3) nearest pond at different time.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

24 Fish - Predators Small Prey

Biomass: based on density (barrier seine) and wet weight (measured directly).

Production/Biomass Ratio: P/B = $(0.002368) W^{-0.26}$ (Banse and Mosher 1980; Peters 1983; for fish); Wet weight per individual in kg measured directly.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: based on gut content analysis (GCA; Carr and Adams 1972, 1973; Luczkovich and Stellwag 1993). Various taxa and size classes make up a compartment, and binary GCA data are used for aggregation. Representative taxa/size are selected to provide quantitative values of diet for a compartment. Selection is based on largest biomass of compartment and largest number of guts analyzed. When no representation occurs, data are used from: 1) adjacent pond at same time; 2) nearest pond at same time; 3) nearest pond at different time.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

25 Fish - Predators Large Prey

Biomass: based on density (barrier seine) and wet weight (measured directly).

Production/Biomass Ratio: P/B = (0.002368) $W^{-0.26}$ (Banse and Mosher

1980; Peters 1983; for fish); Wet weight per individual in kg measured directly.

Consumption/Biomass Ratio: $Q/B = (P/B)/(GE)$ where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from $E=C-(P+R)$.

Diet Composition: based on gut content analysis (GCA; Carr and Adams 1972, 1973; Luczkovich and Stellwag 1993). Various taxa and size classes make up a compartment, and binary GCA data are used for aggregation. Representative taxa/size are selected to provide quantitative values of diet for a compartment. Selection is based on largest biomass of compartment and largest number of guts analyzed. When no representation occurs, data are used from: 1) adjacent pond at same time; 2) nearest pond at same time; 3) nearest pond at different time.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

26 Tadpoles

Biomass: based on density ("edge" quadrats, 0.5-m^2 and "Ruppia" quadrats, 0.0625-m^2) and loss on ignition.

Production/Biomass Ratio: P/B = (0.000631) W$^{-0.179}$ (Banse and Mosher 1980; Peters 1983; for poikilotherms); Dry weight per individual measured directly and converted to wet weight in kg.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency and is based on diet (Christian and Luczkovich 1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated from E=C-(P+R).

Diet Composition: binary diet based on literature (Knowlton 1944; Linzey 1967; Nelson 1972), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa were assumed to reside entirely within the system during the times modeled.

27 Frogs

Biomass: based on density (night surveys) and wet weight.

Production/Biomass Ratio: P/B = (0.000631) $W^{-0.179}$ (Banse and Mosher

1980; Peters 1983; for poikilotherms); Wet weight per individual in kg

measured directly.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food

conversion efficiency and is based on diet (Christian and Luczkovich

1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated

from E=C-(P+R).

Diet Composition: binary diet based on literature (Knowlton 1944; Linzey

1967; Nelson 1972), and used to calculate quantitative diet from biomass

of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with

exception of the post-disturbance model in East and West Ponds where

80% to POC and 20% to DOC were used. This was necessary for

maintaining EE < 1 for DOC.

Migration: although taxa were highly migratory with respect to the size of

the ponds, they were treated as residing within the system during the

modeled times (i.e. both immigration and emigration were zero). This was

accomplished by averaging observed densities for each sampling event

over the entire time being modeled.

28 Snakes

Biomass: based on density (funnel traps) and wet weight (Ulanowicz et al.
2003).

Production/Biomass Ratio: P/B = (0.000631) $W^{-0.179}$ (Banse and Mosher
1980; Peters 1983; for poikilotherms); Wet weight per individual in kg from
Ulanowicz et al. (2003).

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food
conversion efficiency and is based on diet (Christian and Luczkovich
1999).

Egestion: E/Q value from Ulanowicz et al. (2003) where it is calculated
from E=C-(P+R).

Diet Composition: binary diet based on literature (Brown 1979; Collins
1980), and used to calculate quantitative diet from biomass of prey
compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with
exception of the post-disturbance model in East and West Ponds where
80% to POC and 20% to DOC were used. This was necessary for
maintaining EE < 1 for DOC.

Migration: immigration and emigration were assumed to be zero. Taxa
were assumed to reside entirely within the system during the times
modeled.

29 Birds

Biomass: based on density (point counts) and wet weight (Dunning 1993).

Production/Biomass Ratio: P = (0.15 W)/d (Christian and Luczkovich 1999); Wet weight from Dunning 1993.

Consumption/Biomass Ratio: Q/B = (P/B)/(GE) where GE = gross food conversion efficiency = 0.06 (Christian and Luczkovich 1999).

Egestion: E/Q value from Jorgensen et al. (1991).

Diet Composition: binary diet based on field observations and literature (Kushlan 1976, 1986; Master 1992), and used to calculate quantitative diet from biomass of prey compartments and assumption of opportunistic feeding.

Detritus Fate: default values (90% to POC; 10% to DOC) were used with exception of the post-disturbance model in East and West Ponds where 80% to POC and 20% to DOC were used. This was necessary for maintaining EE < 1 for DOC.

Migration: although taxa were highly migratory with respect to the size of the ponds, they were treated as residing within the system during the modeled times (i.e. both immigration and emigration were zero). This was accomplished by averaging observed densities for each sampling event over the entire time being modeled.

30 DOC

Biomass: based on 4 surface water samples; analysis performed by

University of North Carolina, Institute of Marine Science (Richard Weaver).

Detritus Fate: remains as DOC.

Migration: immigration and emigration were assumed to be zero. System

was assumed to be closed with respect to movement across boundaries.

31 POC

Biomass: based on 4 100-ml surface water samples; filtered and analyzed

on Exeter Analytical's Model 440 Elemental Analyzer by Debbie Daniels,

East Carolina University.

Detritus Fate: exported from system (i.e. incorporated into peat underlying

fluff layer in pond).

Migration: immigration and emigration were assumed to be zero. System

was assumed to be closed with respect to movement across boundaries.

Rules Used In Balancing Models

Diet percentages in each model differ from literature source or gut content

data to attain a condition of mass-balance (i.e. flows into each compartment

equal flows out). The approach taken for balancing each model was to obtain

ecotrophic efficiencies (EE) < 1. An EE > 1 indicates predation on that

compartment is greater than production by the compartment. To maintain mass-

balance either consumption by the predators or production by the compartment

needs to be adjusted. Diet is assumed to be the most uncertain variable of these two parameters. Thus, percentages of prey items are adjusted for consumers of compartments with an EE > 1. The following outlines this approach:

- Initially, the diet matrix contains binary relationships based on literature and gut content data.

- The binary matrix is used to construct a quantitative matrix with cells containing the percentage of each prey consumed. The following rules are used to construct the quantitative matrix:

  - Percentages are based on the relative biomass of each prey item.

  - Size is accounted for by small predators not consuming larger prey items (e.g. small predatory Insects do not consume large predatory insects or large herbivorous insects).

- Each compartment with an EE > 1 is balanced individually. The following rules are used:

  - Binary relationships are always maintained.

  - In the diet matrix, row values of a prey compartment (with EE > 1) are decreased incrementally in values no larger than 50% of the original input value until the EE becomes < 1. This ensures the prey item's EE remains relatively high (e.g. ~ 0.9) while obtaining a value less than 1.

    - These adjustments can result in the sum of a consumer compartment's diet dropping below 100%. This is offset by increasing the consumer's intake of prey items with relatively large

biomass. This is also done incrementally in values no larger than 50% of the original input value which ensures the particular prey item's EE is relatively close to its original value.

- Typically one or two consumers "control" a prey item's EE because their biomass is relatively large or their Q/B is relatively large. For example birds are a major player in adjusting fish EE's, frogs for adjusting insect EE's, and snakes for adjusting the EE of both insects and fish.

    - The diet percentages of these "controlling" consumers are identified and adjusted first so as not to unnecessarily change the other consumer-prey relationships.

    - The organism examples given above have ranges larger than the boundaries of the modeled ecosystem. "Imported" material is an important but unknown component of their diet. Therefore, the diet percentages of these organisms are adjusted first.

- Cannibalism is not allowed to exceed 10%.

- After all EE's are brought under 1, "fine adjustments" are made to the diet matrix. The following approach is taken:

    - The diet matrix is reviewed with other ENA models being used as a general guide (St. Marks NWR, Christian and Luczkovish 1999; Neuse River Estuary, Christian et al. 2003; Chesapeake Bay, Baird and Ulanowicz 1989) . For example, diet percentages of microbes and

zooplankton are compared to these models to determine if values are reasonable.

- Diets of Insect compartments are reviewed after noting the specific taxa making up the compartment. For example,

  - Both small and large predatory insects includes Dytiscidae and Odonata. Percentages of Hydrobiidae in the diet of these compartments will be greater when Odonata are a major contributor to their biomass.

  - Piscivorous insects & spiders includes *Dolomedes sp*. and *Belostoma sp*. *Dolomedes sp*. likely utilizes more neuston than *Belostoma sp*. and thus import will be higher when they are present.

Figure 3-1. Visual representation of diet relationships in the ponds during the

three sampling events using East Pond as an example: (a) spring 2002, (b) late

summer 2001, (c) late summer 2002. Circles (or nodes) and numbers represent

model compartments. See Table 3-2 for identification of compartments and taxa

they contain. Reduced size of a node indicates field samping did not detect taxa

for that particular compartment (occurred in late summer 2001 and 2002). These

compartments were retained in the models with an extrememly small biomass

(i.e. 1 x 10^{-5} μgCm^{-2}). Effective trophic levels were calculated by Ecopath.

(a) East Pond Spring 2002.

(b) East Pond Late Summer 2001.

Effective Trophic Level
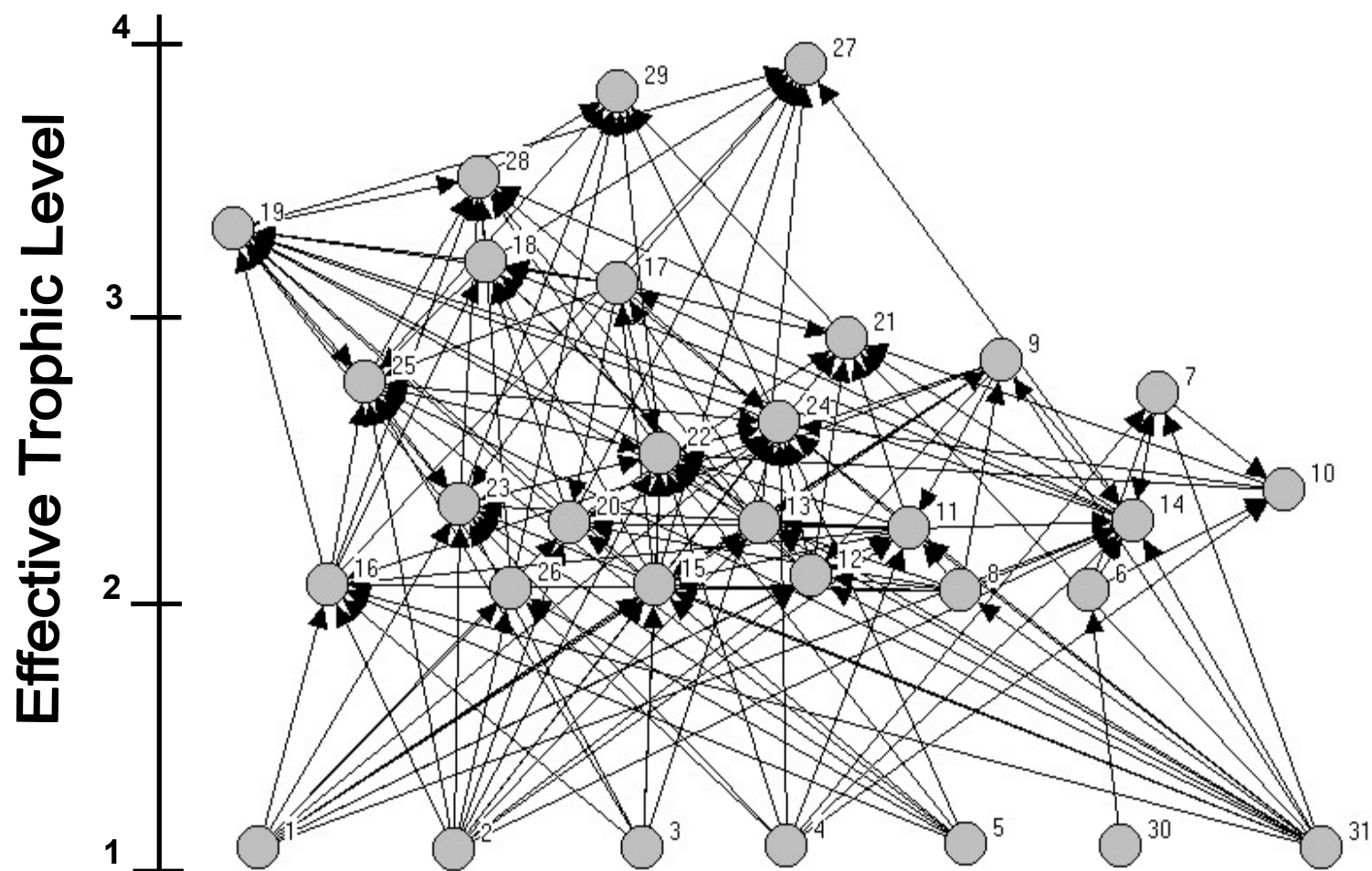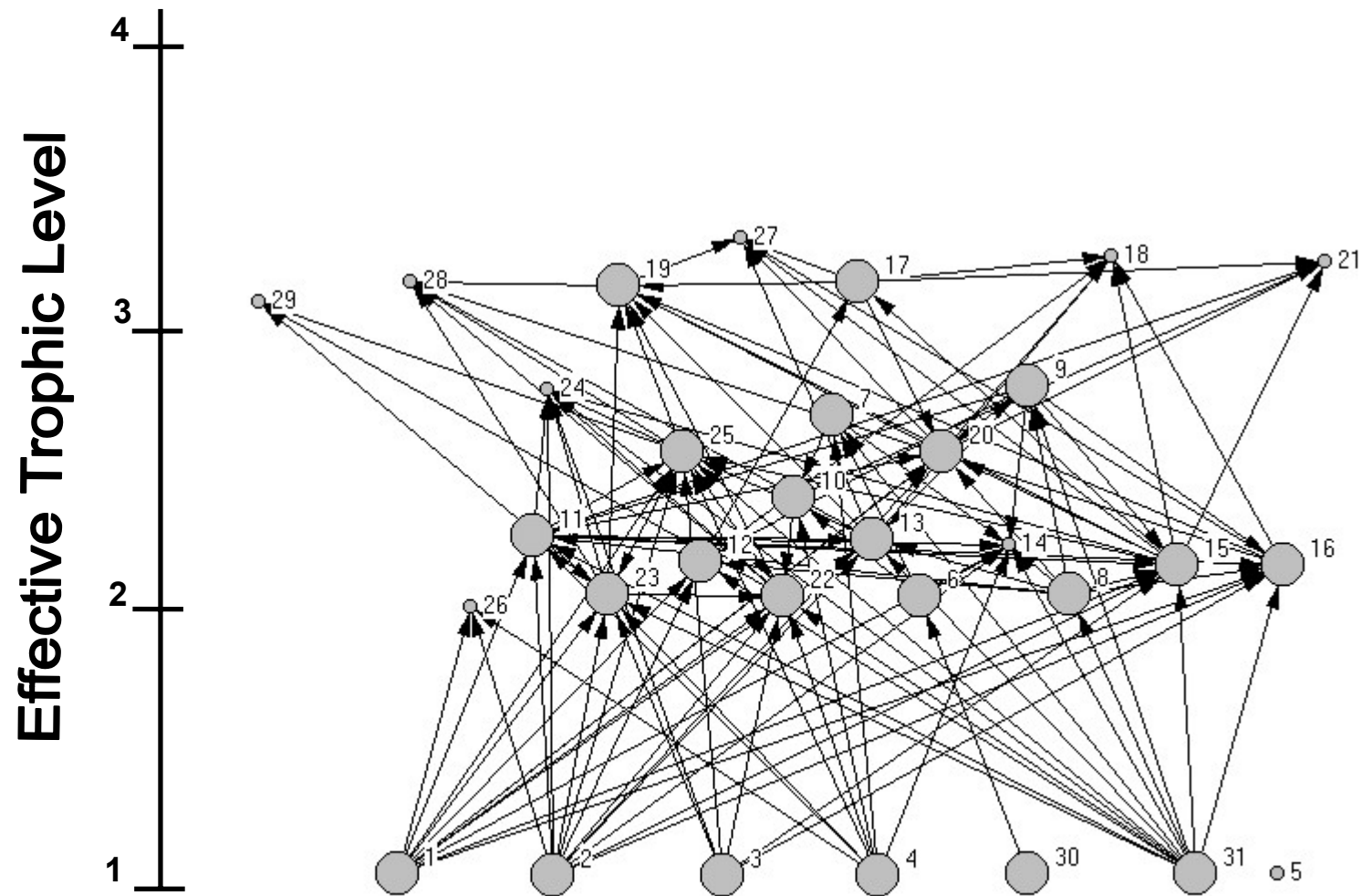
(c) East Pond Late Summer 2002.
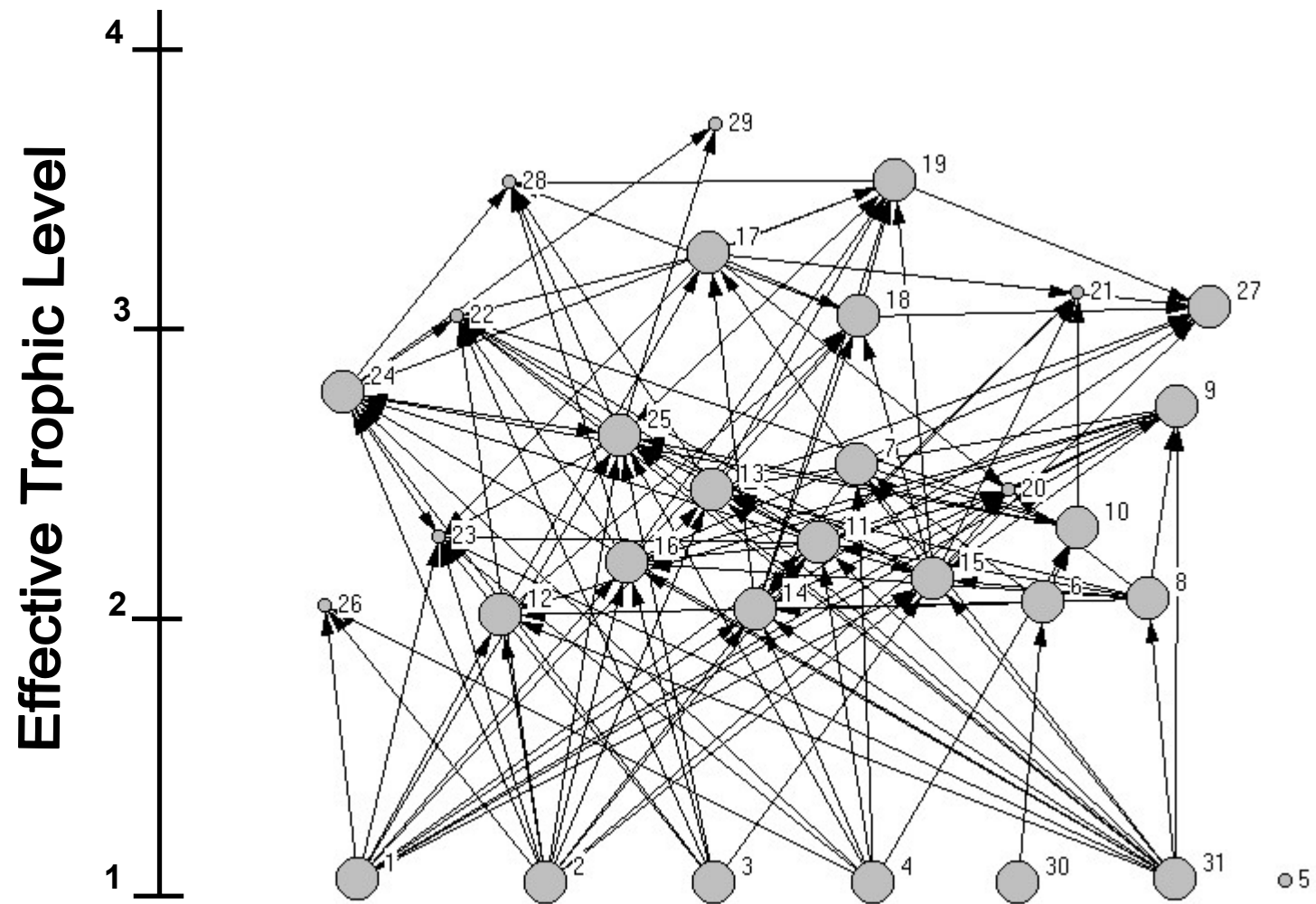
Instrumentation

See field and laboratory methods.

Taxonomy And Systematics

Table 3-2. List of all taxa observed utilizing the ponds and their corresponding

compartments in the Ecopath models. Sources include: producers - Tiner

(1993); meiofauna and zooplankton - Higgins and Thiel (1988);

macroinvertebrates other than insects and spiders - Gosner (1978); insects and

spiders - McCafferty (1981), Merritt and Cummings (1983); fish - Robins et al.

(1986); amphibians and reptiles - Conant and Collins (1991); birds - NGS (1987).

Ecopath Model Compartment	Taxa	Common Name
1. Benthic Microalgae		
2. Epiphytic Algae		
3. Overhanging Vegetation	*Spartina patens*	Saltmeadow Cordgrass
	Distichilis spicata	Salt Grass
4. Phytoplankton		
5. *Ruppia maritima*	*Ruppia maritima*	Widgeon Grass
6. H_2O Column Bacteria		
7. H_2O Column Micro-protozoans		
8. Sediment Bacteria		
9. Sediment Micro-protozoans		

Table 3-2. Continued.

Ecopath Model Compartment	Taxa	Common Name
10. Zooplankton	Ceratopogonidae	Biting Midge Fly Larvae
(defined as in water column)	Chironomidae	Non-Biting Midge Fly Larvae
	Copepod	Calanoid, Cyclopod, Harpacticoid, Nauplii
	Foraminifera	
	Hydracarina	Water Mite Adult
	Hydrobiidae	Mud Snail
	Nematode	
	Ostracod	
	Rotifer	
	Turbellarian	Flatworm
11. Meiofauna	Ceratopogonidae	Biting Midge Fly Larvae
(defined as in sediment fluff layer)	Chironomidae	Non-Biting Midge Fly Larvae
	Copepod	Calanoid, Cyclopod, Harpacticoid, Nauplii
	Foraminifera	
	Hydracarina	Water Mite Adult
	Hydrobiidae	Mud Snail
	Nematode	
	Ostracod	
	Rotifer	
	Turbellarian	Flatworm
12. Hydrobiidae	Hydrobiidae	Mud Snail
13. Amphipods and Decapods	*Callinectes sapidus*	Blue Crab
	Gammarus sp.	Amphipod
	Palaemonetes pugio	Grass Shrimp
	Uca minax	Red-Jointed Fiddler Crab
	Uca pugnax	Mud Fiddler Crab
14. Detritivorous Insects	Culicidae	Mosquitoe Larvae
	Stratiomyidae	Soldier Fly Larvae

Table 3-2. Continued.

Ecopath Model Compartment	Taxa	Common Name
15. Small Herbivorous Insects (<1cm)	Ceratopogonidae	Biting Midge Fly Larvae
	Chironomidae	Non-Biting Midge Fly Larvae
	Corixidae	Water Boatman Adult
	Chrysops sp.	Deer Fly Larvae
	Ephemeroptera	Mayfly Larvae
	Ephydridae	Shore Fly Larvae & Pupae
	Helodidae	Marsh Beetle Adult
	Hydrophilidae	Water Scavenging Beetle Adult
16. Large Herbivorous Insects (>1cm)	*Chrysops sp.*	Deer Fly Larvae
	Dryopidae	Long-Toed Water Beetle Adult
	Ephydridae	Shore Fly Larvae & Pupae
	Hydrophilidae	Water Scavenging Beetle Adult
17. Small Predatory Insects (<1cm)	Dytiscidae	Predacious Diving Beetle Adult
	Hebridae	Velvet Water Bug Adult
	Hydracarina	Water Mite Adult
	Hydrophilidae	Water Scavenging Beetle Larvae
	Naucoridae	Creeping Water Bug Adult
	Odonata	Damselfly & Dragonfly Larvae
18. Large Predatory Insects (>1cm)	Dytiscidae	Predacious Diving Beetle Larvae & Adult
	Hydrophilidae	Water Scavenging Beetle Larvae
	Odonata	Damselfly & Dragonfly Larvae
19. Piscivorous Insects & Spiders	*Belostoma sp.*	Giant Water Bug Adult
	Dolomedes sp.	Fishing Spider Adult
	Gerridae	Water Strider Adult
	Tabanus sp.	Horse Fly Larvae
20. Omnivorous Insects	Haliplidae	Crawling Water Beetle Larvae & Adult

Table 3-2. Continued.

Ecopath Model Compartment	Taxa	Common Name
21. *Anguilla rostrata*	*Anguilla rostrata*	American Eel
22-25. Fish	*Cyprinodon variegatus*	Sheepshead Minnow
(Aggregation variable and based on diet)	*Fundulus sp.*	Mummichog, Spotted and Striped Killifish
	Gambusia sp.	Mosquitofish
	Lucania parva	Rainwater Killifish
	Menidia sp.	Silverside
26. Tadpoles	*Rana utricularia*	Southern Leopard Frog
27. Frogs	*Gastrophryne carolinensis*	Eastern Narrowmouth Toad
	Rana utricularia	Southern Leopard Frog
28. Snakes	*Nerodia sipedon*	Northern Water Snake
29. Birds	*Casmerodius albus*	Great Egret
	Egretta thula	Snowy Egret
30. Dissolved Organic Carbon		
31. Particulate Organic Carbon		

Project Personnel

See acknowledgments.

Metadata Class III. Data Set Status And Accessibility

Status

Latest Update: August 2005

Latest Archive date: August 2005

Metadata status: August 2005, metadata are current

Data Verification

All data entries have been double checked (at a minimum) against the original data sheets and field notes. Figures 3-2 through 3-6 are yield-effort curves used to insure completeness of sampling. Data for these curves were obtained either within the study ponds or from nearby salt marsh ponds to define sampling protocols. For each curve, a vertical dashed line indicates the minimum level of sampling used.

Figure 3-2. Yield effort curve to determine the amount of water to filter for zooplankton sampling. Based on these results, each zooplankton sample represented 75 L of pond water which was filtered through a plankton net (80-μm mesh).

Figure 3-3. Yield effort curve to determine the number of cores needed to adequately sample for meiofauna. A minimum of 4 6.5-cm diameter cores were taken during each sampling event in each pond to quantify meiofauna biomass.

Figure 3-4. Yield effort curve to determine the number of edge quadrats that needed to be sampled. A minimum of 4 0.5-m^2 quadrats along the pond's edge were sampled during each sampling event. During low stress conditions in spring 2002, an additional 4 0.25-m^2 quadrats located within *Ruppia maritima* were also sampled.

Figure 3-2.
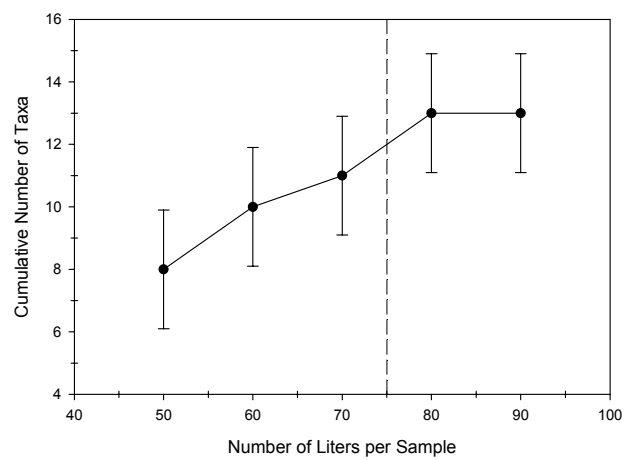
Figure 3-3.
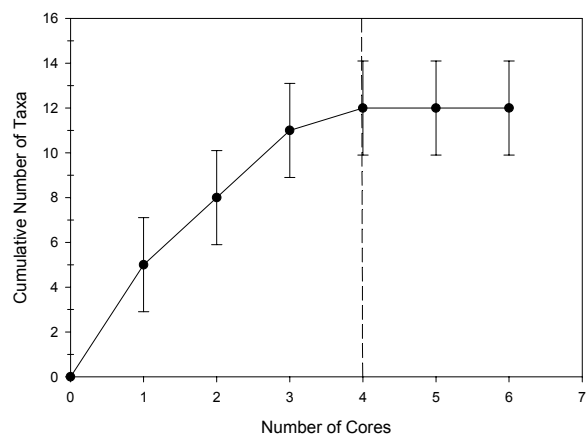
Figure 3-4.
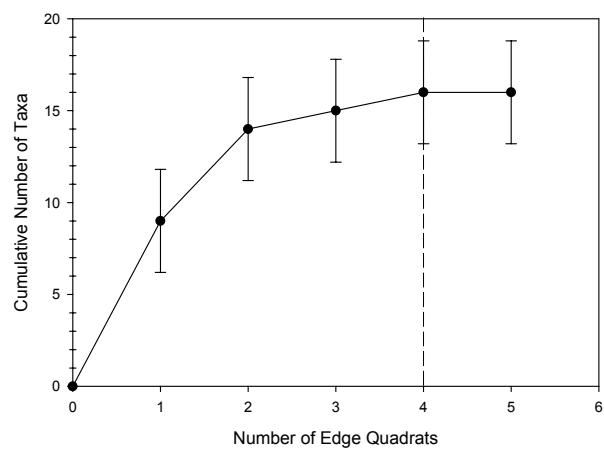
Figure 3-5. Results of an experiment to determine the number of sub-samples
needed to quantify the contents of each seine. The experiment was performed in
a similar size pond as the four ponds modeled for the study. The experiment
consisted of seining a pond, sub-sampling the contents of the entire seine, and
then documenting the cumulative number of taxa with each successive sub-
sample. Based on these data, a minimum of 50% of the total volume of each
seine was sub-sampled and analyzed for the study ponds.

Figure 3-6. Yield effort curve to determine the number of stomachs needed to be
pooled for stomach contents analysis. When available, 15 stomachs were used
in the analysis for each size class of a particular genus. When less were
available, all stomachs within that size class were used.

Figure 3-5.
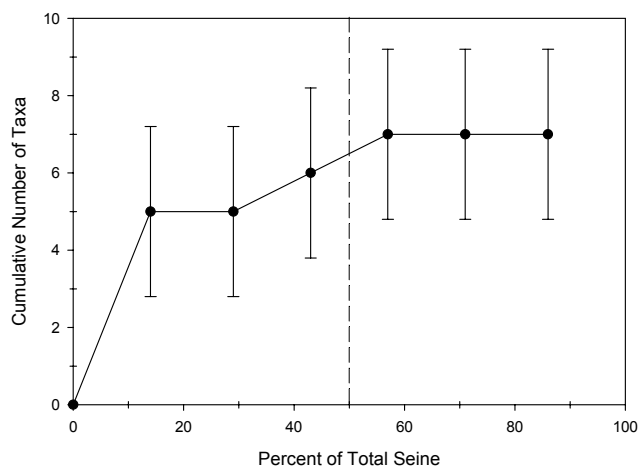
Figure 3-6.
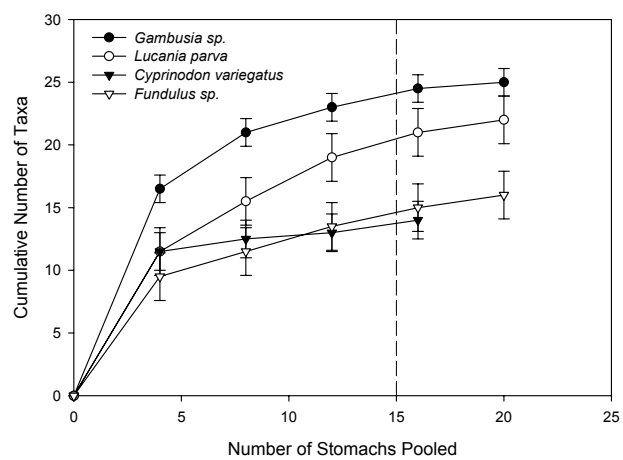
Accessibility

Storage Location And Medium

Original data files exist on primary author's personal computer in Microsoft Excel and Ascii formats, and backup files exist on secondary author's personal computer.

Contact Person

Robert R. Christian, Department of Biology, East Carolina University, Greenville, NC 27858, phone 252-328-1835, email: christianr@mail.ecu.edu

Restrictions

Copyright restrictions: None

Proprietary restrictions: None

Costs: None.

Metadata Class IV. Data Structural Descriptors

Data Set Files

Identity and Size:

Area-Depth.txt (14 records not including header row)

Salinity-Temp.txt (63 records not including header row)

Diurnal_Curve_DO.txt (56 records not including header row)

Light-Dark_Bottle_DO.txt (14 records not including header row)

Abundance-Size.txt (2959 records not including header row)

Stomach_Contents.txt (137 records not including header row)

Ecopath_Input_Parameters.txt (372 records not including header row)

Ecopath_Stacked_Diet_Matrix.txt (384 records not including header row)

Stable_Isotope_Data.txt (641 records not including header row)

Format and Storage mode: Ascii text, tab delimited. No compression schemes used.

Header information: Header, or variable, definitions for each data file are given in Section B Variable Information (below).

Alphanumeric attributes: Mixed

Special characters/fields: "N/A" or "---" indicates not applicable or data not available. AFDW denotes ash free dry weight.

Authentication procedures: Verification of the size of each data file can be performed using the number of records given in the Identity and Size Section above. In addition, the stomach content data file (Stomach_Content.txt) can be verified by checking that each row sums to 1 (cell values represent the percent of material from each prey item).

Variable Information:

Table 3-3. List of variables for each data file.

Header/Variable	Variable Definition	Storage Type
Data File: Area-Depth.txt		
Pond	Data were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	Character
Date	General time represented by data collection.	Character
Average Area (m^2)	Area of pond based on average length and width.	Numeric
H$_2$O Column Thickness (m)	Thickness of water column from air-water interface to top of Fluff Layer. Measured in 6 random locations.	Numeric
Fluff Layer Thickness (m)	Thickness of fluid mud layer at bottom of pond. Measured at same locations as water column.	Numeric
Data File: Salinity-Temp.txt		
Date	Data were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	Character
Time	For consistency, measurements were taken nearly daily during each of the three sampling events between 3-4 pm.	Character
Salinity (ppt)	Surface salinity measurements in ppt for each pond.	Numeric
Temp (^{o}C)	Surface temperature measurements for each pond.	Numeric
Data File: Diurnal_Curve_DO.txt		
Pond	Data were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	Character
Date	Data for two diurnal curves were collected for each pond during Spring 2002.	Character
Time	Time (military) that measurements were taken.	Character
Surface Temp (^{o}C)	Surface temperature was measured at 2 locations.	Numeric
Surface DO (mg/l)	Surface dissolved oxygen was measured at 2 locations.	Numeric

Table 3-3. Continued.

Header/Variable	Variable Definition	Storage Type
Data File: Light-Dark_Bottle_DO.txt		
Pond	Data were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	
Date	Date light-dark bottle experiment was performed.	Character
Time Fill	Time (military) that bottles were filled with pond water.	Character
Time Fix	Time (military) that experiment ended.	Character
Initial DO (mg/l)	Initial dissolved oxygen measurements at time fill.	Numeric
Light Bottle DO (mg/l)	Light bottle dissolved oxygen measurements at time fix.	Numeric
Dark Bottle DO (mg/l)	Dark bottle dissolved oxygen measurements at time fix.	Numeric
Data File: Abundance-Size.txt		
Field Sample	Identifies type and number of sample collected in field.	Character
Taxa	Taxonomic classification of organisms.	Character
Development Stage	Stage is given where pertinent (e.g. larvae vs. adult).	Character
Pond	Data were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	Character
Date	General time period represented by sample.	Character
Location	Location in pond where sample was collected.	Character
Abundance	Density of organisms.	Numeric
Abundance Units	Various units depending on sample methodology.	Character
Size	Precise size or range of sizes represented.	Numeric
Size Units	Various units depending on sample methodology.	Character
Wet Weight	Weight used in production calculations.	Numeric
Wet Weight Units	Various units depending on sample methodology.	Character

Table 3-3. Continued.

Header/Variable	Variable Definition	Storage Type
Data File: Stomach_Contents.txt		
Pond	Data were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	Character
Date	General time period represented in each model.	Character
Taxa	Taxonomic classification of organisms in each sample.	Character
Size Class	Size range given by length in centimeters.	Character
n	Number of stomachs pooled for each sample.	Numeric
Remaining Headers	The remaining column headers represent prey items. All should be self explanatory except for Fluffy White Stuff (FWS). FWS is non-identifiable material that represents partially digested material, stomach lining, or some combination.	Numeric
Data File: Ecopath_Input_Parameters.txt		
Pond	Data were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	Character
Date	General time period represented in each model.	Character
Compartment Number	Each model contains 31 compartments.	Numeric
Compartment Name	Compartments represent aggregations of taxa with common prey and predators.	Character
Biomass (μg C m^{-2} d^{-1})	Biomass of all taxa in a compartment.	Numeric
P/B	Production to biomass ratio for each compartment.	Numeric
Q/B	Consumption to biomass ratio for each compartment.	Numeric
E/Q	Fraction of consumption not assimilated for each compartment.	Numeric

Table 3-3. Continued.

Header/Variable	Variable Definition	Storage Type
Data File: Ecopath_Stacked_Diet_Matrix.txt		
Pond	Data were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	Character
Date	General time period represented in each model.	Character
Compartment Number	Each model contains 31 compartments.	Numeric
Prey Item	Identification of prey items.	Character
Remaining Headers	The remaining column headers represent consumers identified by their compartment number. Cell values represent the percent of each prey item (row) in a consumer's (column) diet.	Numeric
Data File: Stable_Isotope_Data.txt		
Pond	Samples were collected from 4 salt marsh ponds: North Pond, South Pond, East Pond, and West Pond.	Character
Date	General time period samples were collected.	Character
Taxa	Taxonomic classification of organisms in each sample.	Character
Size Class	Size range given by length in cm.	Character
n	Number of whole body individuals pooled for sample.	Numeric
µg N	Micrograms of nitrogen contained in sample.	Numeric
Delta Air	δN value.	Numeric
µg C	Micrograms of carbon contained in sample.	Numeric
Delta Pee Dee Belemnite	δC value.	Numeric
Delta Canon Diablo Meteorite	δS value. Two separate analyses for each sample.	Numeric

Metadata Class V. Supplemental Descriptors

Quality Assurance/Quality Control Procedures

Data were entered into computer files directly from field and laboratory notes and values were double checked. After complete entry of data and during analysis, all data points were checked against original field and laboratory notes.

Computer Programs And Data Processing Algorithms

The following software was utilized for manipulating original data: Excel for general data organization and management; Ucinet 6 (http://www.analytictech.com) and Mage 3D software (http://kinemage.biochem.duke.edu) for correspondence analysis used in aggregating taxa into compartments; NetDraw 2.2 (http://www.analytictech.com) for drawing food web diagrams; Ecopath (http://www.ecopath.org) for model documentation.

Publications And Results

Dame, J. K. and R. R. Christian. In Review. Uncertainty and the use of network analysis for ecosystem-based fishery management. Fisheries XX:XXX-XXX.

Dame, J. K. and R. R. Christian. In Prep. Analysis of trophic networks: How effective is network analysis in quantifying differences in food webs? Ecological Applications XX:XXX-XXX.

Dame, J. K. and R. R. Christian. In Prep. Validation of ecological network analysis using independent techniques. Ecological Applications XX:XXX-XXX.

CHAPTER 4. ANALYSIS OF TROPHIC NETWORKS: HOW EFFECTIVE IS
NETWORK ANALYSIS IN QUANTIFYING DIFFERENCES IN FOOD WEBS?

Abstract

Ecological network analysis (ENA) is a promising approach increasingly

being cited in the literature and employed by decision makers for ecosystem-

based management. However, few studies have critically examined model

output. The objective of this study is to evaluate the effectiveness of ENA in

detecting change in trophic conditions using replicated networks. Quantitative

trophic networks (n=12) representing four high marsh ponds during three

different time periods were constructed from an extensive field sampling program

augmented by literature values. Sampling times corresponded to periods of low

stress (spring 2002), high stress (late summer 2001), and post-disturbance (after

recovery of water levels from severe drought in summer 2002). A null hypothesis

was tested for how values of each of twelve indices given in ENA output differ

among the three stress/disturbance conditions (H_o: Low Stress = High Stress =

Post-Disturbance). Statistical differences were determined using comparable

parametric (repeated measures ANOVA with contrasts) and nonparametric

(Friedman's Test with Ryan's Q multiple comparison test) statistics. Covariance

of each pair of indices was evaluated with Spearman's Rank Correlation Tests.

ANOVA results indicated mean values in 10 of 12 indices were

significantly different among the three stress/disturbance levels, and results from

Friedman's Tests were generally in agreement as mean rankings in 11 of 12

indices showed significant differences. There was little covariance among indices as significant correlation was found in only 7 of 66 possible pairs. These results compared favorably to differences in community level indices among the three stress/disturbance levels. Both ENA output and community indices made a distinction between low stress conditions and the other two stress levels. However, ENA indices identified additional differences that were beyond the scope of the community indices. Additional studies repeating this approach are needed to clarify patterns of trophic response to stress and identify limitations of the application of ENA.

Introduction

There have been continued calls from the scientific community for an ecosystem-based approach to managing our marine resources (e.g. Christensen et al. 1996; ESA 1998; Link 2002; Inter-Research 2004; Pikitch et al. 2004), and two highly regarded commissions recently made similar recommendations (POC 2003; USOC 2004). Change has been slow but ecosystem-based management is increasingly becoming an explicitly stated, even mandated, goal of policy makers (e.g. NOAA 1996, 2003). However as managers struggle with how to implement these lofty goals (e.g. EPAP 1999), most management practices remain focused on parts of a community or a single population. One reason for this disparity is the complexity of quantifying and analyzing whole ecosystems.

An ecosystem is made up of interacting biotic and abiotic components linked by energy flow and cycling of materials (e.g. Odum and Barrett 2005). A community makes up the biotic component of an ecosystem (e.g. Smith 1996). Several well accepted indices are available for quantifying community properties (e.g. Pielou 1975), and the most widely used include richness (number of species or taxa), evenness (relative abundance among species or taxa), and diversity (measure which takes into account both richness and evenness). Unfortunately, few widely accepted indices are available for ecosystems. Ecosystem properties describe processes and interactions that characterize the ecosystem level of organization (i.e. the flow and cycling of material including energy and nutrients). The community level indices described above are based on biologic structure. In a similar manner, food webs provide a framework for quantifying ecosystem properties because they incorporate the exchange of food (i.e. energy) among components of the system and their surroundings.

Food webs have provided the basis for numerous contributions to our understanding of natural systems (for overviews see Pimm 1982; Polis and Winemiller 1996; Belgrano et al. 2005). Two separate paths have emerged from these efforts: the energetic perspective originating with Lindeman (1942) and developed by ecosystem ecology, and the demographic perspective initiated by May (1973) and grounded in community ecology. Network ecology represents the latest stage in the evolution of the energetic perspective (Loreau 2005). Because it focuses on interactions among components of an ecosystem, network

ecology provides a tool for quantifying ecosystem properties and is the foundation for the application of trophic networks to ecosystem-based management.

Network ecology is the discipline, and ecological network analysis (ENA) the practice, of constructing and analyzing networks (i.e. models) that depict the structure and flow of energy and matter within ecosystems (Wulf et al. 1989; Christensen and Pauly 1993a; Christian and Ulanowicz 2002). ENA is most often used in food web studies, and incorporates numerous analyses that can be classified into trophic structure analysis, pathway analysis, and information analysis. Model output from assorted algorithms includes matrices and indices that quantify direct and indirect interactions between compartments, energy flow characteristics, and network size and organization. Results from the analysis of a network model are then used to make inferences about the corresponding ecosystem (Kay et al. 1989; Ulanowicz 2005).

Table 4-1 provides examples of output from the different analyses of ENA given in Ecopath software (Christensen and Pauly 1992). Trophic structure analysis is based on the trophic concepts of Lindeman (1942). Effective trophic levels are not necessarily integers, but are fractional as suggested by Odum and Heald (1975) because they incorporate degrees of omnivory. For calculation of aggregated trophic chain length and trophic efficiency, the entire system is collapsed into discrete trophic levels sensu Lindeman based on an approach suggested by Ulanowicz (1995). This essentially reverses the routine used to

calculate effective trophic levels. Ecotrophic efficiencies calculated by Ecopath are based on the energy balance of each compartment (Christensen et al. 2002). Values must be between 0 and 1 to satisfy the mass-balance requirements of ENA. If a calculated ecotrophic efficiency is greater than 1, then it is typically adjusted downward using the diet matrix. Calculation of an omnivory index is based on the variance of the trophic level of a consumer's prey groups (Pauly et al. 1993).

Pathway analysis characterizes the pathway of flows, and is based on a routine suggested by Ulanowicz (1986). Average path length, as calculated in Ecopath software (Christensen and Pauly 1992), is based on a steady state version of a relationship given by Finn (1976). Primary production required to sustain consumers is scaled by net primary production so that it can be compared among networks. This was initially developed to determine what level of production is required to sustain fisheries harvest (Christensen and Pauly 1993b). Indices from information analysis quantify the growth and development of a system (Ulanowicz 1986). Total system throughput is the sum of all flows occurring in a system, and ascendency is calculated from the average mutual information (or information content) and is scaled by system throughput. Relative ascendency (ascendency/capacity) is the realized development of a system relative to its potential (i.e. capacity). Connectance is an index from food web research (Dunne et al. 2002), and is calculated as the ratio of realized connections to the number of potential connections.

ENA has been used to characterize single ecosystems (e.g. Baird and Ulanowicz 1989), compare multiple ecosystems (e.g. Christian et al. 2005), and evaluate the magnitude of stress imposed on ecosystems (e.g. Monaco and Ulanowicz 1997). Some authors recommend the application of ENA to resource management (e.g. Christensen 1991), and ENA has been used to examine changes in trophic dynamics associated with a wide range of management issues including: introduction of invasive species (Moreau et al. 1993; Kitchell et al. 2000), changes in hydrologic regime (Baird and Heymans 1996; Ulanowicz et al. 2003), onset of hypoxic conditions (Baird et al. 2004), impacts of oil spills (Okey and Pauly 1999) and fish kills (Christian et al. 2003), establishment of marine reserves (Watson et al. 2000), consequences of habitat restoration (Ulanowicz and Tuttle 1992), and the quantification of ecosystem health (Mageau et al. 1995) and integrity (Ulanowicz, 2000). However evaluation of the effects of fisheries harvest is where ENA is most often applied (e.g. Jarre-Teichmann 1998; Pauly et al. 1998; Christensen and Maclean 2004), and fishery managers are increasingly utilizing ENA for planning and decision making (e.g. SAFMC 1998; Okey and Pugliese 2001; ASMFC 2003; NCBO 2003).

Many of the management issues mentioned above are often considered as a stress or disturbance to the ecosystem. Insight into the effects of stress/disturbance on food webs comes from two areas of research. A bottom-up perspective suggests stress/disturbance determine trophic structure through their effect on primary productivity (Rosenzweig 1973; Fretwell 1977; Oksanen et

al. 1981), and a top-down perspective is based on studies documenting the effects on upper trophic levels (Connell 1975; Menge and Sutherland 1976; Lubchenco and Gaines 1981). Using the latter, Menge and Sutherland (1987) provide a conceptual model that predicts greater stress and/or disturbance corresponds to a greater potential for decreased taxa at upper trophic levels, diminished number of interactions, and a reduction in the strength of interactions that exist.

Both community level and ecosystem level indices have been shown to respond to stress/disturbance. Community level indices, including richness, evenness, and diversity, are believed to increase with intermediate levels of disturbance (eg. Connell 1978; Huston 1994) and/or decreased environmental stress (e.g. Gray 1989). From an ecosystem perspective, Ulanowicz (1996) and Monaco and Ulanowicz (1997) used indices from ENA output to conclude stress decreases trophic efficiencies (i.e. shorter aggregated trophic chains, lower bottom-up transfer efficiencies), degrades a system's pathway structure (i.e. lower number of paths, shorter average path length), and negatively impacts system size and organization (i.e. lower total systems throughput and ratio of ascendency to capacity). However, neither of the ecosystem level studies involved statistical analysis of replicated systems.

The objective of this study was to examine the effectiveness of ENA in detecting differences in food web properties. Trophic networks (n=12) representing low stress, high stress, and post-disturbance conditions in four salt

marsh ponds were compared. Salt marsh ponds were used as study systems because they are relatively well-defined, have somewhat simplistic food webs, and allow for adequate replication (Earp 1974; Christian 1981). Differences in trophic dynamics during the three time periods were evidenced by changes in the presence/absence and biomass of pond taxa. A null hypothesis (low stress = high stress = post-disturbance) was statistically tested for each of 12 indices from ENA output to determine if differences were detected between the stress/disturbance conditions. The same hypothesis testing was also performed on community level indices from the ponds (richness, evenness, and diversity), and results from the two types of indices (ecosystem vs. community) were compared. This study represents the first systematic effort to examine the output of ENA using a priori hypotheses and replicated networks.

It should be emphasized that this study was designed to test if ENA can detect differences in food web properties, and not to examine how stress affects food webs. This study utilized a natural experiment sensu Diamond (1986) where observations were made of natural systems and their response to normal processes. Levels of stress and disturbance were not regulated nor comparisons of stressed systems made to controls. Therefore predictions of directionality for index values between the sampling events (e.g. low stress > high stress > post-disturbance) were beyond the scope of this study, and hypotheses were limited to inequalities among the stress/disturbance levels.

Methods

Study Design

Food web networks (n=12) representing three stress/disturbance levels in four salt marsh ponds were constructed using extensive field data augmented with literature values. Ecopath software was then used to analyze and compare the networks. Field work was conducted in a mainland marsh at the headwaters of Upper Phillips Creek within the Virginia Coast Reserve Long Term Ecological Research site on Virginia's Eastern Shore (37.48^o North Latitude, 75.66^o West Longitude).

All four ponds were located in the high marsh community, and had average dimensions of 30-m long x 1 to 5-m wide x 0.5-m deep. Pond walls were nearly vertical, and their bottoms contained a 5 to 15-cm layer of fluid organic muck (or fluff layer) underlain by marsh peat. North and South Ponds were immediately adjacent to each other in a hummock and hollow zone dominated by *Spartina patens* and *Distichilis spicata*. East and West Ponds were located further up-gradient in a forest transition zone, and are also connected. Within each set of ponds, a plywood barrier was constructed to inhibit the direct exchange of water.

Separate sampling events were performed (late summer 2001 - September 3rd to October 10th, early spring 2002 - May 20th to June 21st, and late summer 2002 - July 25th to August 17th) to allow construction of three different models representing a range of trophic conditions for each pond. The times

correspond to different levels of stress and disturbance, and correlate with changes in pond food webs. Late summers are characterized by high stress conditions because of relatively high salinity, high temperature, and low dissolved oxygen. The opposite is found in early spring when low stress conditions prevail. The late summer 2002 sampling event was performed immediately after recovery from an anomalous drop in water level due to drought. The food web in each pond at this time represented a post-disturbance condition.

Sampling And Network Construction

Each of the 12 models represent food web conditions averaged over approximately a one month period. Sampling for the post-disturbance models began approximately 5 days after water levels were restored due to precipitation. For the purposes of sampling and enumerating organisms the ponds were conceptually separated into 3 communities: benthic, epiphytic, and pelagic. Spatial boundaries were defined by the sides of each pond and the base of the fluff layer at the bottom of each pond. A description of sampling protocols and laboratory analyses used for making biomass estimates of taxa utilizing the ponds is included in a complementary data paper (see Chapter 3). Yield-effort curves were used to insure completeness of sampling (Cohen et al. 1993). For each model, taxa were aggregated into 31 compartments. Taxa smaller than macro-invertebrates were aggregated based on diet and the community they occupied. Correspondence analysis was used to aggregate macro-invertebrates

and fish into compartments based on similarities in both diet and predators as outlined in Luczkovich et al. (2002). A breakdown of taxa for each compartment can also be found in Chapter 3.

Ecopath software (Christensen and Pauly 1992) was used in the analysis. Model inputs included the biomass, production/biomass (P/B) ratio, consumption/biomass (Q/B) ratio, fraction of consumption not assimilated (egestion/consumption or E/Q), and diet for each compartment. Biomass, in µg C m^{-2}, was estimated from field sampling. When no representatives of a compartment were detected in the field, a biomass value of 1.00×10^{-5} µg C m^{-2} was used as a place holder in each model. P/B ratios for producers and microbes were obtained from the literature and all others were derived from allometric relationships to body mass as summarized by Peters (1983). Q/B ratios for all compartments were then calculated from gross food conversion efficiencies [GE=(P/B)/(Q/B)] with the only exception being birds which were assumed to have P/B of 1.5% per day with a GE of 6%. This approach followed the general rules used by Christian and Luczkovich (1999). Consumer P/B and Q/B ratios were corrected for temperature differences between sampling events using a Q_{10} of 1.6 (with the exception of Birds - compartment 29). E/Q for all compartments was obtained from Ulanowicz et al. (2003) who calculated egestion from estimates of consumption, production, and respiration [E=C-(P+R)].

In Ecopath, diet relationships are input in the form of a matrix, and unique diet matrices were used in each model for this study. With the exception of *Anguilla rostrata*, the diet of all fish compartments was based on stomach content analysis using the sieve fractionation method (Carr and Adams 1972, 1973; Luczkovich and Stellwag 1993). The diet for all other taxa (including *A. rostrata*) was derived from binary feeding relationships found in the literature. An assumption of opportunistic feeding was used to calculate a quantitative diet matrix from the binary feeding relationships and the biomass of prey compartments in each model. Documentation of model inputs, including literature sources, and rules used in balancing can be found in Chapter 3.

Water quality parameters in each pond including salinity, temperature, and depth were recorded daily during the sampling periods. Surface and bottom dissolved oxygen was measured hourly over one 24 hr period in each pond during each sampling event. Each pond was also surveyed using plane table and alidade techniques. Area and depth measurements were used in biomass estimates to compute μg C m^{-2}, temperature values in estimating Q_{10} adjustments of P/B and Q/B, and all four water quality parameters were used for defining stress conditions.

Hypotheses And Statistical Analyses

Hypothesis testing was performed on the 12 indices from ENA output described in Table 4-1. Based on the degree of stress (i.e. high salinity, high

temperature, low dissolved oxygen) and disturbance (i.e. decreased water level due to drought) in the ponds between the three sampling events, values for each index were predicted to have the following relationship: Low Stress ≠ High Stress ≠ Post-Disturbance. The reasoning for this hypothesis was that environmental stress and disturbance leads to changes in the pond community including presence/absence of taxa, abundance of taxa, and differences in behavior which result in variation of food web interactions.

Similar hypothesis testing was performed on three community level indices representing each pond during the three sampling events. Richness was calculated as the number of taxa detected. Taxa were not always identified to species, but were distinguished to a level where differentiation of diet could be made. Thus, the same taxonomic classifications were made for each pond. A list of taxa is given in Chapter 3. Evenness, or relative abundance among taxa, was calculated from the following (Pielou 1975): $J = - \Sigma \, p_i \, \ln p_i / \ln t$, where J is evenness, p_i is the proportion of biomass of the total belonging to taxa p, and t is the number of taxa or richness. Diversity, which takes into account both richness and evenness, was given by the following (Pielou 1975): $H = - \Sigma \, (p_i) \, (\log_2 p_i)$, where H is the Shannon-Weaver Index..

Both parametric and nonparametric statistics were used for hypothesis testing to provide confidence in the interpretation of results. The parametric approach involved using a split plot design (blocking North-South and East-West Ponds) to run a repeated measures ANOVA with contrasts for each of the

indices. The repeated measures ANOVA was used to determine if there were significant differences among stress levels (or times) and if the interaction between stress level and block was significant. For each ANOVA, contrasts were used to compare stress levels and identify the source of any differences among sampling times (i.e. low stress vs. high stress, low stress vs. post-disturbance, high stress vs. post-disturbance).

Although the use of salt marsh ponds as a study system allowed for replication, satisfaction of the assumptions of parametric tests (e.g. normality) could not be verified because of low n values. Therefore hypotheses were also tested using the Friedman's Test, a nonparametric equivalent to the repeated measures ANOVA (Zar 1984). Values for each index were blocked by pond, ranked, and then analyzed to determine if statistical differences in the rankings exist among the three stress/disturbance levels. Ryan's Q post hoc multiple comparison test (Day and Quinn 1989) was then used to identify relationships between the mean rankings. Separate Friedman's Tests/Ryan's Q tests were performed for each of the indices.

Two other analyses were also performed. Descriptive statistics including mean, standard deviation, and coefficients of variation were calculated for each of the 12 indices described in Table 4-1. This provided a sense of the variability of ENA output among the three stress/disturbance levels. A final analysis involved a series of Spearman's Rank Correlation Tests. Correlation coefficients and their significance were calculated for each combination of indices because

index values potentially vary together. Stepwise error rate was controlled with a Bonferroni correction. SAS System for Windows Version 8 was used for all procedures.

Results

Changes In Abiotic Variables

To determine the effectiveness of ENA in detecting change in trophic conditions it must first be shown that the real-world ecosystems, and inputs for their models, do indeed change. Water quality measurements suggest significant differences in the pond's environmental conditions among sampling events (Table 4-2). Afternoon salinities and temperatures were lowest during low stress conditions and highest the other two times. Although highly variable, the extent of hypoxic conditions was also lowest in each pond during low stress conditions. The degree of disturbance due to drought was greatest in East and West Ponds (which went completely dry), while North and South Ponds were affected to a less extent.

Community Response

Corresponding to the changes in environmental conditions were differences in the number of taxa and their abundance. Community level indices of richness, evenness, and diversity illustrate these differences. Richness is

greatest during low stress conditions (Figure 4-1a), but the opposite is true for evenness (Figure 4-1b) and diversity (Figure 4-1c).

Results from both repeated measures ANOVA (Table 4-3) and Friedman's Tests (Table 4-4) indicate all three community indices were significantly different during low stress conditions relative to the other two times (i.e. Low Stress ≠ High Stress = Post-Disturbance). Although ANOVA contrasts were not significant for richness, a multiple comparison associated with the Friedman's Test indicated mean rankings of the low stress condition were different. Block effects were not significant for richness, but were for the other two indices. This indicates evenness and diversity were more similar in North-South Ponds relative to East-West Ponds (Figure 4-1b and 4-1c), and suggests a stronger relationship between the two indices compared to between diversity and richness.

Food Web Response

After aggregating all taxa into compartments based on diet and common predators, the number of active compartments (i.e. those with taxa present) per food web model differed between time periods. Low stress conditions had the highest number of active compartments with between 27-29 (22-24 consumers; 5 producers). High stress conditions had between 21-23 compartments (17-19 consumers; 4 producers), and post-disturbance networks had 20-21 (16-17 consumers; 4 producers).

Table 4-5 illustrates differences in biomass among the three time periods for two of the four ponds. The model compartment with the largest biomass was typically particulate organic carbon (POC; 31) except during low stress conditions when *Ruppia maritima* (5) was greater. POC was primarily associated with the fluff layer. Epiphyitic algae (2) had the second largest biomass among producers during low stress conditions because of the presence of *R. maritima*. At other times, benthic microalgae (1) were the most abundant producer. For all ponds primary producer biomass was 1-2 orders of magnitude greater during low stress conditions (Figure 4-2a) because *R. maritima* was only present during this time. High temperatures likely inhibit its growth during the other sampling events (Swiderek 1982).

Among consumers, detritivorous insects (14) and zooplankton (10) typically had relatively low biomass except during post-disturbance when they had some of the highest levels (e.g. Table 4-5 and text below). In comparison, meiofauna (11) was consistently one of the larger compartments. Zooplankton and meiofauna were composed of similar taxa (see Chapter 3), and separation into different compartments was based on habitat: zooplankton were located in the water column and meiofauna in the fluff layer. Omnivorous insects (20) had relatively low biomass values during all times. Other differences included an increased biomass of amphibians (26-27), snakes (28), and wading birds (29) during low stress conditions. Tadpoles (26) were an important herbivore and prey item found only during this time.

Differences in consumer biomass between sampling events for all ponds are shown in Figure 4-2b. The largest changes occurred in response to drought conditions, and appeared to differ depending on the degree of drop in water levels. For example, East and West Ponds went completely dry and subsequently experienced a shift from fish-dominated to insect-dominated communities (Figure 4-2c). The reduction in predatory fish (compartments 21-22, 24-25) not only corresponded with a dramatic increase in insects (e.g. mosquito larvae - compartment 14, and herbivorous beetles - compartments 15 & 16) but also other prey items (e.g. zooplankton). The result was an overall increase in consumer biomass in these two ponds. A much less dramatic shift to an insect dominated community occurred in North Pond, and no similar shift occurred in South Pond. Neither of these ponds went completely dry, and predatory fish biomass during post-disturbance was similar to other time periods in both ponds. Although insect and zooplankton biomass increased, there was an overall decrease in consumer biomass during post-disturbance in North and South Ponds.

Differences in biomass among the three sampling periods not only represent variation in the fundamental variable of model input, but indirectly result in changes in diet information used in model construction. Biomass of prey items were used to weight binary feeding relationships to calculate quantitative diet matrices. Thus, variation in biomass generates differences in the diet matrices used in the models.

Changes In ENA Output

Differences in ENA output were found to correspond with changes in abiotic variables (used to characterize stress/disturbance in the ponds) and biotic variables (used to construct trophic networks of the ponds) between the three sampling events. Histograms of five indices from Trophic Structure Analysis are presented in Figure 4-3, four from Pathway Analysis in Figure 4-4, and three from Information Analysis in Figure 4-5. Index values are provided in Appendix 4-I.

Results from repeated measures ANOVA indicate 10 of the 12 indices had significant differences among the three stress/disturbance levels (Table 4-6). Trophic efficiency and number of paths were the exceptions. The former had similar mean values among stress levels, and both had large coefficients of variation indicating relatively high variability between ponds (Table 4-7). Eight of the 10 significant indices had p-values ≤ 0.0184. P-values for average path length and the ratio of primary production required to net primary production were slightly less than the 0.05 alpha level. Thus, differences between stress levels for most of the indices were highly significant. Only one index, ecotrophic efficiency, had a significant interaction between stress level and block indicating most indices exhibited no change in pattern of their values for North and South Ponds relative to East and West Ponds.

As part of the repeated measures ANOVA, three contrasts were run for each index to identify the source of any significant differences among times (i.e.

low stress vs. high stress, low stress vs. post-disturbance, high stress vs. post-disturbance). Alpha levels were adjusted to 0.017 (0.05/3) to control for type I error (i.e. finding a significant difference between stress levels when one did not exist). At the adjusted alpha, significant contrasts between stress levels were found in only four indices and no indices had significant block effects (Table 4-6). Means of average path length, ascendency/capacity, and connectance differed between low stress conditions and post-disturbance. Ascendency/capacity also differed between low stress and high stress conditions, and aggregated trophic chain length differed between high stress and post-disturbance. Therefore, although ANOVA results indicated 10 indices differed between stress levels, ANOVA contrasts were insufficient at identifying which stress levels were different (likely because of relatively low degrees of freedom).

Friedman's Tests coupled with Ryan's Q Multiple Comparisons provided a better understanding of how the indices differed with stress/disturbance in each pond. Results from Friedman's Tests were generally in agreement with those of the repeated measures ANOVA. Mean rankings in 11 of 12 indices exhibited significant differences with stress/disturbance level (Table 4-8). As with the parametric approach, trophic efficiency was an exception. However the Friedman's Test indicated the number of paths index differed between sampling events. Of the 11 significant indices only ecotrophic efficiency had a p-value > 0.002. Thus similar to ANOVA results, differences identified with Friedman's Tests were highly significant.

Multiple comparisons associated with the Friedman's Tests provided a more effective method (relative to ANOVA contrasts) of identifying which stress levels were significantly different for each index (Table 4-8). Results from the multiple comparisons were in agreement with all five significant contrasts from the repeated measures ANOVA. In addition, multiple comparisons indicated the following relationships in the mean rankings of indices among stress levels:

- For number of paths, average path length, ascendency/capacity, connectance: Low Stress ≠ High Stress = Post-Disturbance.

- For aggregated trophic chain length, primary production required/net primary production, and total systems throughput: Low Stress ≠ High Stress ≠ Post-Disturbance.

- For mean effective trophic level, system omnivory index: Post-Disturbance ≠ Low Stress = High Stress.

- For the ratio of herbivory to detritivory: High Stress ≠ Low Stress = Post-Disturbance.

- For mean ecotrophic efficiency: Low Stress ≠ High Stress with Post-Disturbance being similar to both Low and High Stress.

Therefore, although only 3 of the 11 significant indices differed among each of the stress levels (i.e. Low Stress ≠ High Stress ≠ Post-Disturbance), values for low stress conditions were significantly different from those of at least one other stress level in all 11 indices.

There is a high likelihood for correlation between indices because the various algorithms used for their calculation utilize many of the same variables. However, only 7 of 66 possible pairs of indices (or 16%) were found to be significantly correlated (Tables 4-9 and 4-10). No pair of indices from a single suite of analysis (i.e. Trophic Structure Analysis, Pathway Analysis, Information Analysis) had a significant correlation among their rankings. Rankings of all three indices from Information Analysis had a strong correlation with rankings of other indices from Pathway Analysis. Indices from Trophic Structure Analysis with a strong correlation were ecotrophic efficiency and system omnivory whose rankings correlated with those of average path length and the amount of primary production required respectively. Significant correlations between rankings of average path length and those of other indices were always negative.

Summary Of Results

My results show responses in community level indices, food web characteristics, and ENA output accompanied changes in environmental conditions in the ponds. Differences in the presence/absence of taxa and their biomass were likely the basis for these responses. Community level indices were significantly different during low stress conditions relative to high stress and post-disturbance (i.e. Low Stress ≠ High Stress = Post-Disturbance). A comparable relationship between the three stress levels was found for the number and biomass of primary producers, herbivores, and upper trophic levels

making up the food webs. Most ENA indices examined had a similar response in that values for low stress conditions were singled out as being different. However, the ENA indices also showed differences among the three stress levels not observed with the community level indices (e.g. Low Stress ≠ High Stress ≠ Post-Disturbance; Post-Disturbance ≠ Low Stress = High Stress; High Stress ≠ Low Stress = Post-Disturbance).

Discussion

Ecology Of Salt Marsh Pond Ecosystems

An outcome of this study was insight into the ecology of salt marsh pond ecosystems. As with most ecosystems (Huggett 1995), physical forcings apparently regulated biotic interactions in the study ponds. At one scale, this was evident in the presence of three semi-distinct communities in each pond: benthic, epiphytic, pelagic. Although meiofauna and microbes were abundant in the "fluff" layer at the bottom of the ponds, the benthic community was almost entirely depauperate of macrofauna. This was likely a result of constant anoxic conditions in the "fluff" layer, and it was possibly related to relatively high sulfide concentrations. While no analyses were performed to support the latter, all "fluff" samples contained intense sulfide odor. Most macrofauna were associated with the epiphytic community presumably due to the presence of abundant microhabitat along the nearly vertical walls of the ponds. This provided refugia in the form of craggy overhanging peat surfaces with protruding root matter.

An enormous amount of additional refugia was provided by the presence of *R. maritima*. When present it covered between 25 and 90 percent of a pond's surface, resulted in macrofauna dispersing into the center of the ponds, and allowed for increased numbers of taxa and biomass. However, *R. maritima* was only present in spring apparently due to the effects of heat stress during late summer (Swiderek 1982). High stress conditions not only resulted in diminished refugia, but also likely altered behavior of consumers. Hypoxic conditions are known to force fish to gulp air at the water surface, and this potentially results in fish being more susceptible to predation by wading birds (Smith 1995). Increased predation is possibly exacerbated by relatively lower water levels during this time. Wading bird use of a particular pond was highly sporadic (usually 1-2 feeding events per sampling period), but typically involved large congregations (up to 30 birds per set of ponds). Wading birds also utilized the ponds during low stress conditions presumably due to greater amounts of prey and the need to feed young during the breeding season. Thus, the influence of stress on non-trophic factors (i.e. increased refugia from R. maritima and altered behavior of fish taxa) potentially had important affects on trophic interactions in the ponds.

Although differences among the sampling events focused on stress and disturbance, seasonal effects were also a factor. Low stress conditions of spring (relatively low salinity, low temperature, high dissolved oxygen) coincided with movements associated with the reproductive cycle of many organisms that

utilized the ponds. For example, *N. sipedon* (Northern Water Snake) congregate during the spring breeding season and disperse later in the summer (Brown 1940; Conant and Collins 1991), the elver stage of *A. rostrata* (American Eel) typically moves into estuaries and up freshwater systems in late winter and early spring (Van Den Avyle 1984), and tadpoles of *R. utricularia* (Southern Leopard Frog) emerge in spring (Conant and Collins 1991; McDiarmid and Altig 1999). Moreover Smith (1995), working in salt marsh pools, noted seasonal changes in the abundance of young-of-the-year for the same fish species found in my study ponds, and Layman et al. (2000) documented seasonal variation in abiotic and biotic factors that structure fish communities in nearby salt marsh ponds. Thus life history characteristics, seasonal changes, and different environmental conditions combined to produce changes in pond ecology between sampling events, and their effect was particularly dramatic in spring.

Geomorphic setting was also a major factor in pond ecology. North and South Ponds were situated in the hummock and hollow zone of the mainland salt marsh, while East and West Ponds were located up gradient in the forest transition zone. As a result, the edges of North and South Ponds were more irregular and there was greater potential for connectivity with the surrounding marsh surface. The most striking example of a pond's location impacting its food web involves post-disturbance conditions. During drought conditions East and West Ponds went completely dry while North and South Ponds did not. This caused dramatic decreases in fish biomass during post-disturbance in East and

West Ponds, and resulted in a change from a fish dominated to an insect

dominated community (Figure 4-2c).

Ecosystem Level Indices vs. Community Level Indices

Community level indices examined in this study indicate the ponds differed

during low stress conditions relative to the other two times. Indices from ENA

output mostly agreed with the community level indices, but they also suggested

additional differences in the ponds between the three sampling events. This was

because ENA indices were tracking properties other than just biologic structure

given by the number and abundance of taxa.

Average path length, ascendency/capacity, and connectance showed the

same relationships as the community indices (i.e. Low Stress ≠ High Stress =

Post-Disturbance). Each of these is a function of energy flow, number of

interactions, and interaction strength. A fourth ENA index, number of paths, also

had this relationship. It quantifies the number of interactions in a food web, but

unlike the other three, the number of paths is not related to the amount of energy

flow. Thus, although these ENA indices showed similar results as the community

indices, they apparently were tracking a system level response to increased taxa

and biomass. That response was an increased number and strength of

interactions between taxa.

Trends in number of paths, ascendency/capacity, and connectance

(Figures 4-4a, 4-5b, 4-5c) were in agreement with the conceptual model of

Menge and Sutherland (1987). Their model suggests low stress conditions are associated with a greater number and strength of interactions, and this would lead to an increased value of these indices during low stress conditions. However, values for average path length in the ponds were opposite (Figure 4-4b). Average path length was calculated using the following formula: APL = [TST/(Sum of Exports + Sum of Respirations)], where TST = total system throughput (Christensen and Pauly 1992). As expected, total systems throughput was smaller with increasing stress/disturbance (see below) and respiration was larger. However, greater exports (from POC to the peat layer underlying the ponds) during low stress conditions resulted in decreased average path length during this time.

Three indices, each from a separate set of analyses that make up ENA, were different among all three stress levels (i.e. Low Stress ≠ High Stress ≠ Post-Disturbance). These included total systems throughput (from information analysis), ratio of primary production required to net primary production (from pathway analysis), and aggregated trophic chain length (from trophic structure analysis). Total systems throughput is the sum of all flows in a system, and was found to decrease with increased stress/disturbance (Figure 4-5a). This follows the findings of Ulanowicz (1996) and Monaco and Ulanowicz (1997) who gave evidence of stress negatively impacting system size and organization. Opposite of this trend, the ratio of primary production required to net primary production was greater with increasing stress/disturbance (Figure 4-4c). This was likely due

to relatively large decreases in primary production relative to changes in consumer biomass with increasing stress (Figures 4-2a and 4-2b). Thus, even though these indices track different aspects of energy flow in the system, each indicated differences among all three stress levels.

The same was true for aggregated trophic chain length which was calculated as the highest trophic level with ≥ 1 percent of the total systems throughput. However unexpectedly, it was greatest during high stress conditions (Figure 4-3c). This was counter to Menge and Sutherland's (1987) model, as well as the findings of Ulanowicz (1996) and Monaco and Ulanowicz (1997). They indicated increased stress would decrease the number of taxa at upper trophic levels and shorten the Lindeman spine or aggregated trophic chain. However, these studies did not account for the influence of non-trophic factors that were evident in the ponds (e.g. increased stress altering behavior and enhancing predation - see Pond Ecology Section in this Chapter). An increased susceptibility of fish and macro-invertebrates to predation by wading birds during high stress conditions (see Pond Ecology Section in this Chapter) could have contributed to my results.

Unlike the community level indices, two ENA indices were different during post-disturbance relative to the other sampling events. Mean effective trophic level was lowest during this time (Figure 4-3a), and system omnivory index was highest (Figure 4-3e). Post-disturbance was distinguished by an absence of the top predators snakes and birds even though adult frogs were present. Snakes

were present only during low stress conditions, and as mentioned above, birds

were a large player in energy flow during both low stress and high stress

conditions. Post-disturbance was also characterized by lower numbers of taxa

and biomass. Thus the removal of top predators probably resulted in decreased

effective trophic levels, and less diversity likely led to less selectivity among

feeding preferences. Although the community level indices did identify

differences in diversity, they could not distinguish its effect on energy flow.

Results from two other ENA indices highlight additional areas where the

two types of indices differed. First, the ratio of herbivory to detritivory was lowest

during high stress and similar during the other two stress conditions (Figure 4-

4d). The index, calculated as the ratio of the flow from primary producers to the

flow from detritus, is a function of the relationship between herbivore and

detritivore biomass. Detritivory was greatest during high stress conditions due to

a relatively large biomass of sediment bacteria, the largest consumer of detritus

in the ponds. Low stress and post-disturbance conditions were characterized by

relatively greater herbivory due to a large biomass of tadpoles in spring and

relatively low sediment bacteria biomass during post-disturbance. These

differences in biomass, and their effect on energy flow, were not apparent with

the community level indices.

A second index, mean ecotrophic efficiency, was greater during low stress

conditions relative to high stress with values for post-disturbance being similar to

both (Figure 4-3b). Post-disturbance variation in this index suggests a distinction

between North-South Ponds relative to East-West Ponds. A similar pattern was found with other ENA indices (e.g. aggregated trophic chain length during low stress, average path length during high stress and post-disturbance, ascendency/capacity during low stress and post-disturbance). Although evenness and diversity (Table 4-3 and Figures 4-1b and 4-1c) indicated a distinction between the two sets of ponds, significant block effects (i.e. North-South vs. East-West) were found only with mean ecotrophic efficiency among the ENA indices (Table 4-6). The community indices were potentially identifying differences in biologic structure related to variation in the geomorphic setting of the two sets of ponds within the marsh (as well as their response to drought; see Pond Ecology Section in this Chapter). Due to ecological equivalents in the system, it is likely these differences did not lead to significant variation in energy flow. Ecological equivalents are different taxa capable of carrying out similar functions or providing simmilar links in the food web (Odum and Barrett 2005). For example, the shift from a fish dominated to insect dominated community that occurred during post-disturbance was detected as a change in biologic structure. However, indices of energy flow reflected little change because the additional insects filled the same functional role as the fish that were no longer present. Thus in this case, variability detected in the community level indices was not observed in the ENA output.

All 11 of the 12 ENA indices that showed significant differences among stress levels made a distinction between low stress conditions and at least one

other stress level. A common factor was that these indices, with one exception (number of paths), were tracking some aspect of energy flow. Low stress conditions were characterized by an order of magnitude greater producer biomass (Figure 4-2a) and approximately ¼ more active compartments (both producers and consumers; see Results Section). These dramatic differences were due to *R. maritima* whose abundance during low stress conditions provides both a food source and refugia. In comparison, these variables were more similar between high stress conditions and post-disturbance. The major differences between these high stress and post-disturbance were a roughly twofold change in consumer biomass (Figure 4-2b) and the types of consumers (Figure 4-2c). Therefore primary producer biomass, specifically the presence of *R. maritima*, appears to have been a major factor of energy flow in these systems and resulted in distinguishing low stress conditions among most of the ENA indices.

Finally, only 1 of the 12 ENA indices (trophic efficiency) was not significantly different among stress levels. These results were not in agreement with Ulanowicz (1996) and Monaco and Ulanowicz (1997) who found stress lowers trophic efficiency. Both parametric and nonparametric statistics (Tables 4-6 and 4-8 respectively) indicated the null hypothesis could not be rejected for trophic efficiency in the ponds, and this was likely due to similar means and high variability among sampling events (Table 4-7). However with two exceptions, values for trophic efficiency were in agreement with the patterns observed in the

previous studies. Other than South Pond and East Pond during post-

disturbance, trophic efficiency was greatest during low stress conditions and

decreased with increasing stress/disturbance (Figure 4-3d). Thus of 12 ENA

indices, the single index that did not show significant differences among stress

levels displayed trends that corresponded to the findings of other studies.

Practical Implications

The catalyst for undertaking this study is the increasing use of ENA and its

application to ecosystem-based management. Resource managers have begun

to add ENA to their decision making tool box (e.g. NCBO 2003), and ENA output

is being employed to parameterize simulation models used in exploring

management scenarios (e.g. Walters et al. 1997, 2000). Many management

issues such as the effects of fishing (e.g. oyster dredging: Lenihan and Peterson

1998), invasive species (e.g. Great Lakes: Mills et al. 1994), pollution (e.g.

Chesapeake Bay: Kemp et al. 2005), and hydrologic regime shifts (e.g.

Everglades: Davis and Ogden 1994) have been characterized as involving a

stress/disturbance to the natural system. ENA shows great potential in helping

us to understand the ecological affects of these issues to manage them

effectively. Although network ecology has a strong theoretical foundation (e.g.

Kay et al. 1989; Ulanowicz 2005) with numerous empirical studies (e.g. Wulf et

al. 1989; Christensen and Pauly 1993a), there is a need to critically examine

model output and take a more comparative approach to ecological networks (e.g. see "Webs on the Web" at http://galas.sfsu.edu/index_page/wow2.html).

This study is the first systematic effort to statistically evaluate ENA output using replicated networks under different environmental conditions. Using stress/disturbance as a mechanism for driving trophic change, results indicate ENA was effective in detecting a variety of differences in the pond food webs. Both ENA output and community level indices made a distinction between low stress conditions and the other two sampling events. However, the ENA indices identified additional differences between the three stress/disturbance levels that were beyond the scope of the community indices. This is because ENA indices quantify different aspects of energy flow and interactions among components of the food web, whereas community indices are related to different factors of biologic structure (i.e. number and biomass of taxa). In the organizational hierarchy, communities make up food webs and food webs are a major component of ecosystems. This study suggests ENA is more suited for quantifying and evaluating ecosystem properties compared to community level indices.

The concern of covariance among ENA indices was largely unsupported as a relatively small number of significant Spearman's Rank Correlation coefficients were detected. Covariance among indices could lead to similar relationships among the stress/disturbance levels. Therefore in spite of similar variables being used in the various algorithms for calculating the ENA indices,

the relatively small number of significant correlations gives confidence in the independence of the indices and the validity of the results from hypothesis testing. It further indicates the ENA indices were tracking different aspects of the food web.

The implications for using ENA to address management issues should be apparent, but not overstated. Certainly, my results strongly support these efforts as ENA apparently provides the manager with a variety of output to evaluate the functioning of a system. However, it should be noted that my study systems (salt marsh ponds) were unlike most that are analyzed with ENA. The scale of the ponds permitted a relatively thorough documentation of the major taxa involved in energy flow, and they allowed for sources of uncertainty associated with ENA (see Chapter 2) to be addressed and their effects kept to a minimum. This situation is not typical of studies involving larger ecosystems, particularly those undertaken for resource management. Additional studies along the lines of my approach are needed to clarify patterns of trophic response to stress/disturbance and identify limitations of the application of ENA. With respect to the latter, future work should focus on the affects of relying heavily on literature values for model input and specifically whether quantification of flows (e.g. production/biomass) from algorithms and body size estimates is adequate. Moreover, efforts to validate models are needed before relying too heavily on the ecological interpretation of the output.

Table 4-1. Examples of indices provided by three major categories of ENA output (Trophic Structure Analysis, Pathway Analysis, and Information Analysis). These 12 indices were used to evaluate the effectiveness of ENA in detecting differences in food web properties. Full definitions of each index can be found in Christensen and Pauly (1992) and Christensen et al. (2002).

TROPHIC STRUCTURE ANALYSIS

● Mean Effective Trophic Level – mean of each compartment's effective trophic level (which includes degrees of omnivory) weighted by that compartment's biomass.

● Mean Ecotrophic Efficiency - mean of the proportion of production from each compartment that is passed up the food chain.

● Aggregated Trophic Chain Length - number of discrete trophic levels calculated by collapsing the entire food web into a trophic chain.

● Trophic Efficiency of System - geometric mean of the proportion of the input to each trophic level that is passed on to the next trophic level.

● System Omnivory Index - index of how the feeding interactions are distributed between trophic levels.

PATHWAY ANALYSIS

● Number of Paths - sum of the number of pathways from primary producers to all consumers.

● Average Path Length - average number of compartments that a flow passes through.

● PPR/NPP - ratio of the primary production required to sustain the consumption of all consumers to the total net primary production of the system.

● Herbivory:Detritivory Ratio - quantifies the ratio of flow along grazing and detrital food webs.

INFORMATION ANALYSIS

● Total System Throughput - measure of system size given by the sum of all flows.

● Ascendency/Capacity - index of the size and development of a system relative to its potential.

● Connectance - index of food web complexity.

Table 4-2. Water quality parameters used to characterize three different environmental conditions in the ponds.

	Low Stress	High Stress	Post-Disturbance
North Pond			
Salinity (ppt)[1]	14.6	19.2	23.2
Temperature (^{o}C)[1]	23.8	29.5	29.7
Hypoxia Index (hrs)[2]	6	12	14
Water Level Index (%)[3]	100	90	100 (30)
South Pond			
Salinity (ppt)[1]	14.8	19.5	23.4
Temperature (^{o}C)[1]	23.7	29.3	29.7
Hypoxia Index (hrs)[2]	12	16.5	13
Water Level Index (%)[3]	100	90	100 (50)
East Pond			
Salinity (ppt)[1]	10.7	19.3	16.7
Temperature (^{o}C)[1]	22.8	29.5	29.6
Hypoxia Index (hrs)[2]	0	6	12
Water Level Index (%)[3]	100	80	100 (0)
West Pond			
Salinity (ppt)[1]	11.1	19.2	16.1
Temperature (^{o}C)[1]	22.5	29.3	29.4
Hypoxia Index (hrs)[2]	0	6	11
Water Level Index (%)[3]	100	80	100 (0)

[1] Salinity and temperature represent mean daily values measured between 3-4 pm.

[2] The number of hours over a 24 hr period that dissolved oxygen was below 2 mg/l.

[3] Water level in the ponds during sampling periods relative to bank full conditions (i.e. full pond = 100%). For Post-Disturbance, the water level prior to sampling is given in parentheses as an indicator of the degree of disturbance in each pond.

Table 4-3. Results from repeated measures ANOVA for community level indices. A separate ANOVA was run for each index and included 3 contrasts (low stress vs. high stress, low stress vs. post-disturbance, high stress vs. post-disturbance). A split plot design was used by blocking North-South Ponds and East-West Ponds. ANOVA model: Stress Level = Block.

Index	ANOVA Test	Source	df	F Value	Pr > F[1]
Richness	Within Pond Effects	Stress	2	24.49	0.0057 x
		Stress Level x Block	2	3.09	0.1542
	Contrast Low vs. High	Mean	1	25.14	0.0376
		Block	1	2.79	0.2366
	Contrast Low vs. Post-Disturbance	Mean	1	32.40	0.0295
		Block	1	0.40	0.5918
	Contrast High vs. Post-Disturbance	Mean	1	4.76	0.1607
		Block	1	9.94	0.0876
Evenness	Within Pond Effects	Stress	2	388.17	<0.0001 x
		Stress Level x Block	2	18.05	0.0100 x
	Contrast Low vs. High	Mean	1	384.55	0.0026 xx
		Block	1	19.53	0.0476
	Contrast Low vs. Post-Disturbance	Mean	1	1069.99	0.0009 xx
		Block	1	14.14	0.0640
	Contrast High vs. Post-Disturbance	Mean	1	42.45	0.0228
		Block	1	16.58	0.0554
Diversity	Within Pond Effects	Stress	2	163.19	0.0001 x
		Stress Level x Block	2	7.51	0.0443 x
	Contrast Low vs. High	Mean	1	150.87	0.0066 xx
		Block	1	7.48	0.1118
	Contrast Low vs. Post-Disturbance	Mean	1	370.85	0.0027 xx
		Block	1	10.33	0.0847
	Contrast High vs. Post-Disturbance	Mean	1	34.79	0.0276
		Block	1	5.26	0.1488

[1] Significance: X = significant at the 0.05 level for Within Pond Effects; XX = significant at the 0.017 level for Contrasts (Bonferroni correction = 0.05 alpha/3 contrasts).

Table 4-4. Results from Friedman's and Ryan's Q Multiple Comparison Tests for community level indices. Index values were blocked by pond and ranked among the three stress/disturbance levels. Separate tests were run for each index. Friedman's Model: Ranked Index = Stress Level.

Index	Source	df	F Value	Pr > F	Grouping	Mean Rank	n	Stress Level
			Friedmans Test[1]			Multiple Comparison Test		
Richness	Model	2	19.5	0.0005	A	3.00	4	Low
	Error	9			B	1.75	4	High
	Total	11			B	1.25	4	Post
Evenness	Model	2	19.5	0.0005	A	2.75	4	High
	Error	9			A	2.25	4	Post
	Total	11			B	1.00	4	Low
Diversity	Model	2	Infinity	< 0.0001	A	3.00	4	High
	Error	9			B	2.00	4	Post
	Total	11			C	1.00	4	Low

Table 4-5. Example of biomass changes from two of the four study ponds. North and South Ponds are located adjacent to each other in the hummock and hollow zone of a mainland salt marsh, while East and West Ponds are juxtaposed further up gradient in the forest transition zone. This results in similarities within each pair of ponds among the sampling times. Values of 1.00E-05 indicate no individuals of taxa making up that compartment were observed during sampling, and the value is used as a place holder in the model.

	Compartment	North Pond Biomass (ug C m^{-2})			East Pond Biomass (ug C m^{-2})		
		Low Stress	High Stress	Post-Disturbance	Low Stress	High Stress	Post-Disturbance
1	Benthic Microalgae	8.80E+06	2.40E+07	1.79E+07	9.80E+06	3.29E+07	9.41E+06
2	Epiphytic Algae	6.26E+07	1.98E+06	1.27E+05	2.61E+07	1.27E+06	2.16E+05
3	Overhanging Vegetation	1.42E+07	1.12E+07	1.40E+07	1.84E+07	1.44E+07	5.28E+07
4	Phytoplankton	9.93E+05	1.73E+05	1.78E+06	1.40E+06	1.40E+06	4.89E+05
5	*Ruppia maritima*	3.91E+08	1.00E-05	1.00E-05	1.36E+09	1.00E-05	1.00E-05
6	H$_2$0 Column Bacteria	1.84E+05	6.66E+04	1.04E+05	5.66E+05	6.75E+04	8.39E+04
7	H$_2$0 Column Micro-Protozoans	5.39E+04	5.92E+03	5.99E+04	1.22E+05	4.17E+03	7.93E+04
8	Sediment Bacteria	2.88E+06	2.77E+06	5.68E+05	1.53E+06	3.42E+06	2.64E+05
9	Sediment Micro-protozoans	4.46E+05	1.57E+05	5.68E+04	8.36E+04	1.14E+05	2.38E+04
10	Zooplankton	4.16E+03	3.13E+03	5.94E+04	1.84E+03	8.36E+02	2.53E+05
11	Meiofauna	7.14E+05	2.04E+06	1.66E+06	9.76E+05	1.70E+06	3.39E+06
12	Hydrobiidae	4.73E+05	2.61E+04	5.04E+02	9.15E+05	1.47E+05	3.62E+04
13	Amphipods and Decapods	2.12E+05	2.34E+05	1.00E-05	1.21E+03	2.34E+05	2.89E+03
14	Detritivorous Insects	3.64E+02	1.00E-05	6.61E+06	7.35E+03	1.00E-05	2.28E+07

	Compartment	North Pond Biomass (ug C m^{-2})			East Pond Biomass (ug C m^{-2})		
		Low Stress	High Stress	Post-Disturbance	Low Stress	High Stress	Post-Disturbance
15	Small Herbivorous Insects (<1cm)	2.40E+04	1.63E+04	3.99E+04	1.79E+05	2.05E+03	1.77E+05
16	Large Herbivorous Insects (>1cm)	3.66E+04	3.36E+03	5.67E+04	4.19E+05	5.58E+03	1.05E+05
17	Small Predatory Insects (<1cm)	9.24E+03	2.13E+03	6.94E+03	1.79E+04	3.14E+03	1.40E+04
18	Large Predatory Insects (>1cm)	3.65E+04	1.00E-05	8.06E+03	1.45E+05	1.00E-05	4.56E+04
19	Piscivorous Insects & Spiders	4.52E+04	1.33E+03	2.35E+03	3.30E+03	3.56E+03	5.33E+03
20	Omnivorous Insects	1.00E-05	4.75E+01	1.00E-05	5.78E+02	1.03E+03	1.00E-05
21	*Anguilla rostrata*	3.89E+04	1.00E-05	1.00E-05	7.52E+05	1.00E-05	1.00E-05
22	Fish - Pelagic Feeders	1.00E-05	1.00E-05	1.00E-05	1.00E+05	1.23E+06	1.00E-05
23	Fish - Herbivores	1.71E+04	1.77E+05	3.13E+03	1.34E+04	9.57E+04	1.00E-05
24	Fish - Small Prey	3.62E+05	2.59E+05	2.99E+04	9.26E+03	1.00E-05	1.13E+04
25	Fish - Large Prey	1.21E+05	4.84E+05	6.75E+05	5.59E+03	2.10E+05	4.68E+04
26	Tadpoles	2.45E+06	1.00E-05	1.00E-05	1.03E+06	1.00E-05	1.00E-05
27	Frogs	4.54E+04	1.00E-05	3.40E+04	4.86E+04	1.00E-05	6.48E+04
28	Snakes	7.86E+05	1.00E-05	1.00E-05	3.74E+05	1.00E-05	1.00E-05
29	Birds	1.23E+07	1.23E+07	1.00E-05	8.17E+06	8.17E+06	1.00E-05
30	Dissolved Organic Carbon	5.26E+06	5.26E+06	3.21E+07	9.78E+06	9.78E+06	4.93E+07
31	Particulate Organic Carbon	5.00E+07	5.00E+07	2.67E+08	6.38E+07	6.38E+07	3.29E+08

Table 4-6. Results from repeated measures Anova for ENA indices[1].

Index	Anova Test	Source	df	F Value	Pr > F[2]
Mean Effective Trophic Level	Within Pond Effects	Stress	2	22.26	0.0068 x
		Stress x Block	2	2.65	0.1849
	Contrast Low vs. High	Mean	1	8.22	0.1032
		Block	1	14.61	0.0621
	Contrast Low vs. Post-Disturbance	Mean	1	52.52	0.0185
		Block	1	5.19	0.1503
	Contrast High vs. Post-Disturbance	Mean	1	12.22	0.0730
		Block	1	0.00	0.9886
Mean Ecotrophic Efficiency	Within Pond Effects	Stress	2	20.99	0.0076 x
		Stress x Block	2	7.62	0.0432 x
	Contrast Low vs. High	Mean	1	51.52	0.0189
		Block	1	0.59	0.5240
	Contrast Low vs. Post-Disturbance	Mean	1	14.67	0.0619
		Block	1	21.24	0.0440
	Contrast High vs. Post-Disturbance	Mean	1	7.56	0.1108
		Block	1	5.74	0.1389
Aggregated Trophic Chain Length	Within Pond Effects	Stress	2	21.00	0.0076 x
		Stress x Block	2	1.00	0.4444
	Contrast Low vs. High	Mean	1	25.00	0.0377
		Block	1	1.00	0.4226
	Contrast Low vs. Post-Disturbance	Mean	1	1.00	0.4226
		Block	1	1.00	0.4226
	Contrast High vs. Post-Disturbance	Mean	1	Infinity	< 0.0001 xx
		Block	1	-	-
Trophic Efficiency	Within Pond Effects	Stress	2	0.06	0.9406
		Stress x Block	2	0.22	0.8144
	Contrast Low vs. High	Mean	1	0.13	0.7496
		Block	1	1.37	0.3623
	Contrast Low vs. Post-Disturbance	Mean	1	0.02	0.9028
		Block	1	0.20	0.7016
	Contrast High vs. Post-Disturbance	Mean	1	0.14	0.7457
		Block	1	0.01	0.9301
System Omnivory Index	Within Pond Effects	Stress	2	17.09	0.0110 x
		Stress x Block	2	1.36	0.3537
	Contrast Low vs. High	Mean	1	9.06	0.0949
		Block	1	14.04	0.0644
	Contrast Low vs. Post-Disturbance	Mean	1	25.68	0.0368
		Block	1	1.90	0.3020
	Contrast High vs. Post-Disturbance	Mean	1	11.67	0.0760
		Block	1	0.01	0.9162

Table 4-6 continued.

Index	Anova Test	Source	df	F Value	Pr > F^2
Number of Paths	Within Pond Effects	Stress	2	4.49	0.0950
		Stress x Block	2	0.19	0.8376
	Contrast Low vs. High	Mean	1	3.82	0.1899
		Block	1	0.08	0.8022
	Contrast Low vs. Post-Disturbance	Mean	1	5.67	0.1402
		Block	1	0.30	0.6369
	Contrast High vs. Post-Disturbance	Mean	1	0.02	0.8973
		Block	1	1.00	0.4235
Average Path Length	Within Pond Effects	Stress	2	6.96	0.0499 x
		Stress x Block	2	3.04	0.1572
	Contrast Low vs. High	Mean	1	8.49	0.1003
		Block	1	1.51	0.3446
	Contrast Low vs. Post-Disturbance	Mean	1	225.00	0.0044 xx
		Block	1	81.00	0.0121 xx
	Contrast High vs. Post-Disturbance	Mean	1	3.65	0.1963
		Block	1	4.31	0.1735
Primary Production Required/Net Primary Production	Within Pond Effects	Stress	2	7.37	0.0456 x
		Stress x Block	2	5.01	0.0813
	Contrast Low vs. High	Mean	1	18.95	0.0489
		Block	1	0.15	0.7379
	Contrast Low vs. Post-Disturbance	Mean	1	7.70	0.1091
		Block	1	4.93	0.1566
	Contrast High vs. Post-Disturbance	Mean	1	6.99	0.1182
		Block	1	5.12	0.1520
Herbivory:Detritivory	Within Pond Effects	Stress	2	15.38	0.0126 x
		Stress x Block	2	0.37	0.7138
	Contrast Low vs. High	Mean	1	19.83	0.0469
		Block	1	0.42	0.5819
	Contrast Low vs. Post-Disturbance	Mean	1	33.81	0.0283
		Block	1	31.50	0.0303
	Contrast High vs. Post-Disturbance	Mean	1	12.27	0.0727
		Block	1	0.00	0.9786

Table 4-6 continued.

Index	Anova Test	Source	df	F Value	Pr > F[2]
Total Systems Throughput	Within Pond Effects	Stress	2	12.74	0.0184 x
		Stress x Block	2	4.06	0.1088
	Contrast Low vs. High	Mean	1	10.77	0.0816
		Block	1	3.71	0.1938
	Contrast Low vs. Post-Disturbance	Mean	1	14.75	0.0616
		Block	1	4.46	0.1690
	Contrast High vs. Post-Disturbance	Mean	1	22.26	0.0421
		Block	1	1.58	0.3357
Ascendency/Capacity	Within Pond Effects	Stress	2	32.40	0.0034 x
		Stress x Block	2	3.47	0.1337
	Contrast Low vs. High	Mean	1	54.74	0.0178 xx
		Block	1	0.22	0.6848
	Contrast Low vs. Post-Disturbance	Mean	1	233.38	0.0043 xx
		Block	1	35.28	0.0272
	Contrast High vs. Post-Disturbance	Mean	1	0.62	0.5126
		Block	1	2.22	0.2745
Connectance	Within Pond Effects	Stress	2	22.23	0.0068 x
		Stress x Block	2	2.19	0.2273
	Contrast Low vs. High	Mean	1	29.23	0.0326
		Block	1	0.61	0.5161
	Contrast Low vs. Post-Disturbance	Mean	1	94.61	0.0104 xx
		Block	1	4.52	0.1674
	Contrast High vs. Post-Disturbance	Mean	1	0.06	0.8288
		Block	1	2.96	0.2276

[1] A separate Anova was run for each index using a split plot design (i.e. blocking North-South Ponds and East-West Ponds).

[2] Significance: X = significant at the 0.05 level for Within Pond Effects; XX = significant at the 0.017 level for Contrasts (Bonferroni correction = 0.05 alpha/3 contrasts).

Table 4-7. Descriptive statistics for the 12 indices from ENA output used in this study. Mean, standard deviation, and coefficient of variation were calculated from trophic networks representing the four salt marsh ponds during three stress/disturbance conditions.

Index	n	Low Stress			High Stress			Post-Disturbance		
		Mean	StDev	CV	Mean	StDev	CV	Mean	StDev	CV
Mean Effective Trophic Level	4	2.88	0.22	8	2.72	0.20	8	2.17	0.10	5
Mean Ecotrophic Efficiency	4	0.534	0.024	5	0.372	0.038	10	0.457	0.089	19
Aggregated Trophic Chain Length	4	2.0	1.2	58	3.5	0.6	16	2.8	0.5	18
Trophic Efficiency	4	4.09	0.88	22	3.81	1.63	43	4.41	3.59	81
System Omnivory Index	4	0.146	0.007	5	0.158	0.018	11	0.208	0.029	14
Number of Paths	4	53449	35474	66	7040	4739	67	6408	5121	80
Average Path Length	4	2.2	0.1	3	3.1	0.7	24	2.5	0.2	8
PPR/Total PP	4	1.5	1.6	110	4.9	1.2	24	48.5	50.6	104
Herbivory:Detritivory	4	1.0826	0.0153	1	0.8524	0.0998	12	1.0495	0.0531	5
Total System Throughput ($ugCm^{-2}d^{-1}$)	4	2.17E+08	1.48E+08	68	4.01E+07	8.32E+06	21	1.70E+07	5.50E+06	32
Ascendency/Capacity	4	63	8	12	32	10	33	36	5	15
Connectance	4	0.207	0.007	4	0.167	0.013	7	0.169	0.009	5

Table 4-8. Results from Friedman's and Ryan's Q Multiple Comparison Tests for ENA indices[1].

Index	Source	df	F Value	Pr > F	Grouping	Mean Rank	n	Stress Level
			Friedman's Test[2]			Multiple Comparison Test		
Mean Effective	Model	2	19.50	0.0005	A	2.28	4	Low
Trophic Level	Error	9			A	2.25	4	High
	Total	11			B	1.00	4	Post
Mean Ecotrophic	Model	2	5.79	0.0242	A	2.75	4	Low
Efficiency	Error	9			AB	2.00	4	Post
	Total	11			B	1.25	4	High
Aggregated Trophic	Model	2	21.50	0.0004	A	2.875	4	High
Chain Length	Error	9			B	1.875	4	Post
	Total	11			C	1.25	4	Low
Trophic Efficiency	Model	2	0.30	0.7479	A	2.25	4	Low
	Error	9			A	2.00	4	Post
	Total	11			A	1.75	4	High
System Omnivory	Model	2	19.50	0.0005	A	3.00	4	Post
Index	Error	9			B	1.75	4	High
	Total	11			B	1.25	4	Low
Number of Paths	Model	2	13.50	0.0020	A	3.00	4	Low
	Error	9			B	1.50	4	High
	Total	11			B	1.50	4	Post
Average Path Length	Model	2	19.50	0.0005	A	2.75	4	High
	Error	9			A	2.25	4	Post
	Total	11			B	1.00	4	Low
Primary Production	Model	2	Inifinity	<0.0001	A	3.00	4	Post
Required/Net Primary	Error	9			B	2.00	4	High
Production	Total	11			C	1.00	4	Low
Herbivory:Detritivory	Model	2	19.50	0.0005	A	2.75	4	Low
	Error	9			A	2.25	4	Post
	Total	11			B	1.00	4	High
Total Systems	Model	2	Inifinity	<0.0001	A	3.00	4	Low
Throughput	Error	9			B	2.00	4	High
	Total	11			C	1.00	4	Post
Ascendency/Capacity	Model	2	13.50	0.0020	A	3.00	4	Low
	Error	9			B	1.50	4	High
	Total	11			B	1.50	4	Post
Connectance	Model	2	19.50	0.0005	A	3.00	4	Low
	Error	9			B	1.75	4	Post
	Total	11			B	1.25	4	High

[1] Index values were blocked by pond, ranked among the three stress/disturbance levels, and separate tests were run for each index.

[2] Model: Ranked Index = Stress Level.

Table 4-9. Matrix of correlation coefficients from the Spearman's Rank Correlation Tests of ENA indices[1,2].

	ETL	EE	TCL	TE	OI	Paths	APL	PPR	H/D	TST	A/C	CI
ETL	1	0.21	-0.30	0.06	0.04	0.63	-0.38	-0.82	-0.66	0.80	0.62	0.43
EE	---	1	-0.62	0.23	0.54	0.76	*-0.86*	-0.29	0.07	0.40	0.78	0.79
TCL	---	---	1	0.09	-0.71	-0.45	0.78	0.52	0.28	-0.46	-0.69	-0.57
TE	---	---	---	1	-0.22	0.24	0.04	0.01	0.20	0.00	0.10	0.06
OI	---	---	---	---	1	0.25	-0.62	-0.04	0.06	0.09	0.40	0.35
Paths	---	---	---	---	---	1	-0.78	-0.69	-0.39	0.77	*0.90*	*0.89*
APL	---	---	---	---	---	---	1	0.57	0.29	-0.61	*-0.90*	*-0.87*
PPR	---	---	---	---	---	---	---	1	*0.87*	*-0.94*	-0.71	-0.67
H/D	---	---	---	---	---	---	---	---	1	-0.77	-0.41	-0.42
TST	---	---	---	---	---	---	---	---	---	1	0.69	0.73
A/C	---	---	---	---	---	---	---	---	---	---	1	0.83
CI	---	---	---	---	---	---	---	---	---	---	---	1

[1] A total of 66 correlations were performed and only seven were found to be significant. A Bonferoni adjustment was made that lowered the 0.05 alpha level to 0.00076 (BF=0.05 alpha/66 tests). Coefficients for the seven significant correlations are italicized in bold.

[2] Index abbreviations: ETL = Mean Effective Trophic Level; EE = Mean Ecotrophic Efficiency; TCL = Aggregated Trophic Chain Length; TE = Mean Trophic Efficiency; OI = System Omnivory Index; Paths = Number of Paths; APL = Average Path Length; PPR = Primary Production Required/Net Primary Production; H:D = Ratio of Herbivory to Detritivory; TST = Total Systems Throughput; A/C = Ascendency/Capacity; CI = Connectance Index.

Table 4-10. Results from the Spearman's Rank Correlation Tests for ENA Indices showing the summary statistics for significant correlations[1,2].

		n	t	df	P-Value	r_s
Mean Ecotrophic Efficiency	Average Path Length	12	-5.33	10	0.00033	-0.86
System Omnivory Index	Primary Production Required/Net Primary Production	12	5.51	10	0.00026	0.87
Number of Paths	Ascendency/Capacity	12	6.35	10	8.4E-05	0.90
Number of Paths	Connectance	12	6.11	10	0.00011	0.89
Average Path Length	Ascendency/Capacity	12	-6.35	10	8.4E-05	-0.90
Average Path Length	Connectance	12	-5.69	10	0.0002	-0.87
Primary Production Required/Net Primary Production	Total Systems Throughput	12	-8.49	10	7E-06	-0.94

[1] A total of 66 correlations were performed and only these seven were found to be significant. A Bonferoni adjustment was made that lowered the 0.05 alpha level to 0.00076 (BF=0.05 alpha/66 tests).

[2] Abbreviations: n = number of values correlated; t = test statistic; df = degrees of freedom; P-Value is for 2 tailed test; r_s = Spearman's Correlation Coefficient.

Figure 4-1. Community level indices: a) Richness, calculated as the number of

taxa; b) Evenness calculated as the relative biomass of taxa; c) Diversity

calculated as the Shannon-Weaver Index. See text for details on algorithms

used for finding J and H.

(a) Richness.
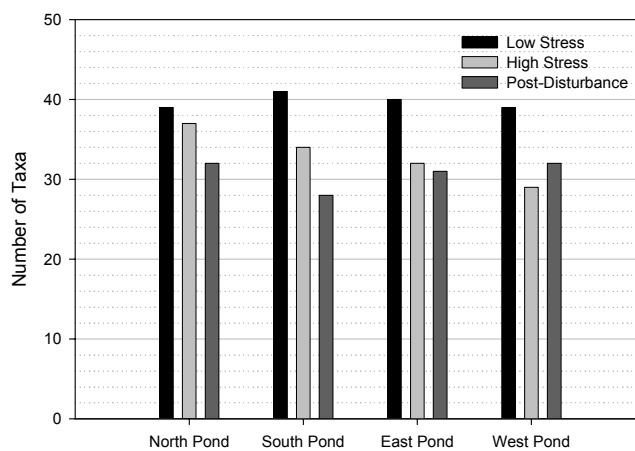
(b) Evenness.
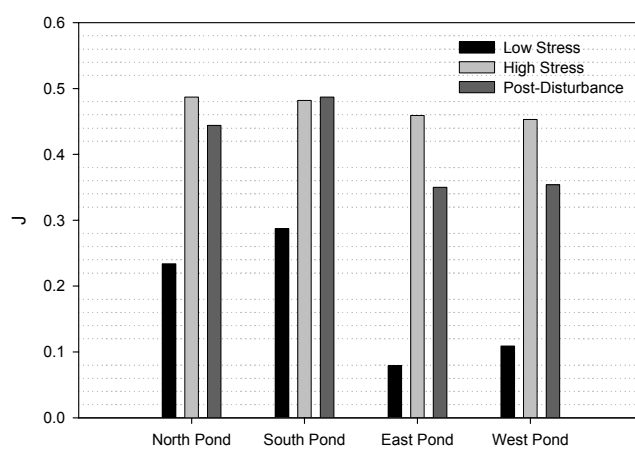
(c) Diversity.
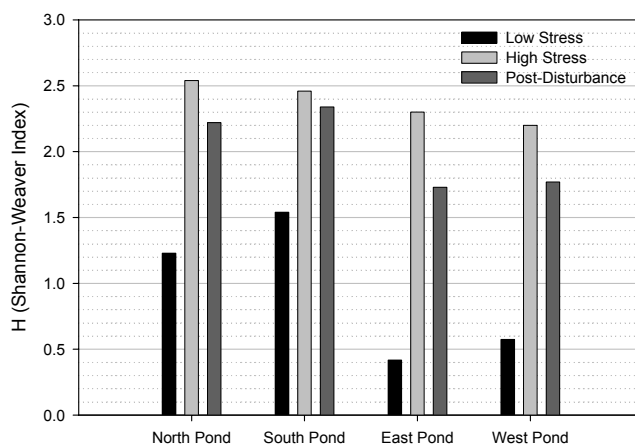
Figure 4-2. Differences in biomass during the three time periods: a) Primary

Producers in All Ponds; b) Consumers in All Ponds; c) Predatory Fish and

Insects in East and West Ponds.

(a) Producer Biomass in All Ponds.
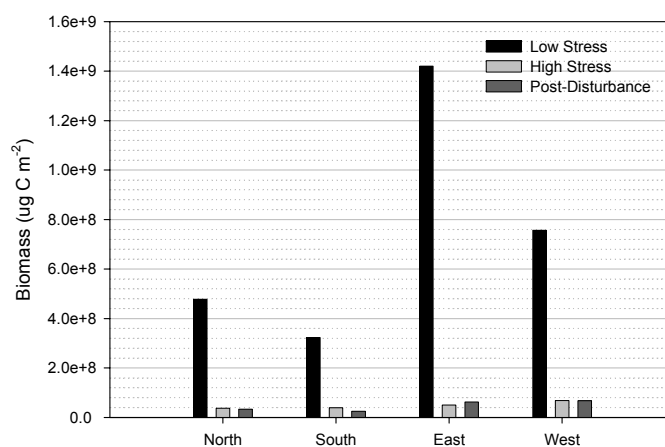
(b) Consumer Biomass in All Ponds.
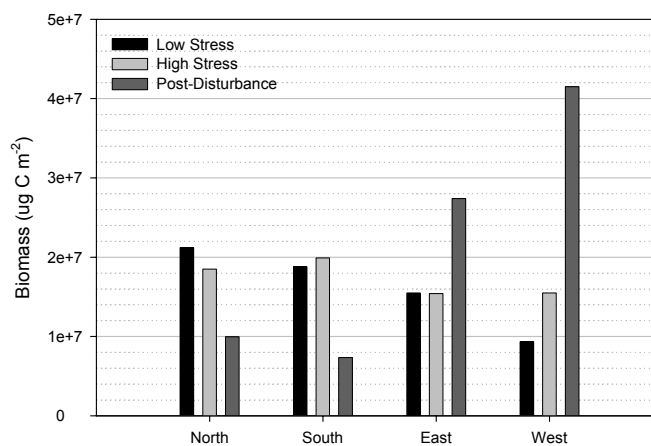
(c) Predatory Fish & Insect Biomass in East and West Ponds.
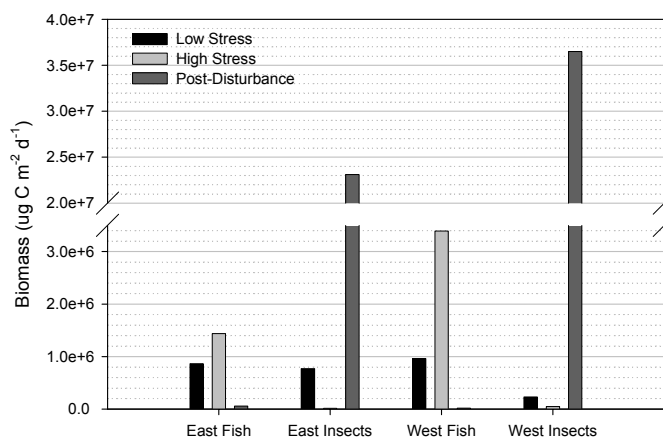
Figure 4-3. Indices from trophic structure analysis: a) Mean effective trophic

level of all consumer groups weighted by their biomass; b) Mean ecotrophic

efficiency of all active groups excluding top predators; c) Aggregated trophic

Chain Length calculated as the highest trophic level with ≥ 1% of total

throughput; d) Trophic efficiency of all flows calculated as the geometric mean of

Lindeman spine; e) Omnivory index for whole system.

(a) Mean Effective Trophic Level of Consumer Groups.
(weighted by biomass)
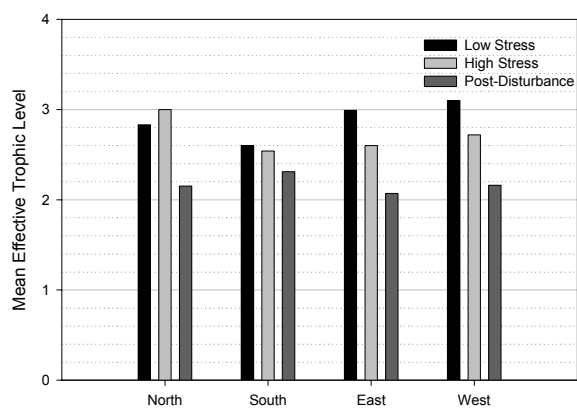
(b) Mean Ecotrophic Efficiency.
(all active groups except top predators)
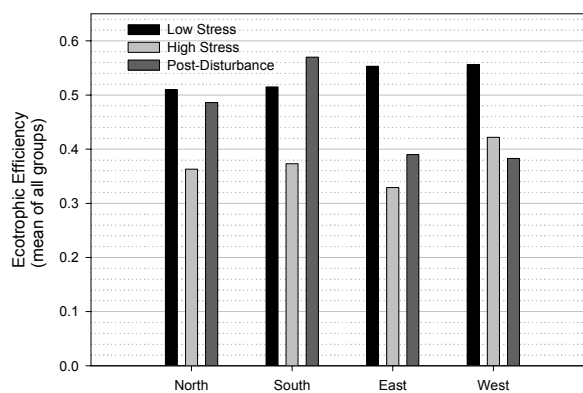
(c) Aggregated Trophic Chain Length.
(Highest TL with >/= 1% Total Throughput)
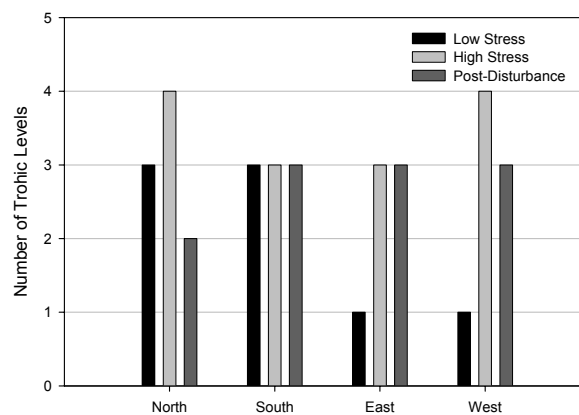
(d) Trophic Efficiency of All Flows Combined.
(calculated as geometric mean of Lindeman spine)
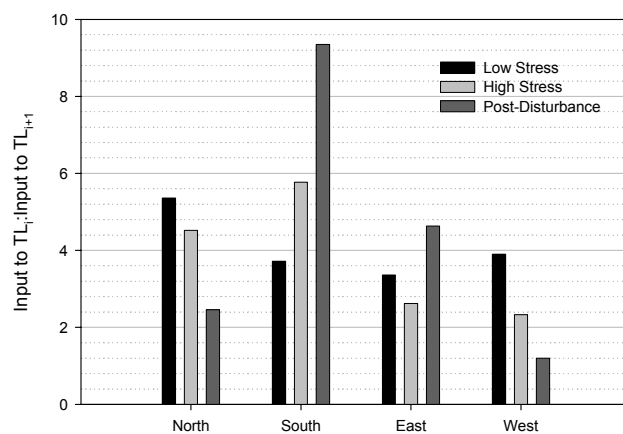

(e) System Omnivory Index.
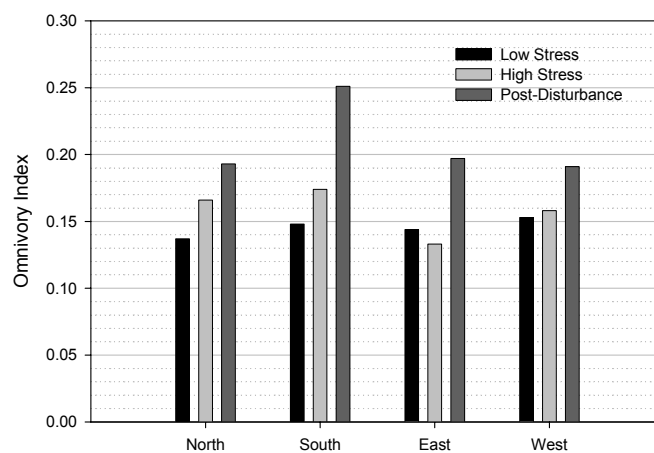
Figure 4-4. Indices from pathway analysis: a) Total number of paths from trophic level 1 to consumers; b) Average Path Length; c) Ratio of primary production required to net primary production; d) Ratio of herbivory to detritivory.

(a) Total Number of Paths from Trophic Level 1 to Consumers.
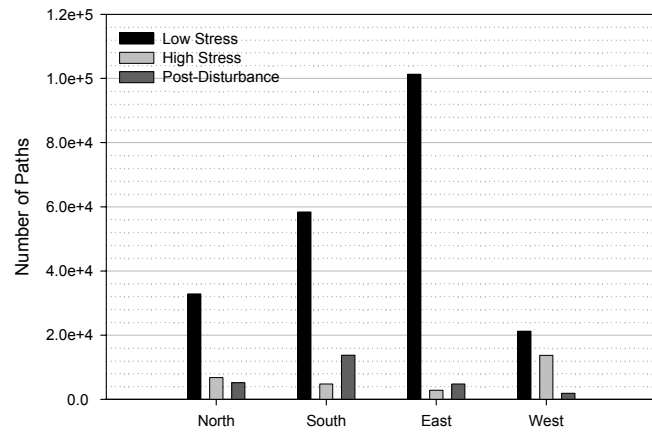
(b) Average Path Length.
(TST/Sum of Exports + Sum of Respirations)
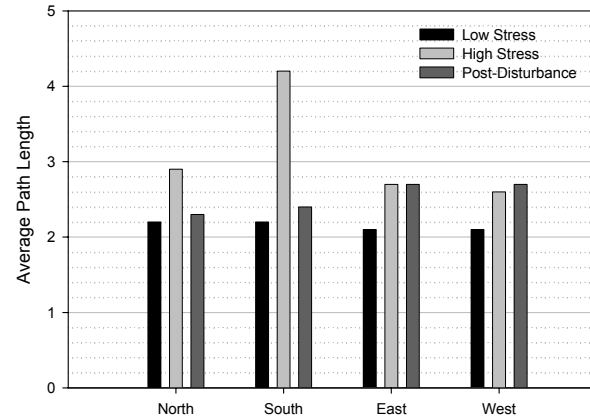
(c) Primary Production Required/Net Primary Production.
(Primary Production Required from producers only)
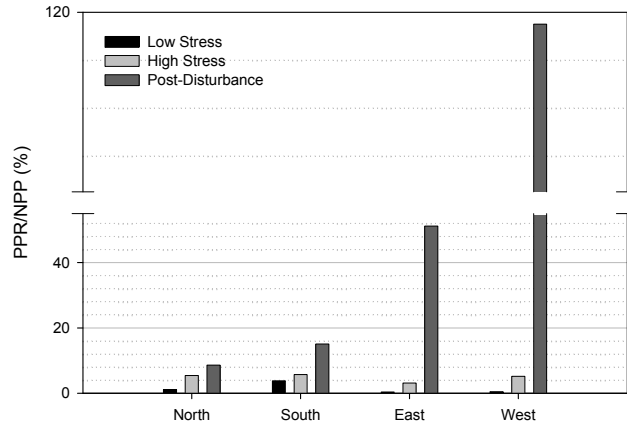
(d) Herbivory:Detritivory.
(TL 1 throughput from Producers/TL 1 throughput from detritus)
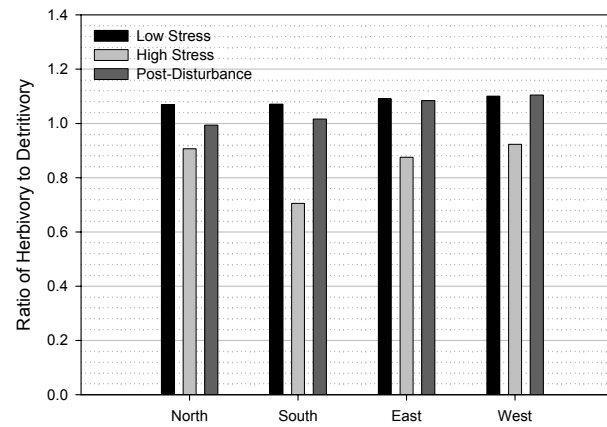
Figure 4-5. Indices from information analysis: a) Total Systems Throughput (µg

C m^{-2} d^{-1}); b) Ascendency/Capacity; c) Index of connectance.

(a) Total Systems Throughput.
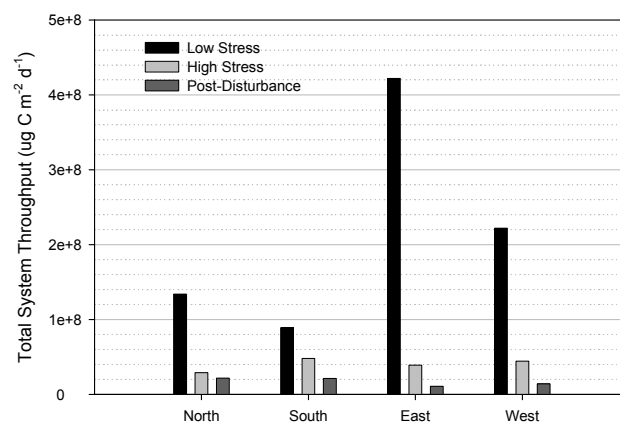
(b) Ascendency/Capacity.
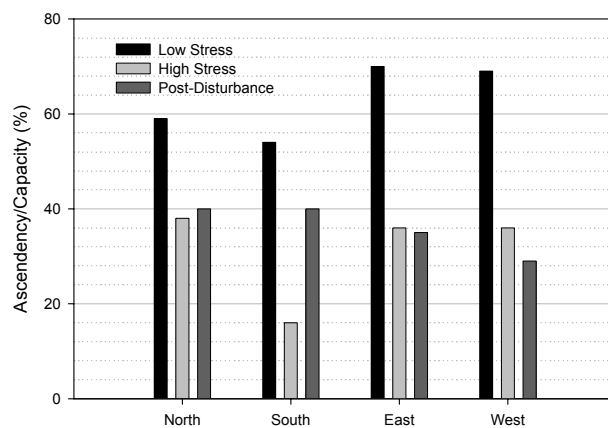
(c) Connectance.
(Number of Actual Links/Number of Possible Links)
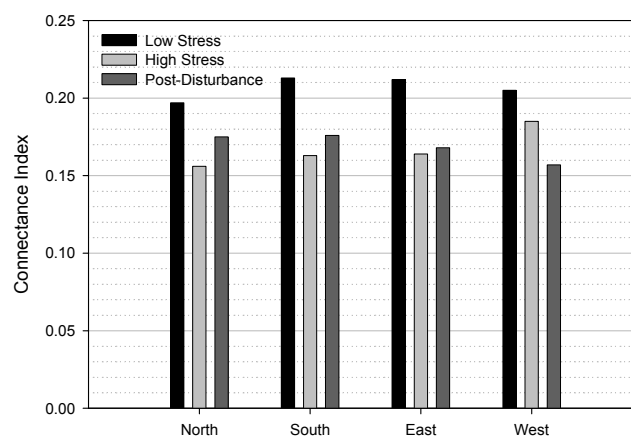
Appendix - Values of the 12 ENA indices examined in this study[1].

	North Pond			South Pond			East Pond			West Pond		
	Low	High	PD	Low	High	PD	Low	High	PD	Low	High	PD
ETL	2.83	3.00	2.15	2.60	2.54	2.31	2.99	2.60	2.07	3.10	2.72	2.16
EE	0.510	0.363	0.486	0.515	0.373	0.570	0.553	0.329	0.390	0.556	0.422	0.383
TCL	3	4	2	3	3	3	1	3	3	1	4	3
TE	5.36	4.52	2.46	3.72	5.77	9.35	3.36	2.62	4.63	3.90	2.33	1.20
OI	0.137	0.166	0.193	0.148	0.174	0.251	0.144	0.133	0.197	0.153	0.158	0.191
Paths	32877	6769	5185	58384	4803	13769	101300	2856	4776	21233	13732	1900
APL	2.2	2.9	2.3	2.2	4.2	2.4	2.1	2.7	2.7	2.1	2.6	2.7
PPR	1.1	5.4	8.6	3.8	5.7	15.1	0.4	3.1	51.2	0.5	5.2	119.0
H:D	1.0686	0.9064	0.9935	1.0712	0.7056	1.0162	1.0902	0.8749	1.0837	1.1004	0.9225	1.1046
TST	1.3E+8	2.9E+7	2.2E+7	8.9E+7	4.8E+7	2.1E+7	4.2E+8	3.9E+7	1.1E+7	2.2E+8	4.4E+7	1.4E+7
A/C	59	38	40	54	16	40	70	36	35	69	36	29
CI	0.197	0.156	0.175	0.213	0.163	0.176	0.212	0.164	0.168	0.205	0.185	0.157

[1] Index Abbreviations: ETL = Mean Effective Trophic Level; EE = Mean Ecotrophic Efficiency; TCL = Aggregated Trophic Chain Length; TE = Mean Trophic Efficiency; OI = System Omnivory Index; Paths = Number of Paths; APL = Average Path Length; PPR = Primary Production Required/Net Primary Production; H:D = Ratio of Herbivory to Detritivory; TST = Total System Throughput (ug C m^{-2} d^{-1}); A/C = Ascendency/Capacity; CI = Index of Connectance.

CHAPTER 5. VALIDATION OF TROPHIC NETWORKS ANALYZED WITH ECOLOGICAL NETWORK ANALYSIS

Abstract

Validation is an important step in the modeling process, but one that has mostly been overlooked with ecological network analysis (ENA). The objective of this study was to validate quantitative food web models analyzed with ENA. Mass balance models representing food webs in four salt marsh ponds during two times (spring 2002 and late summer 2002) were constructed and analyzed using common methods. Four separate aspects of model output were validated: respiration, aggregation of taxa, trophic levels, and extended diet. Values of community and plankton respiration derived from field measurements of dissolved oxygen were compared to Ecopath output. Dual isotope plots ($\delta^{13}C$ vs. $\delta^{15}N$) were used to compare how taxa grouped according to their isotopic signatures relative to their aggregation based on correspondence analysis. Trophic levels of individual taxa derived from $\delta^{15}N$ data were compared to the effective trophic level of their corresponding model compartment calculated by Ecopath. Carbon sources for four consumer compartments in one model were validated by comparing the results of dual isotope mixing models ($\delta^{13}C$ vs. $\delta^{34}S$ and $\delta^{34}S$ vs. $\delta^{15}N$) to the total dependency matrix from NETWRK output. Validation was based on paired t-tests for trophic levels, and on graphical comparison of results for respiration, aggregation, and carbon source.

Validation results were mixed. Model estimates of both community and plankton respiration (corrected to include primary producers) were typically greater than values calculated from dissolved oxygen measurements. Aggregation of taxa based on correspondence analysis compared well with stable isotope data in only one of four model compartments. Trophic levels were in agreement in three of the four models examined, and carbon source results were consistent in two of the four compartments evaluated. Plausible explanations could be made in each case where validation results were not in agreement. These included invalid assumptions associated with the models and differences in the methods used for validation relative to ENA. To date, this study represents the most extensive attempt to validate ENA models relative to the number of networks and scope of output assessed. It demonstrates that validation, as part of an iterative process, should be utilized with ENA to test assumptions and improve models.

Introduction

Quantitative models are essential to our understanding and management of coastal and marine ecosystems. Examples include models to assess fish stocks (e.g. Hilborn and Walters 1992; Funk et al. 1998), to evaluate nutrient loading and water quality (e.g. Thomann et al. 1994; Bowen and Hieronymus 2000), and to identify critical habitat (e.g. DeLong and Collie 2004; Steel et al. 2004). Commonly these and other issues can only be addressed through

modeling, and regardless of the type of model or its use, validation is arguably
the most important step in the modeling process (Overton 1977; Mankin et al.
1979; Haefner 1996). However, it can also be one of the most overlooked. This
has been particularly evident in the case of mass balance food web models and
the subsequent development of ecological network analysis (ENA).

ENA is a modeling technique for examining the structure and flow of
material in ecosystems (Wulf et al. 1989; Christensen and Pauly 1993). ENA
incorporates a suite of analyses that include input-output analysis, trophic
structure analysis, pathway analysis, biogeochemical cycle analysis, and
information analysis (Table 5-1). ENA is mostly used to evaluate food webs (e.g.
Belgrano et al. 2005), and two software packages have most commonly been
employed by ecologists: Ecopath (http://www.ecopath.org) and NETWRK
(http://www.cbl.umces.edu/~ulan/ntwk/network.html). The use of Ecopath in the
literature alone has increased over 20 fold in the last 15 years (see Chapter 2).

ENA has been applied, or recommended for application, to various
management issues. These include invasive species (Moreau et al. 1993;
Kitchell et al. 2000), fresh water delivery (Baird and Heymans 1996; Ulanowicz et
al. 2003), hypoxia (Baird et al. 2004), oil pollution (Okey and Pauly 1999), fish
kills (Christian et al. 2003), marine reserves (Watson et al. 2000), habitat
restoration (Ulanowicz and Tuttle 1992), and quantifying ecosystem health
(Mageau et al 1995) and integrity (Ulanowicz 2000). However ENA is most often
applied to evaluating the effects of fisheries harvest (e.g. Jarre-Teichmann 1998;

Pauly et al. 1998; Christensen and Maclean 2004), and fisheries managers are increasingly utilizing ENA for planning and decision making (e.g. SAFMC 1998; Okey and Pugliese 2001; ASMFC 2003; NCBO 2003).

ENA is one of several modeling techniques with potential for application to ecosystem-based fisheries management (Hallowed et al. 2000; Whipple et al. 2000; Latour et al. 2003). By quantifying direct and indirect interactions, ENA provides the ability to evaluate an entire food web rather than a single component. Moreover, ENA can be used for a quantitative comparison of food webs that incorporates fishery harvest information into the analysis. The public need for ecological modeling is accelerating (Ludwig et al. 2001; Clark et al. 2002; Rose and Cowan 2003), and an increased emphasis on ecosystem-based approaches, and its inherent complexities, will require an even greater reliance on quantitative models for making policy decisions. Application of trophic networks and ENA to ecosystem-based management introduces an added dimension related to the potential use of model output for justifying highly controversial decisions. This heightens the need for confidence in the models which can only be accomplished through validation.

In spite of numerous examples of ENA in the literature and its increasing application to management issues, few attempts have been made to validate ENA models. These only examine trophic levels calculated for model compartments (Kline and Pauly 1998; Mathisen and Sands 1999). In Chapter 2 several sources of uncertainty are discussed including natural variability of input

parameters, data collection methods, model construction techniques, and assumptions of algorithms used in the analyses that make up ENA. Others identify potential limitations of ENA associated with the quality and quantity of input data (Ruesink 1999; Morissette et al. 2003; Plaganyi and Butterworth 2004), as well as methods of aggregating taxa for model construction (Luczkovich et al. 2002). Few studies have systematically examined model behavior or evaluated output under different conditions (Abarca-Arenas and Ulanowicz 2002; Allesina and Bondavalli 2003), and only one utilizes replicated networks and hypothesis testing (see Chapter 4). Given the issues of uncertainty and our lack of understanding how it effects model output, additional efforts toward validating ENA models are necessary.

The objective of this study is to validate quantitative models of salt marsh pond food webs analyzed with ENA. To date, it represents the most extensive attempt to validate ENA models relative to the number of networks and scope of output assessed. The study is part of a larger effort to critically evaluate ENA. A comprehensive data set for construction of 12 trophic networks (4 salt marsh ponds at 3 times) is presented in Chapter 3, and the effectiveness of ENA in detecting differences in trophic conditions among the 12 models is examined in Chapter 4. A third component focusing on model validation is presented in this paper. Validation is attempted by comparing selected models and their output to independent data/techniques. Field measurements of dissolved oxygen and stable isotope data are used to corroborate results from specific portions of ENA

output from both Ecopath (calculations of respiration and effective trophic level from trophic structure analysis) and NETWRK (extended diet estimates from input-output analysis) software, as well as aggregation of taxa into model compartments performed using correspondence analysis. The level of agreement necessary to make a statement of validation is addressed separately for each analysis.

A word about terminology. There has been much discussion on the meaning of validation of ecological models (e.g. Rykiel 1996). This mostly comes from literature addressing dynamic or predictive modeling (i.e. simulation). Differentiation is made between validation, verification, and calibration (Oreskes et al. 1994), as well as corroboration (Caswell 1976). Some consider validation of dynamic models impossible (e.g. Starfield and Bleloch 1986). ENA differs from dynamic modeling in that it is not predictive of a timeline, but focuses on the analysis of a model representing a snapshot in time (see Chapter 2). Nevertheless validation (or whatever term is appropriate) is a necessary step in both types of modeling, and its meaning needs to be clarified. For the purposes of this study, I use the term validation in the sense of "confirming" or "corroborating" the output of ENA by comparing it to independent data and techniques.

Methods

Study Design

This study represents the model validation component of a larger effort to critically evaluate ENA. Overall, trophic networks representing four salt marsh ponds (North Pond, South Pond, East Pond, and West Pond) during three times (late summer 2001 and 2002, and spring 2002) were constructed using an extensive field sampling program augmented with literature values. Fieldwork was conducted in a mainland marsh at the headwaters of Upper Phillips Creek within the Virginia Coast Reserve Long Term Ecological Research site on Virginia's eastern shore (37.48^o North Latitude, 75.66^o West Longitude). Selected models representing all four ponds during two of the sampling events, spring 2002 and late summer 2002, were utilized for validation. Output from both Ecopath and NETWRK software were evaluated.

Model construction was at least equivalent in rigor to that used in other ENA studies. Standing stock biomass of all major taxa and diets of selected taxa (fish) were determined from field sampling. Taxa were separated into different size classes and then aggregated into compartments using correspondence analysis (Luczkovich et al. 2002). Body size estimates of consumers, also based on field sampling, were used to calculate production (Peters 1983) which was then used to calculate consumption (Christian and Luczkovich 1999). Information from the literature was used to fill gaps in the field data. Additional description of the ponds, sampling events, and model construction is given in

Chapter 4. Field data of biologic and physical variables characterizing the ponds,

model input, and model documentation can be found in Chapter 3.

In several aspects, model construction for this study exceeded that used

in other studies. For example unlike most ecosystems analyzed with ENA, the

scale of the salt marsh ponds (roughly 30 m x 3-5 m x 0.5 m) allowed for

relatively thorough documentation of the major taxa involved in energy flow.

Many trophic networks are based on data from numerous studies collected at

different times and places within the boundary conditions of the model (e.g. Baird

and Ulanowicz 1989). Others are based on data from an entirely different

system, time of year, and/or different environmental conditions from those

represented in the model (e.g. Haflon et al. 1996). Although information from the

literature was used in this study (e.g. production/bioomass ratio for producers

and binary diet data for some consumers), the majority of model input came from

data collected from the ponds during the times represented by the models.

Moreover, yield-effort curves suggest completeness of sampling for this study

(see Chapter 3), and these are typically not found in food web studies (Cohen et

al. 1993). The implications of the exhaustive approach to field sampling and

model construction is that these models are as appropriate as any to attempt

validation.

Four separate approaches were used for model validation:

1. Field measurements of respiration (diurnal studies and light-dark

   experiments) were compared to calculated values for various model

compartments. Respiration is part of Ecopath software output (Christensen and Pauly 1992).

2. Network construction involved aggregation of taxa into compartments, based on stomach contents analysis and information from the literature, using correspondence analysis (Luczkovich et al. 2002). These aggregations were compared to groupings based on stable isotope analysis (SIA).

3. Trophic levels obtained from SIA for various taxa were compared to effective trophic levels of model compartments (Christensen and Pauly 1992) given in the trophic structure analysis component of ENA (part of Ecopath output).

4. Information from SIA on carbon source for consumer compartments were compared to values provided by matrices of total dependency (Szyrmer and Ulanowicz 1987) in the input-output component of ENA (part of NETWRK software output).

Respiration

Calculated values of respiration were validated for trophic networks representing all four ponds during spring 2002. These networks were analyzed with Ecopath software which is typically relied upon to calculate respiration of each consumer compartment in a model. This reflects Ecopath's focus on application to fisheries analysis where respiration rarely is measured and other variables are more readily available (Christensen et al. 2002). Respiration of the pond community, and plankton community, calculated by Ecopath were

compared to values estimated from field measurements of dissolved oxygen concentration (DO). DO was measured using diurnal studies (for estimates of pond community respiration) and light-dark bottle experiments (for estimates of plankton community respiration).

Estimates of community respiration were made from two separate diurnal studies for each pond during spring 2002. One was immediately proceeding (25-26 May) and one immediately following (24-25 June) field sampling that documented presence/absence and abundance of taxa making up each pond's food web. DO measurements were made approximately every three hours over a 24 hour period using a Model 55 YSI DO Meter at two locations in each pond. A diurnal curve of DO was plotted for each location, and estimates of the negative slope corresponding to the rate of respiration were obtained. Respiration rates were corrected for diffusion of oxygen across the air-water surface using the following relationship (Caffrey 2003): O_2 Diffusion = [1 - (DO_{peak} + DO_{valley})/200] x dt x D, where DO_{peak} and DO_{valley} are the percent saturation of dissolved oxygen at the peak and valley of the diurnal curve, dt is the elapsed time between the peak and valley of curve, and D is a diffusion coefficient of 500 mg O_2 m^{-2} d^{-1}.

The average corrected rate of respiration in each pond was then converted from units of oxygen to units of carbon to facilitate comparison with Ecopath model output. The following relationship from Elliot and Davison (1975) was used: R (μg C m^{-2} d^{-1}) = negative slope of DO curve (mg l^{-1} hr^{-1}) x (12/32) x

RQ x 1000 μg/mg x 24 (hr d^{-1}) x Liters per Pond x 1/Pond Area (m^{-2}), where R is respiration, 12/32 a conversion constant when dealing with mass, and RQ a respiratory quotient. RQ is the ratio of the amount of CO_2 produced per O_2 consumed. An RQ of 0.85 was used which represents a mixed diet of carbohydrates, proteins, and lipids (Withers 1992).

Community respiration estimated from the diurnal curves were then compared graphically to the sum of respiration calculated for all model compartments except Frogs (compartment 27), Snakes (compartment 28), and Birds (compartment 29). The diurnal curve estimates were assumed to correspond to all model compartments with the exception of those representing taxa not living entirely within the ponds. For each pond, respiration estimates from diurnal curves were considered to be a range of potential values because they represented two temperature extremes. Values from 25-26 May were the lower limit (i.e. lower temperatures), and those from 24-25 June the upper limit (i.e. higher temperatures). Validation of community respiration was considered successful if the calculated value from Ecopath, corrected for primary producers (see below), fell within the range given by the diurnal curves.

Estimates of plankton respiration were made from 3 to 5 light-dark bottle experiments (Valiela 1995) for each pond during the spring 2002 sampling event. DO measurements were made using a modified Winkler titration method (Strickland and Parsons 1972), and plankton respiration was calculated as the difference between initial DO measurements and dark bottle DO measurements

(n = 2 per experiment). An average value for plankton respiration was then converted to equivalent units in the Ecopath models using the same method described above. These estimates were assumed to correspond to the sum of respiration calculated for the following compartments in each Ecopath model: Phytoplankton (compartment 4), H_2O column bacteria (compartment 6), H_2O column microprotozoans (compartment 7), and zooplankton (compartment 10). Results from the light-dark bottle experiments were used to calculate a mean and 95% confidence interval for each pond. Validation of plankton respiration was considered successful if the calculated values from Ecopath, corrected for phytoplankton (see below) were within the 95% confidence interval given by the light-dark bottle experiments.

Validation of respiration involved correcting values from Ecopath to include primary producers. Respiration calculated by Ecopath refers to consumers only, and is derived from the following relationship: $R = Q - (P + U)$, where R is respiration, Q is consumption, P is production, and U is the amount of unassimilated food. Model input for consumer compartments consists, in part, of P (as P/B where B is biomass), Q (as Q/B), and U (as U/Q). Ecopath uses adjustments to respiration for balancing the input and output of each consumer compartment (Christensen et al. 2002). Furthermore, Ecopath generally uses net primary production estimates and does not include respiration from primary producers. Therefore, respiration of appropriate primary producer compartments was added to Ecopath values of community and plankton respiration. Estimates

of primary producer respiration were made by considering net primary production roughly equivalent to respiration (e.g. Baird et al. 2004).

Stable Isotope Analysis

Stable isotope analysis (SIA) was performed on samples representing producer and consumer compartments of trophic networks. Material collected during field sampling for presence/absence and abundance was sub-sampled, dried at 60°C a minimum of 24 hours, ground with mortar/pestle or Wiley mill, and frozen until SIA could be performed. Whole bodies of consumers were analyzed with an average of ~5 bodies/sample depending on the quantity of individuals available. The gastrointestinal tract of fishes (except *Anguilla rostrata* - compartment 21) were separated from their body and used for stomach contents analysis with the remaining carcass used for SIA.

Samples from all ponds during spring and late summer 2002 were analyzed for δ^{13}C and δ^{15}N. Late summer 2002 North Pond samples were also analyzed for δ^{34}S. Typically, δ-values were based on averaging results from approximately 5 samples. The following relationship defines the δ-values (Peterson and Fry 1987): $\delta X = [(R_{sample}/R_{reference}) - 1] (10^3)$, where X is ^{13}C, ^{15}N, or ^{34}S and R is the corresponding ratio ^{13}C/^{12}C, ^{15}N/^{14}N, and ^{34}S/^{32}S. Each δ-value is a measure of the amount of heavy and light isotopes in a sample, and represents the difference from a reference in parts per thousand. Increases in δ-values indicate increases in the amount of heavy isotope, and conversely,

decreases represent greater amounts of the light isotope. Reference values
were obtained from carbon in the Pee Dee limestone, nitrogen gas in the
atmosphere, and sulfur in the Canyon Diablo meteorite. Analyses were
performed by University of California at Davis Stable Isotope Facility (δ^{13}C and
δ^{15}N) and Coastal Science Laboratories in Austin, Texas (δ^{34}S).

Aggregation Of Taxa

Model construction involved aggregation of taxa, and their different size
classes, into groups (i.e. compartments) using correspondence analysis as
outlined by Luczkovich et al. (2002). This approach allowed grouping based on
similarities of both prey and predators, and followed the trophospecies concept
(Yodzis and Winemiller 1999). Diets were determined from stomach contents
analysis (Carr and Adams 1972, 1973; Luczkovich and Stellwag 1993) and
information from the literature (see Chapter 3). UCINET 6 software (Borgatti et
al. 2002) was used for computing factor scores of both consumers and prey
items from a binary matrix of diet relationships for each model. The factor scores
were then exported into Mage 3D software (http://kinemage.biochem.duke.edu)
and taxa were grouped visually (i.e. somewhat subjectively) into compartments
based on their proximity in 3-dimensional space.

Aggregation of fish and macroinvertebrate taxa based on correspondence
analysis were compared to SIA data for all ponds during spring and late summer
2002. This was done graphically using separate δ^{13}C vs. δ^{15}N plots for each

model compartment for which SIA data were available. The plots presented the means and standard deviations of the taxa making up a compartment. Validation was based on the presence of overlap in the standard deviations of both $\delta^{13}C$ and $\delta^{15}N$ of taxa within a compartment.

Trophic Level

Ecopath calculates an effective trophic level (ETL) for each compartment in a model. ETL is a fractional value that takes into account degrees of omnivory. In Ecopath, producers and detritus are assigned an ETL of 1, and each consumer's ETL is calculated from the following relationship (Christensen and Pauly 1992): $ETL_i = 1 + \Sigma \, (\%_{ji}) \, (ETL_j)$, where ETL_i is the effective trophic level of a consumer compartment (i), $\%_j$ is the proportion of prey (j) in the diet of consumer (i), and ETL_j is the effective trophic level of prey (j).

ETLs calculated by Ecopath were compared to trophic levels calculated from SIA data. This was done for selected consumer compartments in models from each pond during late summer 2002. Trophic levels for taxa above herbivores were calculated from $\delta^{15}N$ data and a herbivore reference using the following relationship (Hobson and Welch 1992; Kline and Pauly 1998; Mathisen and Sands 1999): $TL_i = [(\delta N_{Mean\,i} - \delta N_{Herbivore})/\Delta\delta N] + TL_{Herbivore}$, where $TL_i =$ trophic level of group (i), $\delta N_{Mean\,i} =$ mean isotopic ratio for taxa making up group (i) and for which SIA data are available, $\delta N_{Herbivore} =$ isotopic ratio of a herbivore group in the system, $\Delta\delta N =$ enrichment factor, and $TL_{Herbivore} = 2$, which was

assigned as the trophic level of the herbivore group. An enrichment factor of +2.3 was used which accounts for fractionation of $\delta^{15}N$ among similar taxa with diets involving a mixture of plant, microbial, and animal material (McCutchan et al. 2003). Paired t-tests were used to determine how well the ETLs calculated by Ecopath matched trophic levels calculated from SIA data. The following null hypothesis was used for each t-test, H_o: $TL_{\delta N}$ - TL_{ENA} = 0, where $TL_{\delta N}$ = trophic level calculated from $\delta^{15}N$ data, and TL_{ENA} = ETL calculated by Ecopath.

<div align="center">Carbon Source (Extended Diet)</div>

ENA output from NETWRK software includes a total dependency matrix as part of the input-output analysis. This matrix quantifies the fraction of ingestion by a compartment that passed through another compartment at some point, and thus, quantifies the extended diet for consumers in the food web (Ulanowicz 1999). The total dependency matrix from model output of North Pond during late summer 2002 was used to determine the carbon sources for selected consumer compartments. These were compared graphically to carbon source information obtained from mixing models using SIA data ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$).

A NETWRK model representing the late summer 2002 food web in North Pond was created using inputs (biomass) and outputs (respirations and exchanges) from a corresponding Ecopath model. Standardized percentages of the main primary producer and detritus groups in the diet of selected consumers were calculated from values obtained in the total dependency matrix.

Producer/detritus groups included benthic microalgae (compartment 1), epiphytic

algae (compartment 2), phytoplankton (compartment 4), and particulate organic

carbon (POC; compartment 31). *Ruppia maritima* (compartment 5) was not

present, and herbaceous vegetation (compartment 4) was considered relatively

unimportant to the diets. Consumers used in the analysis were those with

relatively large biomass and with adequate SIA data ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) were

available. They included Culicidae larvae (compartment 14), adult Hydrophilidae

1-2 cm (compartment 16), Odonata larvae 1-2 cm (compartment 18), *Fundulus*

sp. 3-4 cm and *Gambusia sp.* 2-3 cm (both in compartment 25).

Three-source/dual-isotope mixing models (Phillips and Gregg 2001) were

used to calculate the percentage of each carbon source in the selected

consumer's diet. Phytoplankton and POC were considered a single source for

this analysis because they were obtained by filtering pond water and were not

separated. For each consumer taxa, separate 95% confidence intervals were

provided by a mixing model using $\delta^{13}C$-$\delta^{34}S$ data and by another model using

$\delta^{34}S$-$\delta^{15}N$ data. A resultant confidence interval was calculated as the overlap in

the 95% confidence interval from both mixing models. Resultant confidence

intervals for each consumer taxa were plotted on a ternary plot, and this outlined

a range of potential combinations for the three carbon sources (benthic

microalgae, epiphytic algae, phytoplankton/POC). Results from the total

dependency matrix, representing the carbon source solution for the NETWRK

model consumer compartments, were then plotted on the ternary plots

corresponding to the appropriate taxa. Validation was considered successful if results from the NETWRK model fell within the resultant confidence interval from the mixing models.

Results

Respiration

For all ponds, Ecopath estimates of community respiration (i.e. all compartments except Frogs - 27, Snakes - 28, and Birds - 29) corrected to include primary producers were over an order of magnitude greater than those obtained from diurnal studies of DO (Figure 5-1a). Ecopath estimates not corrected for primary producers (i.e. only consumers with the exception of compartments 27, 28, and 29) were less than the range calculated from DO field data in North, South, and West Ponds, and within the range calculated for East Pond. Results from plankton respiration were closer, but still generally not in agreement (Figure 5-1b). Ecopath estimates of plankton respiration corrected to include phytoplankton were roughly double the mean calculated values from light-dark bottle experiments in North and South Ponds, and over triple the mean value calculated from DO data in East Pond. The estimate for West Pond was the only one that fell within the range calculated from light-dark bottle experiments. Thus, there was poor agreement between values of respiration calculated from field data and Ecopath model estimates corrected for primary producers.

Aggregation Of Taxa

Using the approach outlined above, there was generally poor agreement between aggregation of taxa based on correspondence analysis and SIA data. For example based on overlap of standard deviations in δ^{13}C and δ^{15}N, taxa from compartment 24 (predatory fish that utilize small prey) in North Pond during spring 2002 could be separated into three compartments (Figure 5-2a). In this example, aggregation based on SIA would group different sizes of the same genus together (i.e. one compartment each for *Lucania parva*, *Gambusia sp.*, and *Fundulus sp.*). However, SIA data from the same compartment in South Pond were in agreement with results from correspondence analysis (Figure 5-2b). Although there was more within taxa variation in South Pond relative to North Pond, overlap of standard deviations along both the δ^{13}C and δ^{15}N axes suggested similarity in diets among all taxa from compartment 24 in South Pond. Using identical criteria, SIA data from taxa in compartment 18 (predatory insects) from East and West Ponds during late summer 2002 did not agree with the grouping derived from correspondence analysis. The SIA data suggested the taxa be grouped into two separate compartments in East Pond (Figure 5-2c), and four separate compartments in West Pond (Figure 5-2d). Thus based on the criteria used in this particular analysis, aggregation based on correspondence analysis was in agreement with the SIA data in only one of the four compartments examined.

Trophic Level

There was fairly good agreement between trophic levels calculated by Ecopath and those from $\delta^{15}N$ data for the ponds during late summer 2002. Plots comparing the two methods often showed some scatter around a line representing 100% conformity (dashed line in Figure 5-3). Fairly consistent results from the two methods was found in South and West Ponds (Figure 5-3b and 5-3d, respectively). Plots of North and East Ponds exhibited the same slope as the dashed "line of agreement", but most data points were offset such that trophic levels from $\delta^{15}N$ data were slightly greater than those from Ecopath (Figure 5-3a and 5-3c respectively). Thus, the plots suggest trophic levels from Ecopath were potentially validated by $\delta^{15}N$ data in at least two of the four ponds.

P-values from paired t-tests suggest H_o ($TL_{\delta N}$ - TL_{ENA} = 0) could not be rejected at the 0.05 alpha level for South, East, and West Ponds; however, North Pond's p-value was significant (Table 5-2). Acceptance of H_o is a "tricky" matter (Nickerson 2000) due to the likelihood of a Type II error (i.e. accepting H_o when it is false). Therefore, 95% confidence intervals were used to determine the relative size of disagreement between the two methods (Aberson 2002) by adding the mean difference to the 95% confidence interval for each pond (Table 5-2). This suggested most data points differed by a trophic level of 0.27 in South Pond, 0.39 in West Pond, 0.69 in East Pond, and 0.81 in North Pond. Given the highest trophic level in each pond was between 3.5 and 4, there was fairly good

agreement between the two methods in South and West Ponds. However, results of the two methods from North and East Ponds were less consistent.

Carbon Source (Extended Diet)

Five taxa representing four model compartments were used to validate the extended diet analysis for the North Pond late summer 2002 food web network. Results from stable isotope mixing models ($\delta^{13}C$ vs. $\delta^{34}S$ and $\delta^{34}S$ vs. $\delta^{15}N$) are shown in Table 5-3. Values represent the percent each carbon source (i.e. producer/detritus group) contributes to a particular consumer as identified by the similarity in their isotopic signatures. Percentages are presented as the 95% confidence intervals from each carbon source. Overlap of the 95% confidence intervals between the two mixing models provides a resultant confidence interval that takes into account signatures from all three isotopes. Based on the resultant confidence intervals, benthic microalgae was the major source of carbon for detritivorous and large herbivorous insects (compartments 14 and 16, respectively). In comparison, the mixing models suggest epiphytic algae was a relatively greater source of carbon for large predatory insects and predatory fish that utilized large prey items (compartments 18 and 25, respectively).

Ternary plots for each of the four model compartments allow for comparison of the resultant confidence intervals from stable isotope mixing models and carbon source estimates from ENA output (Figure 5-5). The polygon in each ternary plot delineates a range of solutions representing contributions

from each of the three carbon sources that satisfy the stable isotope mixing models. The solid circle in each ternary plot represents results from the total dependency matrix of the NETWRK model output, and these were typically normalized to 100 percent.

Results from stable isotope mixing models suggest different carbon source percentages for three of the four compartments compared to the ENA output. For compartment 16 (large herbivorous insects), the carbon source estimates were in agreement between the two methods (Figure 5-5b). Results for compartment 14 (detritivorous insects) were relatively close; however, the contribution of benthic microalgae was approximately 10% less in the ENA output compared to the stable isotope mixing models (Figure 5-5a). The contribution of epiphytic algae was considerably less in the ENA output for compartments 18 and 25 (large predatory insects, Figure 5-5c; predatory fish that utilize large prey, Figure 5-5d). Results from the stable isotope mixing models indicated a relatively lower contribution of benthic microalgae for compartment 18, and relatively less phytoplankton/POC for compartment 25. Thus based on the criteria used, carbon source estimates in only one of the four compartments were in agreement between the two methods.

Discussion

Two things must be considered when interpreting the validation results. First, reasonable approaches were used in model construction. The models

were based on extensive field sampling performed within the boundaries of the ponds during specific dates. As mentioned earlier, the scale of the ponds permitted relatively thorough documentation of the taxa involved in energy flow. Literature values were used only to fill gaps in the field data. Moreover, objective techniques were used for aggregating taxa and balancing compartment flows (see Chapter 3). These attributes of model construction are not always found in ENA studies (see Chapter 2).

A second consideration is the validation techniques assumed to be representative of reality have their own issues of uncertainty. While diurnal studies of DO and light-dark bottle experiments are accepted methods for calculating respiration (e.g. D'Avanzo et al. 1996 and Valiela 1995, respectively), variability can result from a number of issues including air-water diffusion and anaerobic metabolism (Kemp and Boynton 1980) and the presence of sulfides (inhibits the Winkler method). Likewise, SIA has potential pitfalls including isotopic fractionation and differential allocation of nutrients to tissues (e.g. Gannes et al. 1997) and stable isotope variability (e.g. Fourqurean et al. 2005). Efforts to control uncertainty in DO measurements included the use of a diffusion correction for the diurnal studies (Caffrey 2003) and an azide modification of the Winkler method for light-dark bottle experiments (APHA 1992). Uncertainty in SIA was addressed by using whole organisms for analysis (as opposed to muscle tissue) and utilizing relatively large sample sizes (n ≥ 5 individuals for most samples). While these efforts helped control uncertainty, they did not totally

eliminate it. Moreover, each area of validation contained its own set of rules (e.g. use of 95% confidence intervals vs. standard deviations vs. range of observed values) that constrained the validation results.

Given these considerations, results from the efforts to validate ENA models were not overly successful. Community respiration estimates from the four Ecopath models analyzed were over an order of magnitude greater than values calculated from DO field measurements. Estimates of plankton respiration were in agreement in only one of the four models. Similarly, aggregation of taxa using correspondence analysis was in agreement with SIA data in only one of four compartments analyzed. Trophic levels calculated from $\delta^{15}N$ data were consistent with those given in Ecopath models of two ponds. Results from two other ponds indicate trophic levels from the $\delta^{15}N$ data were slightly greater than those from the models. Estimates of carbon source from NETWRK output agreed with results from stable isotope mixing models in only one of the four compartments analyzed. Although these results are not encouraging, there are potential explanations for validation results not being in agreement.

Respiration

Among the different aspects of ENA models being validated, results from estimates of community respiration showed the most dramatic discrepancies. When estimates from Ecopath were corrected to include primary producers,

community respiration from models of all ponds were over an order of magnitude greater than values obtained from diurnal DO studies. Community respiration calculated from field data were between 2.1 and 4.3 g C m^{-2} d^{-1}, and were relatively similar to the 1.2 to 2.2 g C m^{-2} d^{-1} found in other salt marsh ponds (Johnston et al. 2003). This suggests differences in results of community respiration from the two methods were, at least for the most part, based in the models.

Results from plankton respiration also showed discrepancies but to a lessor extent. Ecopath plankton respiration estimates, corrected to include phytoplankton, were roughly 2 to 3 times the values calculated from light-dark bottle experiments in three of four models. Corrections to include primary producers, for both community and plankton respiration, involved calculating each producer compartment's respiration from its biomass and P/B since producer respiration is considered roughly equivalent to net primary production (Jorgensen et al. 1991). If this relationship between respiration and net primary production is accurate for the primary producers in the study ponds, then the large discrepancies in respiration estimates could potentially be due to the biomass and/or P/B of the producer compartments being overestimated each of the models.

Other possible explanations exist for the observed differences in respiration estimates from Ecopath and DO measurements. These involve microbes which are considered major contributors to respiration (e.g. Hopkinson

and Smith 2005; Sobczak 2005). First, I calculated respiration as aerobic and no provision was made for anaerobic metabolism. Sediment microbes (particularly bacteria) make up a relatively large portion of consumer biomass in the ponds (see Chapter 3 and 4). If a significant portion of sediment microbe metabolism were anaerobic, then Ecopath estimates of respiration for these compartments (i.e. sediment bacteria - compartment 8 and sediment microprotozoans - compartment 9) would be overestimated.

Secondly, biomass estimates of both H_2O column and sediment microbes involved cell density calculations from direct counts (see Chapter 3). These techniques do not distinguish between live vs. dead cells. If a significant percentage of the cells were non-living, then Ecopath estimates of respiration for these compartments (i.e. H_2O column bacteria - compartment 6, H_2O column microprotozoans - compartment 7, sediment bacteria, and sediment microprotozoans) would be too large. The issue of anaerobic respiration would likely affect Ecopath estimates of community respiration only, whereas the issue of distinguishing live vs. dead cells would affect estimates of both plankton and community respiration from Ecopath. However, in both cases Ecopath estimates would be expected to be greater than those calculated from DO measurements.

Ecopath calculations indicate microbes were the largest contributor to respiration among consumers residing in the ponds (Table 5-4). However, even though overestimates of the microbial compartments in the models may have contributed, they cannot fully explain the large differences between respiration

estimates from Ecopath and DO measurements. Corrections to include primary

producers in the Ecopath estimates resulted in extremely large differences in the

results from the two methods (Figure 5-1a and 5-1b). This suggests the biomass

and/or P/B of producer compartments were overestimated in the models if the

assumption of primary producer respiration approximating net primary production

is accurate.

Aggregation Of Taxa

Discrepancies between aggregation of taxa using correspondence

analysis and SIA data could potentially be due to fundamental differences in the

two methods. Aggregation using correspondence analysis incorporated two

components: use of common prey items and similarities in predators (Luczkovich

et al. 2002). SIA data involved diet only. This difference would likely affect

invertebrate compartments more than fish groups. The invertebrates were

mostly insects, and their predators (mostly fish) probably differed depending on

size (e.g. Norton and Cook 1999). Small insects provide prey for both large and

small predators, and large insects were prey for only the larger predators. This

aspect of predator-prey relationships was accounted for when using

correspondence analysis; however, it was not incorporated into the SIA data.

Aggregation of fish taxa were likely less affected by this difference between the

two methods because their major predators (snakes and birds) probably did not

differentiate to a large degree between prey size. Results from the four

compartments analyzed concur with this reasoning. Agreement between the methods was found in one of two fish compartments, but not in the two insect compartments. Therefore lack of validation was potentially due to SIA only accounting for similarities in diet, and this likely affected the insect compartments to a greater degree than the fish.

Validation results for aggregation of fish taxa were also potentially affected by a second difference between the two methods. For fish taxa, gut content analysis was used to identify similarity in the use of various prey items. Gut content analysis represents feeding behavior over a relatively short time because most fish typically digest their food particles on the order of hours or days (Bond 1996). In contrast, SIA data represents a relatively long history of the diet and not just the latest meal (Peterson and Fry 1987). Changes in the availability of prey items or feeding preferences may not be evident from gut content analysis, but would be incorporated into the isotopic signatures of the fish. Thus variability in diet over time, and whether it is incorporated into the data, potentially contributed to lack of validation of the fish compartments.

The influence of variability in diet may not be as evident in validation of the insect compartments because their diet was based on information from the literature (e.g. McCafferty 1981; Merritt and Cummins 1984). The literature provides a broader source of diet information relative to the more site and time specific gut content analysis. This may have resulted in the literature information being more compatible with the SIA data compared to gut content analysis.

Thus, variability in diet over time likely presented less of a problem for validation of the insect compartments.

Trophic Level

Any discrepancies in trophic level estimates from Ecopath and SIA could potentially be due to incorrect assumptions associated with the methods. Ten of twelve data points in the plots of North and East Ponds were above the "line of agreement" indicating trophic levels from the $\delta^{15}N$ data were higher than those from Ecopath (Figure 5-3a and 5-3c). In South and West Ponds, where no significant difference in trophic levels between the two methods were found, the values from $\delta^{15}N$ data were also slightly higher (Figure 5-3b and 5-3d). Although Ecopath by definition assigns a trophic level of 1 to detritus (Christensen and Pauly 1992), others suggest the trophic level of detritus should be that of the organism which released it (Burns 1989; Burns et al. 1991; Gaedke and Straile 1997). If the latter convention is used, then consumers of detritus as well as their predators (and so on) will take on an elevated trophic level relative to the convention of detritus having a trophic level of 1. Thus, the practice used by Ecopath could potentially result in lower trophic level estimates relative to those calculated from $\delta^{15}N$ data.

It is unclear, however, why a designation of trophic level 1 for detritus would affect results from North and East Ponds more than South and West Ponds. There was no significant difference in the ratio of herbivory to detrivory

among the four models analyzed for validation (see Chapter 4). Thus, the

relative importance of detritivory was no greater in the two models with larger

discrepancies between trophic level estimates than it was in the two models that

showed better agreement. Therefore it is possible, but unclear, if the convention

of using detritus as trophic level 1 was a factor in the validation analysis.

Another issue that could have affected the validation of trophic level

estimates deals with assumptions of diet associated with the models. Diet

matrices in the models were based on an assumption of opportunistic feeding

and used relative abundance (biomass) to produce quantitative diet distributions.

If a more selective feeding mode were utilized by consumers (i.e. based on

nutritional value or handling time), then trophic level estimates from Ecopath may

correspond less to those from the δ^{15}N data. The scatter of data points on plots

of North, East, and West Ponds could be associated with this issue (Figure 5-3a,

5-3c, 5-3d).

Other attempts have been made to validate trophic levels from Ecopath

with δ^{15}N data. Kline and Pauly (1998) worked with a model of Prince William

Sound, Alaska, with 2 producers and 19 total compartments (Dalsgaard and

Pauly 1997), while Mathisen and Sands (1999) used a model of Becharof Lake,

Alaska, with 1 producer and 10 total compartments. Kline and Pauly validated 7

model compartments. Their trophic level estimates from δ^{15}N data had a mean

difference of 0.09 (95% CI = 0.09) from those given in the Ecopath model.

Mathisen and Sands used a similar approach to validate 6 compartments, and

had a mean difference of 0.41 (95% CI = 0.29) between the two methods.

Results from three of the ponds compared favorable with these studies as mean

differences ranged from 0.12 (95% CI = 0.27) to 0.34 (95% CI = 0.35) in South,

East, and West Ponds (Table 5-2). Mean differences from North Pond were

larger at 0.53 (95% CI = 0.28). Therefore in three of four models analyzed, this

study had as much success as others who have attempted validation of trophic

levels using $\delta^{15}N$ data.

Carbon Source (Extended Diet)

An examination of the assumptions associated with total dependency

matrices in the models is necessary to understand validation results from the

extended diet analysis. The total dependency matrices were based on

opportunistic feeding. Binary diets (either a prey item is used or not) were first

determined from gut content analysis (fish compartments other than *A. rostrata*)

and the literature (all other compartments). If a prey item was utilized by a

predator, then the amount consumed was calculated by considering the prey's

biomass. The greater that prey item's biomass then the more important it was in

the predator's diet. This approach only considers prey selectively as a function

of prey biomass. That is, prey biomass was a surrogate for how often that prey

was encountered and consumed. No other elements of prey selectivity (e.g.

catchability, handling time/effort, food quality) were taken into account. Minor

adjustments were made to the diets (see Chapter 3) to balance compartments

(i.e. maintain ecotrophic efficiency < 1) and to make diets ecologically realistic (e.g. so that detritus is not 100% of predatory fish diet). In contrast, the SIA data implicitly incorporated factors of both prey selectivity and assimilation. A consumer's isotopic signature is based on what is actually assimilated into tissue and not just what is ingested (Peterson and Fry 1987). Therefore, discrepancies between model output and SIA data could be due to differences in how prey selectivity was incorporated into the two methods.

Results from three of the four compartments examined suggest consumers were more selective than what was accounted for in the opportunistic feeding approach. Benthic microalgae appear to have been of slightly greater importance to compartment 14 relative to what is defined in the model (Figure 5-5a), and epiphytic algae was considerably more important to compartments 18 and 25 (Figure 5-5c and 5-5d). This greater degree of selectivity could apply to these specific consumer compartments as well as their prey. Therefore as with validation efforts examining aggregation and trophic levels, underlying factors associated with the SIA data may not match assumptions imbedded in the ENA models.

Another characteristic of the SIA data may have contributed to differences in carbon source results between the two methods. The collection of pure samples of benthic microalgae or epiphytic algae for SIA is difficult (Hamilton et al. 2005), and stable isotope values for primary producers may have been contaminated by other taxa. The model for North Pond during late summer 2002

was used for validation of extended diet. The mean $\delta^{15}N$ value used for epiphytic

algae (compartment 2) was 0.91 (st. dev. = 1.44) while values for the other

producers ranged from -1.81 to -3.60. Culicidae (compartment 14) had the

lowest $\delta^{15}N$ value among consumers in North Pond during late summer 2002

(mean = 0.70; st. dev. = 0.17). Because of fractionation, elevated $\delta^{15}N$ values

associated with the epiphytic algae relative to other groups suggests

contamination. This could have resulted from the incorporation of microbes,

meiofauna, or POC into the samples. However, differences in $\delta^{15}N$ values do not

necessarily mean contamination was a problem; only that it was a possibility.

Benthic microalgae (compartment 1) could have been the major carbon source

for Culicidae as is suggested in Figure 5-5a.

Another example of how contamination of SIA samples could have

affected the results involves phytoplankton/POC. Separation of phytoplankton

from POC and other constituents of the H_2O column (e.g. bacteria,

microprotozoans, zooplankton) was not attempted for validation of extended diet.

Based on biomass estimates, phytoplankton and POC would have made up

approximately 95% (38% phytoplankton and 57% POC) of the material collected

on a filter. Therefore, contamination of phytoplankton/POC probably did not

affect results of the extended diet analysis.

Conclusions

This study represents the most extensive attempt to validate ENA models to date relative to the number of models and scope of output evaluated. On the surface, my results may not present a very convincing argument for ENA. However, examination of the results along with details of the methods reveal plausible explanations for why there was not more agreement. My analysis suggests two general explanations. One involves the inherent differences in the methods used for validation relative to ENA. Unfortunately, few techniques are a perfect fit for validating ENA models because virtually none provide the same information. For example, SIA is one of the only methods available for validating trophic levels. However trophic level estimates based on $\delta^{15}N$ data have their own set of assumptions (e.g. Gannes et al. 1997; Post 2002), and these estimates represent specific taxa as opposed to aggregations of taxa. Another example is that SIA incorporates diet information only, whereas the use of correspondence analysis in aggregating taxa combines both similarities in prey and predators (Luczkovich et al. 2002).

A second explanation, not mutually exclusive of the first, for why some of the validation efforts may have failed involves assumptions associated with the models. Ecopath models often disregard primary producer respiration and assume all metabolism is aerobic. This likely affected the validation of respiration to some degree; however, overestimates of producer biomass and P/B probably played a greater role. ENA's assumption of steady state conditions

does not allow it to consider variation in diet over time. Moreover, ENA does not account for selective assimilation and assumes the same nutritional value for all prey items. These factors, coupled with assumptions of opportunistic feeding in my models, likely contributed to the problems of validating trophic levels and carbon source estimates. However, efforts to validate trophic level estimates may have been potentially more limited by Ecopath's assumption of trophic level 1 for detritus. Limitations in the validation methods and assumptions in the models need to be acknowledged and considered when interpreting the validation results.

Validation should be an integral part of the modeling process, and failed attempts at validation are not necessarily a negative development. A major contribution of ecological models is their heuristic value (Odum and Barrett 2005) because the process of modeling often highlights shortcomings in our understanding of the system being modeled. This is especially true for ENA which analyzes trophic networks that synthesize diverse data such as demographics, feeding behavior, physiology, energetics, and environmental factors. ENA should be thought of as more than just a "black box" tool for quantifying trophic relationships. It provides an approach for gaining a better understanding of an ecosystem. Through an iterative process, validation should be utilized to test assumptions and improve the models. This is particularly important as ENA is increasingly being used in resource management. For example as ENA becomes a common tool to address issues such as ecosystem-

based fisheries management, confidence in the models and the decisions that result from them will depend on adequate validation.

Table 5-1. Description of output from ecological network analysis.

Input-Output Analysis[A] - quantifies direct and indirect relationships between compartments. ● Mixed Trophic Impact Matrix - sums the positive and negative impacts of each compartment on every other compartment.	Input-Output Analysis[B] - quantifies direct and indirect relationships between compartments. ● Total Contribution Matrix - gives the percent of flow through a compartment that passes into another. ● Total Dependency Matrix - gives the percent of flow through a compartment that had once passed through another (e.g. extended diet).
Trophic Structure Analysis [C] - provides information based on the trophic concepts of Lindeman (1942). ● Effective trophic level – fractional value of a compartment's trophic level that takes into account degrees of omnivory. ● Trophic efficiency - the proportion of consumption passed up the food chain. ● Omnivory Index - variance of trophic levels in a consumer's diet.	
Pathway Analysis[A] - characterizes the pathway of flows. ● Pathway from any primary producer to a selected consumer through a specified prey. ● Primary production required to sustain the consumption of each group. ● Herbivory:Detritivory Ratio - quantifies the ratio of flow along grazing and detrital food webs.	Biogeochemical Cycle Analysis[B] - evaluates the characteristics of cycles within the system. ● Number of cycles organized by the smallest common flow. ● Length of cycles and distribution of flow along them. ● Finn Cycling Index - amount of flow involved in cycling.
Information Analysis[C] - quantifies attributes characteristic of the growth and development of the system. ● Total System Throughput - sum of all flows occurring in a system. ● Development Capacity - index of the potential of a network to develop given its particular set of connections and throughput. ● Ascendency - index of the size and developmental potential that a system has attained.	

[A] Ecopath software output.

[B] NETWRK software output.

[C] Output of both Ecopath and NETWRK.

Table 5-2. Results of paired t-tests[1] comparing trophic levels calculated from $\delta^{15}N$ data[2] to effective trophic levels calculated by Ecapath[2].

Model	d.f.	t-Statistic	t-Critical	P-Value	Mean Difference	95% Confidence Interval
North Pond	5	3.7385	2.5706	0.0135	0.5309	0.2783
South Pond	6	1.4906	2.4469	0.1866	0.1165	0.1532
East Pond	5	1.8967	2.5706	0.1163	0.3382	0.3495
West Pond	5	0.6861	2.5706	0.5232	0.1006	0.2873

[1] The following null hypothesis was used for each t-test - H_o: $TL_{\delta N}$ - TL_{ENA} = 0; where $TL_{\delta N}$ is the trophic level calculated from $\delta^{15}N$ data, and TL_{ENA} is the trophic level calculated by Ecopath. $TL_{\delta N}$ (Hobson and Welsh 1992; Kline and Pauly 1998; Mathisen and Sands 1999) = $[(\delta N_{Mean} - \delta N_{Herbivore})/\Delta \delta N] + TL_{Herbivore}$; where δN_{Mean} is the average $\delta^{15}N$ of taxa in compartment, $\delta N_{Herbivore}$ is a herbivore reference which contains the lowest $\delta^{15}N$ value among consumers, and $\Delta \delta N$ is a correction for fractionation equal to +2.3 (McCutchan et al. 2003).

[2] Stable isotope data and models are from late summer 2002.

Table 5-3. Results from stable isotope mixing models (Phillips and Gregg 2001): $\delta^{13}C$ vs. $\delta^{34}S$ and $\delta^{34}S$ vs. $\delta^{15}N$. Values represent the percent (given as the 95% confidence interval) a carbon source contributes to each consumer taxa. Resultant confidence intervals represent the interval in common (or overlap) from both models. Data are from North Pond during late summer 2002.

Consumer Taxa[1]	$\delta^{13}C$ vs. $\delta^{34}S$ Confidence Intervals			$\delta^{34}S$ vs. $\delta^{15}N$ Confidence Intervals			Resultant Confidence Intervals		
	BM[2]	EA[2]	Phyto/POC[2]	BM[2]	EA[2]	Phyto/POC[2]	BM[2]	EA[2]	Phyto/POC[2]
Culic (14)	84-100	0	9-29	73-100	0-24	0-13	84-100	0-24	9-13
Hyd A1-2 (16)	71-100	0-33	0-29	55-100	0-82	0-21	71-100	0-33	0-21
Odo 1-2 (18)	3-49	18-70	15-46	0-42	34-100	0-39	3-42	34-70	15-39
Fun 3-4 (25)	5-55	35-82	0-26	10-53	27-83	0-31	10-53	35-82	0-26
Gam 2-3 (25)	16-65	28-62	3-26	28-70	8-43	12-40	28-65	28-43	12-26

[1] Consumer taxa and their corresponding model compartment numbers (in parenthesis) are: Culic = Culicidae larvae; Hyd A1-2 = adult Hydrophilidae 1-2 cm; Odo 1-2 = Odonata larvae 1-2 cm; Fun 3-4 = *Fundulus sp.* 3-4 cm; Gam 2-3 = *Gambusia sp.* 2-3 cm.

[2] Carbon sources considered are: BM = benthic microalgae (compartment 1), EA = epiphytic algae (compartment 2), Phyto/POC = phytoplankton and particulate organic carbon (compartments 4 & 31 respectively).

Table 5-4. Importance of microbial compartments to Ecopath estimates of respiration. Units of respiration estimates are g C m^{-2} d^{-1}.

|	Compartment 6 H$_2$O Column Bacteria	Compartment 7 H$_2$O Column Microprotozoans	Compartment 8 Sediment Bacteria	Compartment 9 Sediment Microprotozoans
North Pond: Total Community Respiration[1,3] = 68; Total Plankton Respiration[2,3] = 1.26				
Compartment Respiration	0.48	0.04	1.58	0.13
% of Consumer Respiration of Community	20.5	1.7	68.0	5.7
% of Consumer Respiration of Plankton	92.3	7.6	---	---
South Pond: Total Community Respiration[1,3] = 45; Total Plankton Respiration[2,3] = 1.14				
Compartment Respiration	0.39	0.02	1.28	0.45
% of Consumer Respiration of Community	16.8	0.9	55.6	19.6
% of Consumer Respiration of Plankton	94.5	5.2	---	---
East Pond: Total Community Respiration[1,3] = 219; Total Plankton Respiration[2,3] = 2.09				
Compartment Respiration	1.47	0.09	0.84	0.03
% of Consumer Respiration of Community	57.5	3.5	32.9	1.0
% of Consumer Respiration of Plankton	94.3	5.7	---	---
West Pond: Total Community Respiration[1,3] = 115; Total Plankton Respiration[2,3] = 1.04				
Compartment Respiration	0.46	0.12	0.35	0.05
% of Consumer Respiration of Community	43.4	11.2	33.1	4.7
% of Consumer Respiration of Plankton	79.4	20.5	---	---

[1] Total community respiration includes all compartments except those that do not live entirely within the ponds: Frogs (27); Snakes (28); and Birds (29).

[2] Total plankton respiration includes: Phytoplankton (4), H$_2$O Column Bacteria (6), H$_2$O Column Microprotozoans (7), and Zooplankton (10).

[3] Respiration estimates are corrected to include primary producers (see text).

Figure 5-1. Comparison of estimates for community respiration (a) and plankton
respiration (b) from all ponds in spring 2002. Model estimates of community
respiration included all consumer compartments except those not living entirely in
the ponds (i.e. Frogs, compartment 27; Snakes, compartment 28; and Birds,
compartment 29). Model estimates of plankton respiration included H_2O column
bacteria (compartment 6), H_2O column microprotozoans (compartment 7), and
zooplankton (compartment 10). Corrections were made to model estimates to
include primary producers, and assumed net primary production (calculated from
biomass and P/B) was roughly equivalent to respiration (Baird et al. 2004).

(a) Estimates of Community Respiration.

Respiration
(g C m^{-2} d^{-1})

●   Diurnal Curve Results from May 25-26 2002 Corrected for Diffusion
○   Diurnal Curve Results from June 24-25 2002 Corrected for Diffusion
▲   Value from Ecopath Model without Corrections for Primary Producers
     Numbers in Parentheses are Values Corrected for Primary Producers

(b) Estimates of Plankton Respiration.

- ● Mean and 95% CI from Light/Dark Bottle Experiments
  (North n = 4; South n = 4; East n = 3; West n = 3)
- ▲ Value from Ecopath Models Corrected to Include Phytoplankton

Figure 5-2. Dual isotope plots comparing how taxa grouped according to their isotopic signatures relative to how they were aggregated based on correspondence analysis. (a) and (b) represent compartment 24, predatory fish that utilize small prey, from North and South Ponds respectively during spring 2002. (c) and (d) represent compartment 18, large predatory insects, from East and West Ponds respectively during late summer 2002. The mean and standard deviation of δ^{13}C and δ^{15}N are shown for individual taxa in each compartment. Ellipsoids delineate how taxa would group based on stable isotope data alone.

(a) Compartment 24 North Pond Spring 2002.
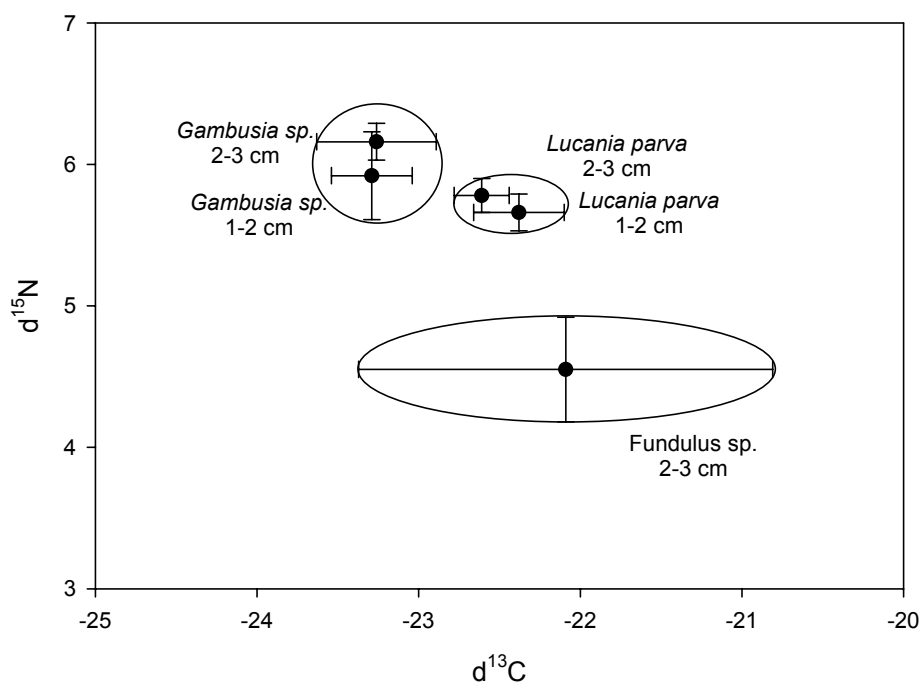
(b) Compartment 24 South Pond Spring 2002.
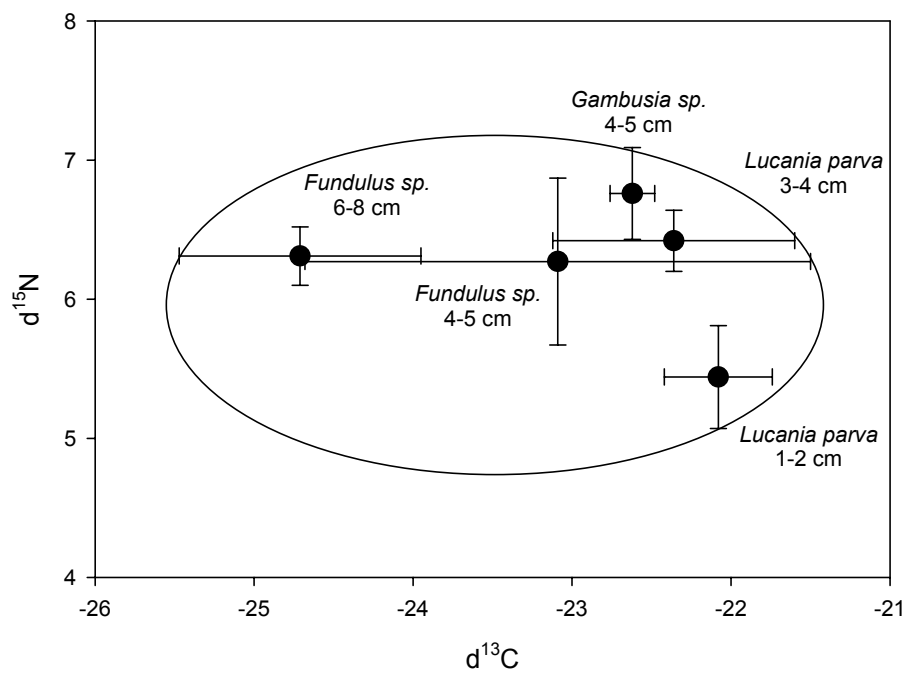
(c) Compartment 18 East Pond Late Summer 2002.
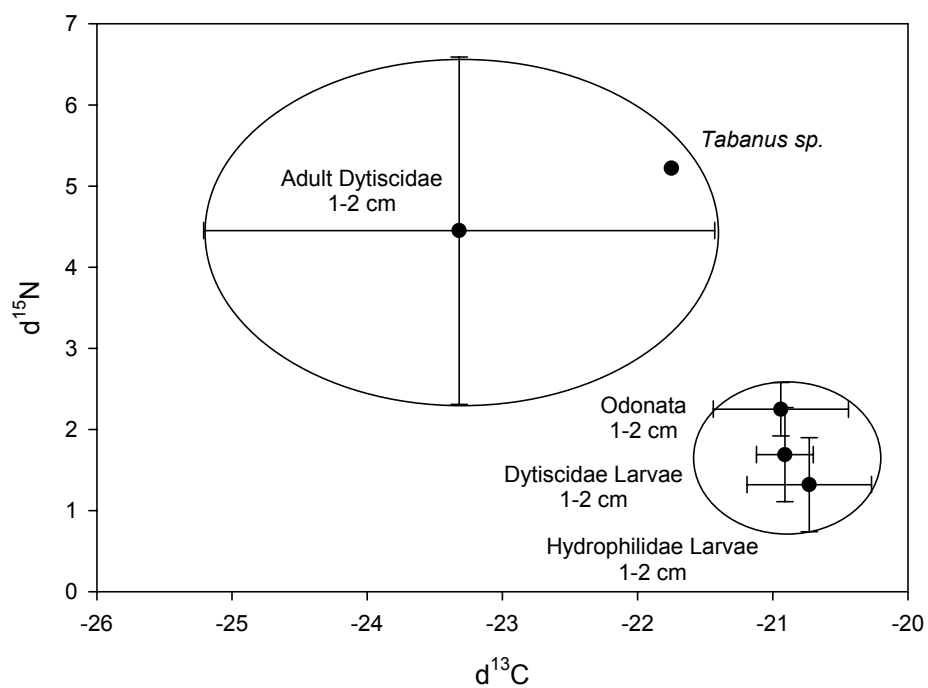

(d) Compartment 18 West Pond Late Summer 2002.
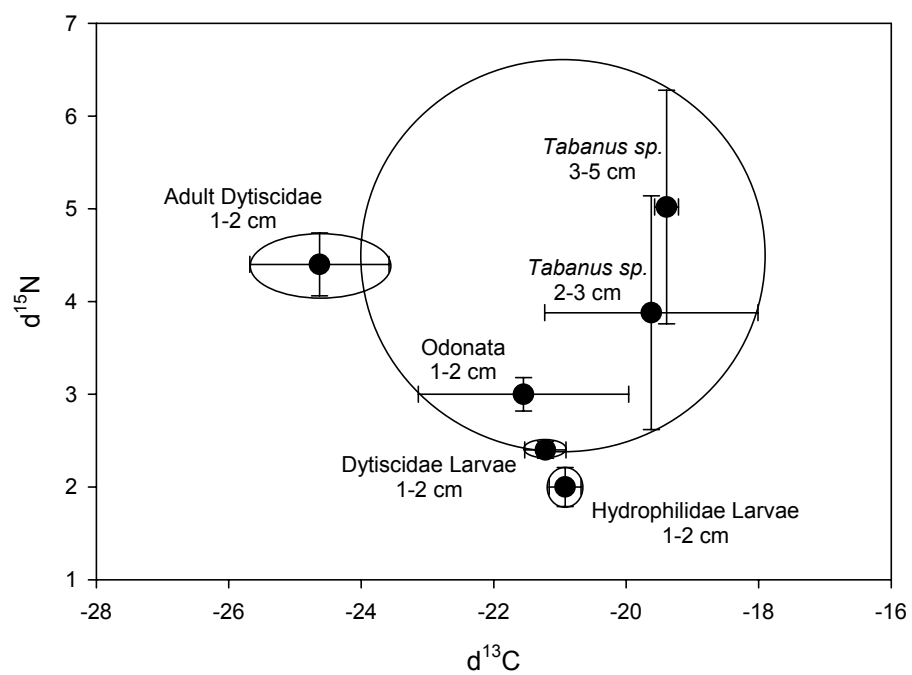
Figure 5-3. Trophic levels of individual taxa derived from $\delta^{15}N$ data compared to the effective trophic level of their corresponding model compartment calculated by Ecopath. The dashed line represents a hypothetical perfect fit of results from the two methods. Data are presented from late summer 2002 and include (a) North Pond, (b) South Pond, (c) East Pond, and (d) West Pond. Validation was based on paired t-tests.

(a) North Pond.
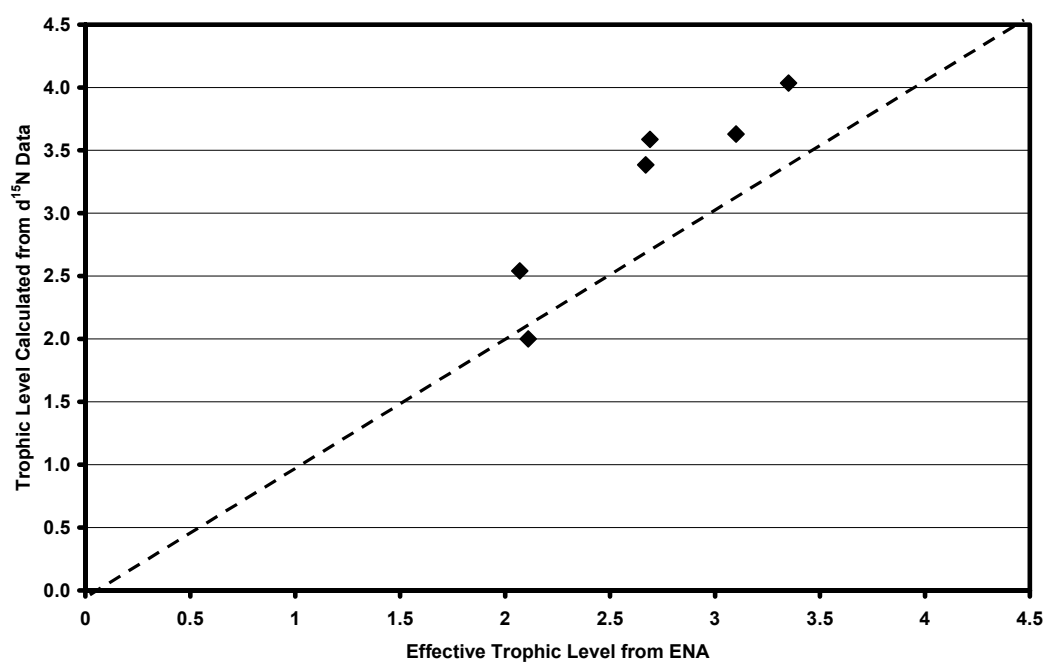
(b) South Pond.
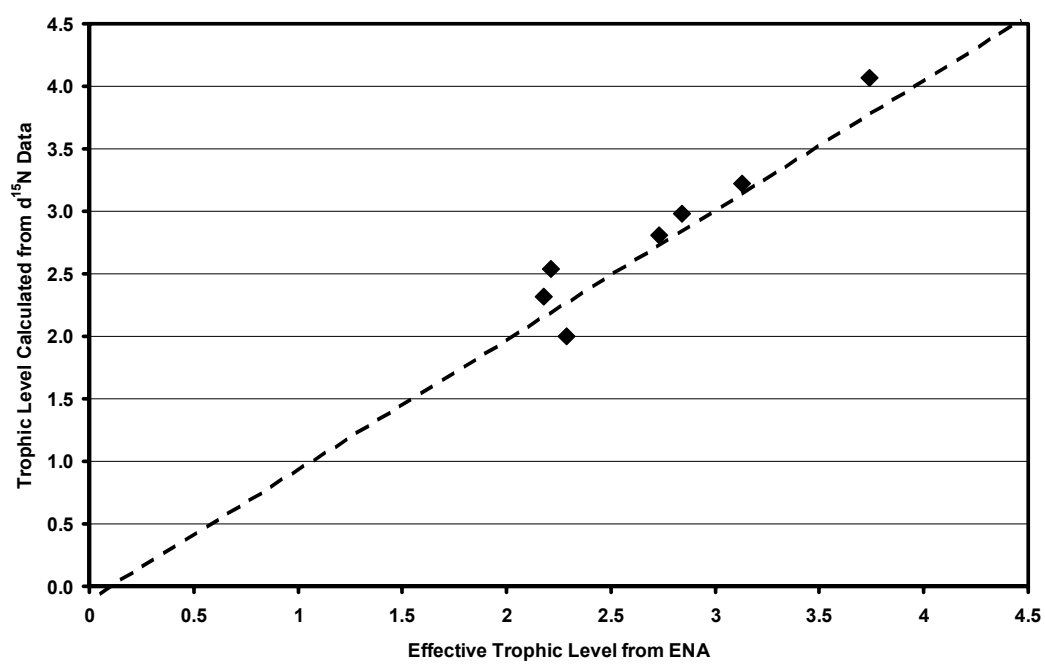
**(c) East Pond.**
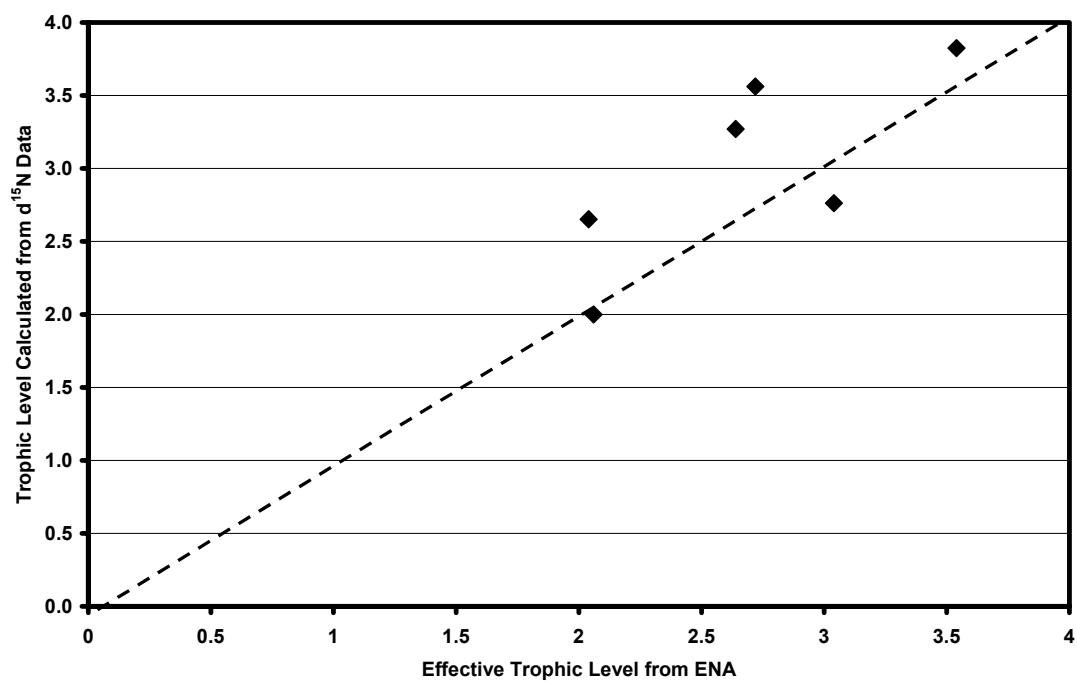
(d) West Pond.
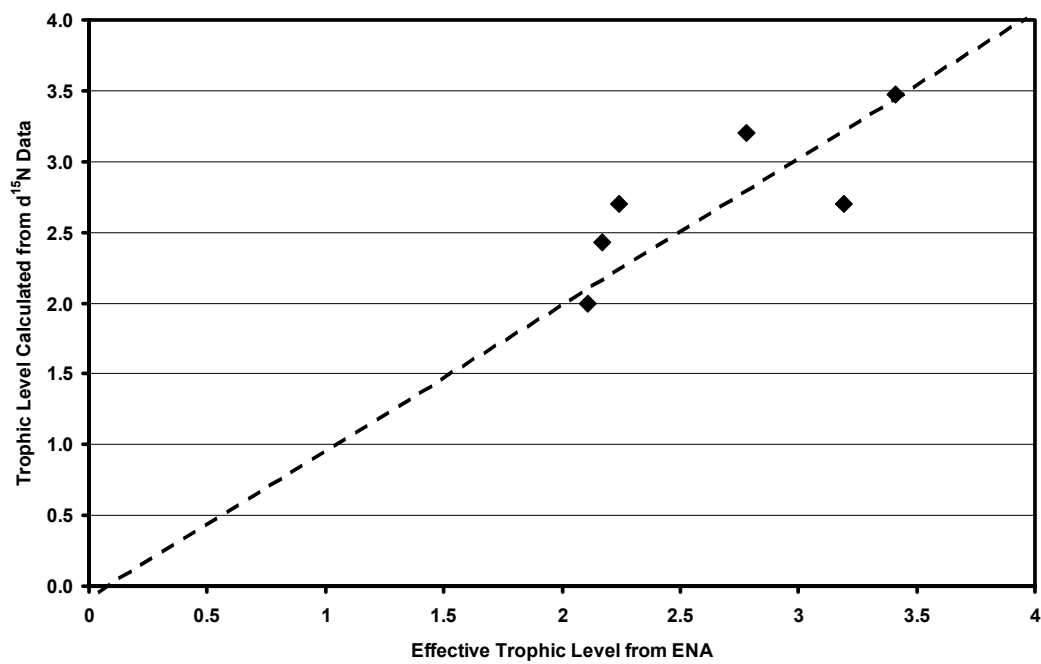
Figure 5-4. Ternary plots comparing carbon source estimates for four consumer compartments from the North Pond late summer 2002 model: (a) detritivorous insects (compartment 14), (b) large herbivorous insects (compartment 16), (c) large predatory insects (compartment 18), (d) predatory fish that feed on large prey (compartment 25). Circles represent results from the total dependency matrix of NETWRK output. Polygons were derived from 95% confidence intervals obtained from dual isotope mixing models (Phillips and Gregg 2001): $\delta^{13}C$ vs. $\delta^{34}S$ and $\delta^{34}S$ vs. $\delta^{15}N$ (see Table 3). Stable isotope data used in the mixing models were from individual taxa contained in each compartment (identified in parenthesis at the top of each plot).

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(a) Detritivorous Insects, Compartment 14.
(Culicidae Larvae)

Benthic Microalgae (1)

Epiphytic
Algae (2)

Phytoplankton
and POC
(4 & 31)

(b) Large Herbivorous Insects, Compartment 16.
(Adult Hydrophilidae 1-2 cm)

Benthic Microalgae (1)

Epiphytic
Algae (2)

Phytoplankton
and POC
(4 & 31)

(c) Large Predatory Insects, Compartment 18.
(Odonata Larvae 1-2 cm)

Benthic Microalgae (1)

Epiphytic
Algae (2)

Phytoplankton
and POC
(4 & 31)

(d) Predatory Fish - Large Prey, Compartment 25.
(*Gambusia sp*. 2-3 cm)

Benthic Microalgae (1)

Epiphytic
Algae (2)
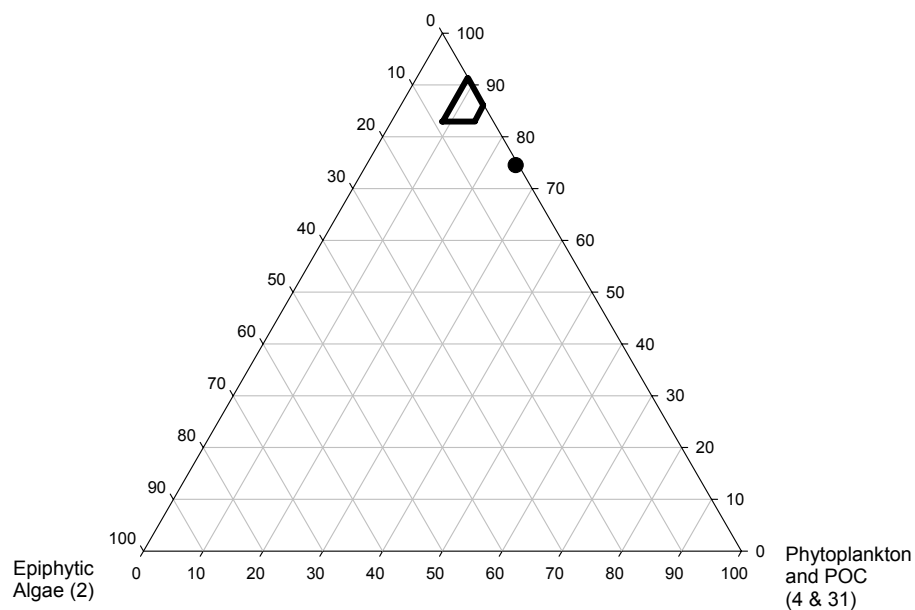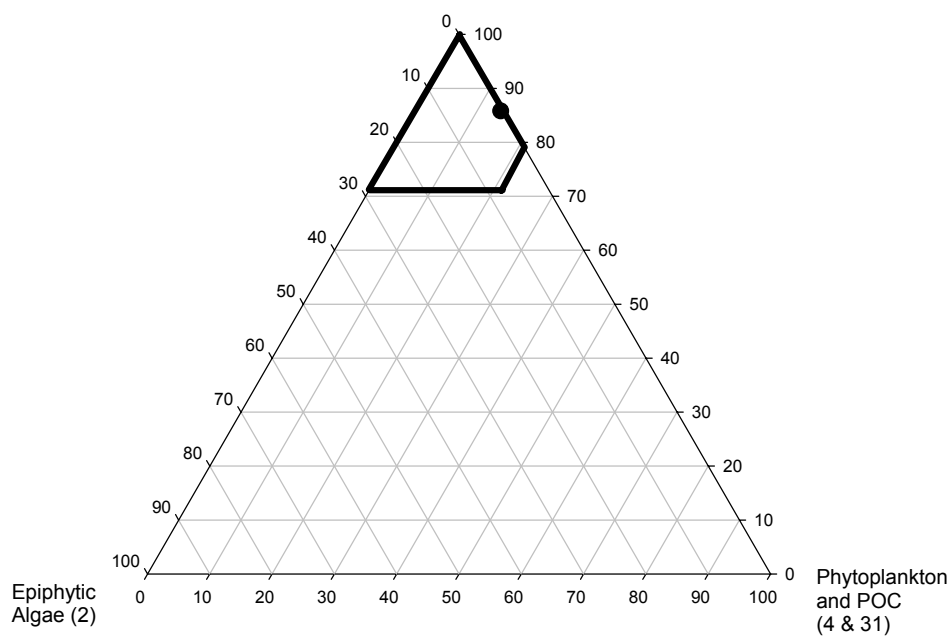
Phytoplankton
and POC
(4 & 31)
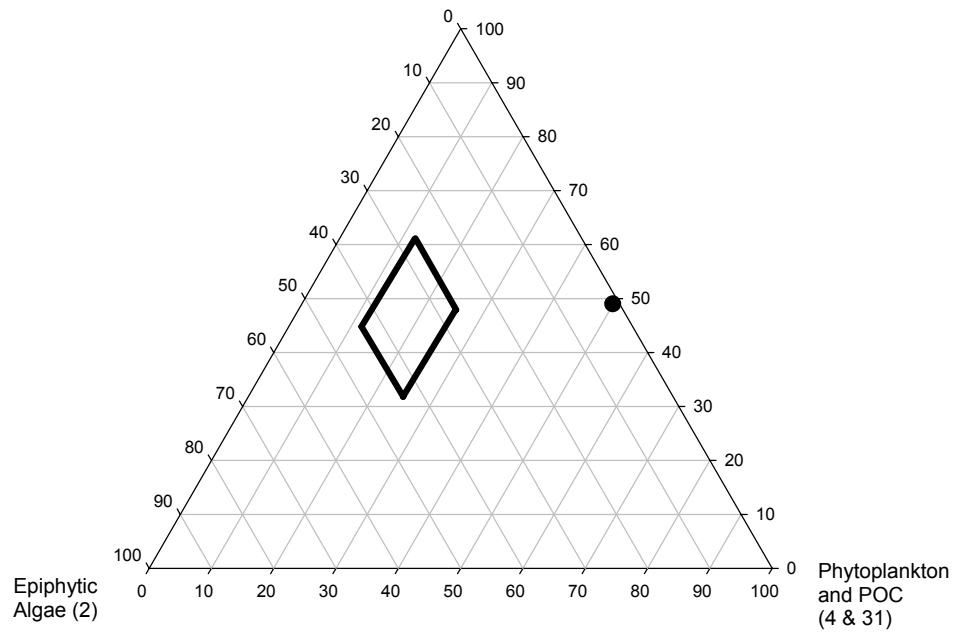
CHAPTER 6. SUMMARY AND SYNTHESIS

Although ecological network analysis (ENA) represents a growing area of ecology, it has received little critical evaluation. A more thorough assessment is necessary if ENA is to meet the challenges of both the scientific and management communities. Thus, the objectives of this study were (1) to present the potential ENA has for assisting in ecosystem-based management, while at the same time, identify some of its limitations; (2) to evaluate the effectiveness of ENA in detecting differences in food web properties; (3) to validate ENA models using independent methods; and (4) to provide a detailed data set documenting trophic relationships in multiple ecosystems replicated in time.

Chapter 2 identified four major sources of uncertainty in the reliability of ENA models: natural variability of input parameters; data collection methods; model construction; and fundamental assumptions of the algorithms. Suggestions for addressing these issues included (1) use of a priori predictions of model output and sensitivity analysis of model input to address variability of input data; (2) incorporation of multivariate techniques into model construction; (3) and validation of model output to estimate how well a model depicts the real-world system. A priori hypotheses were used in Chapter 4 to statistically test the effectiveness of ENA in detecting differences in salt marsh pond food webs, and efforts to validate models of those food webs were made in Chapter 5.

Findings from Chapter 4 suggest ecological network analysis (ENA) was reasonably effective in detecting differences in the food web properties examined. ANOVA results indicated mean values of 10 of 12 ENA indices were significantly different among the three stress/disturbance conditions, and results from the equivalent non-parametric test (Friedman's Test) were generally in agreement (mean rankings in 11 of 12 indices showed significant differences). Confidence in these results was given by a relatively low amount of covariance among the indices.

Results from Chapter 5 indicated mixed success in validating the models in four general areas: respiration, aggregation of taxa, trophic level, and carbon source or extended diet. The implications of these results were examined in Chapter 6. First, the models were modified so they agree with results from the independent methods used for validation. Explanations given for lack of validation in Chapter 5 were used as the basis for making the modifications. Next, a comparison of the output from the unmodified and modified models was performed.

Methods

Modifications To Reach Agreement In Respiration Estimates

Separate models from the validation efforts were modified to reach agreement for community and plankton respiration, aggregation of taxa, and extended diet. Models with the greatest discrepancies were selected for

analysis. For community and plankton respiration, the trophic network of East

Pond during spring 2002 was modified such that values from Ecopath (corrected

to include primary producers) were in agreement with estimates based on field

measurements of dissolved oxygen (DO). These modifications were determined

by considering the adjustments necessary for models of all four ponds to agree

with estimates of community and plankton respiration from DO data (Figure 6-1).

This approach ensured the adjustments were relatively consistent and

reasonable within the variability observed, and that they satisfied the field data

for both the plankton and total community.

Model adjustments for respiration involved two steps that included

modifying primary producer compartments followed by microbe compartments.

Adjustments to primary producer compartments in East Pond were:

- Benthic microalgae (compartment 1) biomass decreased 50%.

- Epiphytic algae (compartment 2) biomass decreased 1 order of magnitude.

- Phytoplankton (compartment 4) biomass decreased 75%.

- *Ruppia maritima* (compartment 5) biomass decreased 1 order of

  magnitude, and P/B decreased from 0.157 to 0.004.

Changes for benthic microalgae, epiphytic algae, and *R. maritima* were

equivalent to those necessary for the other ponds (Figure 6-1). Epiphytic algae

and *R. maritima* were adjusted the same magnitude because they were

associated with each other in the ponds. The adjusted P/B value for *R. maritima*

is similar to that used for *Halodule sp*. in a model by Christian and Luczkovich

(1999). Phytoplankton changes were greater in East Pond (75% decrease vs. 50% decrease in the other ponds) because initial biomass estimates were at least 30% larger there relative to the other ponds. This general approach was taken because primary producer compartments accounted for the largest portion of the respiration estimates in the Ecopath models (i.e. once model output was corrected to include producer respiration).

Adjustments to microbial compartments alone could not explain the differences in respiration estimates. However, they were necessary to bring the models within the range of values calculated from DO data. For the spring 2002 network of East Pond, these adjustments involved:

- H_2O column microbe biomass (compartments 6 & 7) decreased 75%.

- Sediment microbe biomass (compartments 8 & 9) decreased 50%.

Decrease in biomass of H_2O column microbes could be necessary due to assumptions associated with direct count methods used to determine microbe density. These methods do not discriminate between live and dead cells, and biomass calculations assumed all cells were living. Overestimation of sediment microbe biomass could be related to the presence of anaerobic metabolism which ENA does not consider.

Modifications To Reach Agreement In Aggregation Of Taxa

Aggregation of taxa was modified in the network for West Pond during late summer 2002 to be in agreement with stable isotope data (SIA). Modification

involved grouping taxa based on similarity of $\delta^{13}C$ and $\delta^{15}N$ values as opposed to using correspondence analysis. This was done for compartment 18 (large predatory insects) and compartment 25 (predatory fish that utilize large prey). Adequate SIA data were not available for other groups. Five consumer compartments (two insects and three fish) were added to the modified model (Table 6-1). The diet matrix of the modified network was also altered to account for differences in the diet of the "new" compartments relative to their original compartment. SIA data were used as a guide to define the diet of the "new" compartments such that higher $\delta^{15}N$ values correlated with relatively higher effective trophic levels in the model.

Modifications To Reach Agreement In Carbon Source Estimates

The diet matrix in the late summer 2002 North Pond network was modified such that carbon source estimates for three compartments from ENA agreed with those from stable isotope mixing models (Figure 6-2). The three compartments were detritivorous insects (14), large predatory insects (18), and predatory fish that utilize large prey (25). Their diet and their prey's diet were modified. The changes resulted in a slightly increased importance of benthic microalgae (compartment 1) to detritivorous insects, and a much greater importance of epiphytic algae (compartment 2) to large predatory insects and predatory fish that utilize large prey. As with the unmodified results, parameters from an

Ecopath model were used as input for NETWRK to obtain the extended diet

output in the form of the total dependency matrix.

Results and Discussion

Comparison Of Ouput: Modifications For Respiration

Modifications to the spring 2002 network of East Pond to reach agreement

in community and plankton respiration estimates resulted in notable differences

in six of the twelve indices analyzed (Table 6-2). Three indices increased (mean

ecotrophic efficiency, trophic chain length, and average path length increased)

while three others decreased (ratio of herbivory to detritivory, total systems

throughput, and ascendency/capacity).

These differences can be attributed to the modifications decreasing

primary production while keeping consumption relatively constant. This

increased the percent of primary production consumed (i.e. increased ecotrophic

efficiency of consumer compartments) as well as the percent of total throughput

that reached each trophic level. A large portion of producer biomass was

directed into detritus where it was exported from the boundaries of the model (i.e.

settled below the "fluff" layer). As a result, average path length increased

because exports decreased to a greater extent than total systems throughput

(see Table 6-2 for algorithm). The ratio of herbivory to detritivory decreased

because the total flow from detritivory declined less than the flow from herbivory

(i.e. relative importance of detritivory increased). Decreases in total systems

throughput and ascendency/capacity resulted from an overall reduction in flows

due to decreased biomass. Therefore, decreases in primary production (via

biomass of all producer compartments and P/B of *R. maritima*) along with

relatively similar levels of consumption (decreases in microbe biomass had a

relatively minor impact) had a significant affect on the system.

Comparison Of Output: Modifications For Aggregation

Modifications to the late summer 2002 network of West Pond based on

aggregation of taxa using stable isotopes resulted in significant differences in five

of twelve indices (Table 6-3). A greater number of consumer compartments, and

associated adjustments to the diet matrix, produced larger values for the

following indices: ecotrophic efficiency, system trophic efficiency, omnivory index,

number of paths, and the ratio of primary production required to net primary

production. The number of paths and ratio of primary production required to net

primary production increased because of a greater number of consumer

compartments while primary production remained constant. The other three

indices increased as a result of changes in the diet matrix. Diets of the additional

compartments were defined such that relationships given by $\delta^{15}N$ data were

maintained (i.e. higher $\delta^{15}N$ values corresponded to higher trophic levels). The

result was an increased consumption at upper trophic levels which led to

increases in ecotrophic efficiency, system trophic efficiency, and omnivory.

Comparison Of Output: Modifications For Carbon Source

In contrast to the other two comparisons, modifications to the late summer 2002 network of North Pond did not produce notable differences in the twelve ENA indices analyzed (Table 6-4). Adjustments were based on validation of the extended diet analysis and involved minor changes to the diet matrix. Changes did not include the addition of new prey items for a consumer or increased feeding of a consumer at different trophic levels. However, they did involve switching selectivity of a consumer from one primary producer to another. Thus the modifications did not involve significant changes in flow or interactions, and did not result in significant differences in the ENA indices.

Implications Of Findings

ENA was effective in detecting differences in food web properties that accompanied changing environmental conditions. However, the models could not always be validated by independent methods. The lack of total validation could be attributed to three factors. The first involves errors in model parameterization. Although initial parameter estimates were reasonable and well documented (see Chapter 3), validation results identified differences in respiration estimates that could only be explained by overestimates of primary producer biomass and P/B. A second factor involves inherent differences in the methods. For example when aggregating taxa, SIA considers similarities in diet only and correspondence analysis incorporates similarities in diet and predators.

Also, trophic level estimates from SIA are for specific taxa whereas estimates

from ENA are for compartments that often contain multiple taxa. The third

contributing factor to a lack of validation involves assumptions of the methods.

Assumptions associated with ENA that were likely problematic include steady

state conditions, similar assimilation of all prey items, aerobic metabolism, and

trophic level 1 for detritus (see Chapter 5 for discussion). In all cases,

discrepancies in the validation results could be explained by at least one of these

three factors.

Comparison Of Unmodified And Modified Models

Results from this study raise a fundamental question: what kind of

difference in the food web is needed for a significant change in the ENA indices

to be observed? One of the uncertainties identified in Chapter 3 involved the

potential for multiple conditions to satisfy the mass-balance requirements of ENA

and thus provide multiple "solutions" to the food web. This suggests minor

changes to the food web parameters will likely not significantly affect model

output. This is supported by results from the comparison of unmodified and

modified models based on validation of extended diet. Adjustments to the diet

matrix involving a change of 10-40% in consumption of one primary producer

relative to another producer did not significantly affect the indices from ENA

(Table 6-4). Thus, ENA output remained relatively constant with changes in diet

that did not involve additional interactions or adjustments in flow across trophic levels (i.e. did not involve changes to flow structure).

Other results from the comparison of unmodified and modified models provide additional insight. An 89% reduction in primary producer biomass resulted in changes to indices from trophic structure analysis, pathway analysis, and information analysis. Changes to indices included increases between 12-100% and decreases between 33-97%. Additionally, a 15% increase in the number of compartments resulted in changes to indices from trophic structure analysis and pathway analysis. In this case, the indices increased between 13-110%. Information indices were not significantly affected by the greater number of compartments. Thus, ENA output was sensitive to changes in the amount of material available for energy flow (i.e. primary producer biomass) as well as structural aspects of that flow (i.e. number of compartments).

Few studies are available for comparison with these results. Abarca-Arenas and Ulanowicz (2002) documented greater ascendency with an increased number of compartments due to differences in aggregation of taxa. Although I found no significant difference in the ratio of ascendency to capacity with a 15% increase in compartments, the ascendency was slightly greater in the modified model containing more compartments. Abarca-Arenas and Ulanowicz also noted greater differences in ascendency when there was a 20% or more change in the number of compartments. Therefore if more compartments were

added to the model used in this study, changes to the information indices may have been observed.

Application Of ENA To Resource Management

There are several implications of this study to the application of ENA for resource management. As outlined in Chapter 3, ENA has great potential as a tool for ecosystem-based management. There are few techniques that can quantify direct and indirect interactions and index system level attributes. ENA was shown in this study to be effective in detecting differences in food web properties. Differences in ENA indices were documented with changes in environmental conditions (i.e. not between replicated ponds), the amount of primary producer biomass, and the number of model compartments. However it should be recognized that because output appears to remain relatively constant with minor changes in input parameters, differences in the food webs being analyzed need to be dramatic for a distinction to be made.

Consistency in methods and meticulous documentation are needed throughout the field, laboratory, model construction, and model analysis stages of a study involving trophic networks. There needs to be an appreciation of the source, quality, and variability of the input data, and there needs to be a recognition that decisions made during model construction can affect model output. For comparative studies, the same assumptions and techniques should obviously be used in all models.

As shown, decisions concerning aggregating taxa into compartments were particularly influential to model output. However, other issues of model construction not explicitly discussed also potentially affected model output and thus my interpretations. These included:

- Use of "dummy compartments" or providing a biomass of 1.00×10^{-5} µg C m^{-2} for compartments that were not represented in the field sampling.

- Potential effects of previous sampling on subsequent food web models.

- Assumption that immigration and emmigration were insignificant for all model compartments during the sampling events.

- Adjusting the "detritus fate" input during the post-disturbance networks of East and West Ponds such that flow to DOC increased from 10% to 20%.

The use of "dummy" compartments allowed for comparison of the networks (e.g. Baird et al. 2004) without incorporating differences (i.e. a different number of comapartments) that may be due to insufficient sampling. Assumptions concerning minimal effects of previous sampling and immigration/emmigration were based on daily observations of the ponds both during and between sampling events. Finally, the "detritus fate" input was adjusted to satisfy the mass balance requirements of Ecopath. These types of assumptions are reasonable, but potentially have a large effect on model output and should be clearly stated via model documentation.

Validation should be considered as an iterative component of the modeling process (Overton 1977; Mankin et al. 1979; Haefner 1996). In this

study, validation of respiration identified discrepancies in model parameterization. Certainly, the adjustments to primary producer biomass are supported. However decreases in microbial biomass were also suggested as being necessary to validate the models, and this may alter ENA output to an extent that is not wanted. For example, anaerobic metabolism was given as an explanation for overestimates of microbial biomass. Anaerobic microbes, like their aerobic counterparts, process materials in the ecosystem and are available for consumption by organisms that tolerate some degree of hypoxic conditions (e.g. aquatic insect larvae and fish found in the ponds). Decreases in microbial biomass to satisfy respiration estimates could alter model output that is more meaningful. Therefore validation should be used to help "fine tune" a model, but adjustments should be done only after the major objectives of the modeling effort (e.g. to increase understanding of the food web) are considered.

Inherent uncertainties exist with any scientific method, particularly one that involves modeling. The key is to recognize they exist, understand their effects, and minimize them when possible. While ENA is certainly not the "holy grail" for implementing ecosystem-based management, it apparently is adequate for augmenting other approaches (e.g. other multi-species models and single species approaches) to provide a more holistic, multi-species approach. The process of constructing, analyzing, and validating trophic networks gives a keener understanding of the components of an ecosystem and how they interact, as well as a recognition of areas that need further research.

Table 6-1. Number of compartments from unmodified and modified models based on results from validating aggregation of taxa. Modifications involved aggregating taxa using dual isotope plots ($\delta^{13}C$ vs. $\delta^{15}N$).

	West Pond Late Summer 2002 Unmodified (aggregation based on correspondence analysis)	West Pond Late Summer 2002 Modified (aggregation based on stable isotope analysis)
Producers	5	5
Consumers	24	29
Detritus	2	2
Total	31	36

Table 6-2. ENA output from unmodified and modified models based on results from validation of community and plankton respiration. Modifications involved decreasing the biomass of primary producer and microbe compartments (see text). Indices in bold showed significant changes.

	East Pond Spring 2002 (unmodified)	East Pond Spring 2002 Modified (biomass of primary producers and microbes decreased)
Effective Trophic Level[1]	2.99	3.07
Ecotrophic Efficiency[2]	**0.553**	**0.618**
Trophic Chain Length[3]	**1**	**4**
System Trophic Efficiency[4]	3.36	3.36
Omnivory Index[5]	0.144	0.144
Number of Paths[6]	101300	101300
Average Path Length[7]	**2.1**	**2.8**
PPR/NPP[8]	0.4	0.4
Herbivory:Detritivory[9]	**1.0902**	**0.7286**
Total System Throughput[10]	**4.22E+08**	**9.22E+06**
Ascendency/Capacity[11]	**69.9**	**34.7**
Connectance Index[12]	0.212	0.212

Notes:
[1] Mean of consumers weighted by biomass.
[2] Mean of active groups w/o top predators.
[3] Highest trophic level with ≥ 1% of total throughput.
[4] Geometric mean of trophic level 2 through uppermost trophic level.
[5] For system.
[6] From trophic level 1 to all consumers.
[7] Calculated in Ecopath as total system throughput/(Σ exports + Σ respirations).
[8] PPR/NPP = primary production required/net primary production (%).
[9] Calculated as trophic level 1 total system throughput from primary producers/trophic level 1 total system throughput from detritus.
[10] Units = ug C m^{-2} d^{-1}.
[11] Units = %.
[12] Calculated as number of realized links/number of potential links.

Table 6-3. ENA output from unmodified and modified models based on results from validating the aggregation of taxa into compartments. Modifications involved aggregating taxa using dual isotope plots (δ^{13}C vs. δ^{15}N). Indices in bold showed significant changes.

	West Pond Late Summer 2002 Unmodified (aggregation based on correspondence analysis)	West Pond Late Summer 2002 Modified (aggregation based on stable isotope analysis)
Effective Trophic Level[1]	2.16	2.16
Ecotrophic Efficiency[2]	**0.383**	**0.566**
Trophic Chain Length[3]	3	3
System Trophic Efficiency[4]	**1.20**	**2.51**
Omnivory Index[5]	**0.191**	**0.215**
Number of Paths[6]	**1900**	**591781**
Average Path Length[7]	2.7	2.7
PPR/NPP[8]	**119**	**136**
Herbivory:Detritivory[9]	1.1046	1.1048
Total System Throughput[10]	1.40E+07	1.40E+07
Ascendency/Capacity[11]	29	29
Connectance Index[12]	0.157	0.190

Notes:
See Table 6-2 explanation.

Table 6-4. ENA output from unmodified and modified models based on results from validation of carbon source estimates (extended diet). Modifications involved basing the diet of three compartments (#14, #18, #25) on results from stable isotope mixing models (Phillips and Gregg 2001): $\delta^{13}C$ vs. $\delta^{34}S$ and $\delta^{34}S$ vs. $\delta^{15}N$. None of the indices showed significant changes.

|	North Pond Late Summer 2002 Unmodified	North Pond Late Summer 2002 Modified (diet based on stable isotope mixing models)
Effective Trophic Level[1]	2.15	2.15
Ecotrophic Efficiency[2]	0.486	0.480
Trophic Chain Length[3]	2	2
System Trophic Efficiency[4]	2.46	2.46
Omnivory Index[5]	0.193	0.186
Number of Paths[6]	5185	5169
Average Path Length[7]	2.3	2.3
PPR/NPP[8]	8.6	9.0
Herbivory:Detritivory[9]	0.9935	0.9960
Total System Throughput[10]	2.18E+07	2.19E+07
Ascendency/Capacity[11]	39.7	39.7
Connectance Index[12]	0.175	0.174

Notes:
See Table 6-2 for explanation.

Figure 6-1. Comparison of respiration estimates from modified networks with values calculated from dissolved oxygen field data. (a) Community respiration estimates from Ecopath are compared to estimates based on two diurnal studies that were corrected for diffusion. (b) Plankton respiration estimates from Ecopath are compared to estimates based on light-dark bottle experiments. Modifications involved adjusting primary producer compartments and microbe compartments.

(a) Comparison of Community Respiration Estimates from Modified Models.

[All Compartments Except: Frogs (27) + Snakes (28) + Birds (29)]
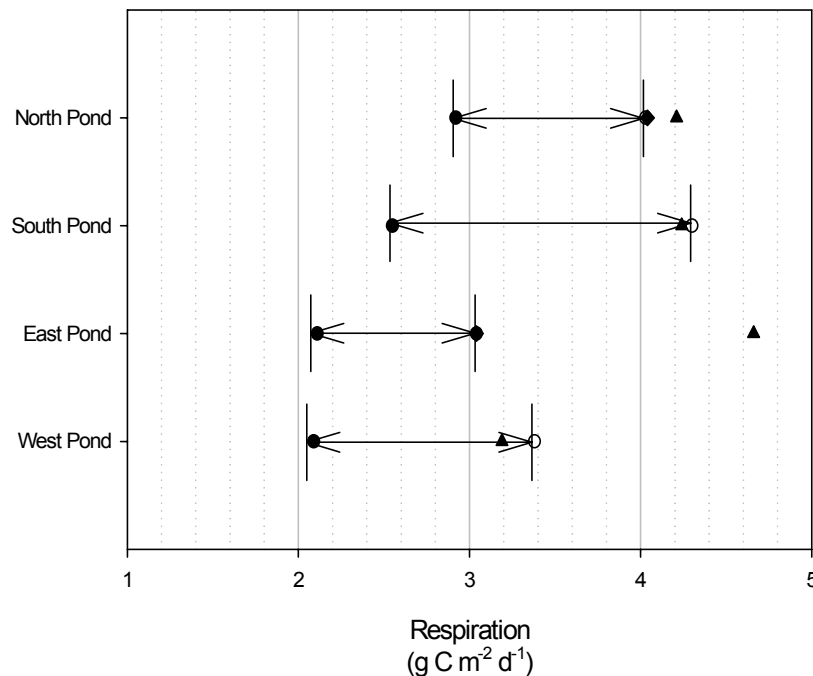
Respiration
(g C m^{-2} d^{-1})

● Diurnal Curve Results from May 25-26 2002 Corrected for Diffusion
○ Diurnal Curve Results from June 24-25 2002 Corrected for Diffusion
▲ Values from Ecopath models with Modifications to Producers
◆ Values from Ecopath models with Modifications to Producers and Microbes

Summary of Modifications for Community Respiration

Primary Producer Compartments

All ponds

- Benthic Microalgae biomass decreased 50%.
- Epiphytic Algae biomass decreased 1 order of magnitude.
- *Ruppia maritima* biomass decreased 1 order of magnitude.
- *R. maritima* P/B decreased from 0.156 to 0.004.

North, South, and West Ponds - Phytoplankton biomass decreased 50%.

East Pond - Phytoplankton biomass decreased 75%.

Microbe Compartments

North Pond

- H$_2$O Column microbe biomass decreased 40%.
- Sediment microbe biomass decreased 10%, but could be as much as 95%.

South Pond – Sediment microbe biomass could be decreased as much as 95%.

East Pond

- H$_2$O Column microbe biomass decreased 75%.
- Sediment microbe biomass decreased 10% but could be as much as 100%.

West Pond – Sediment microbe biomass could be

(b) Comparison of Plankton Respiration Estimates
from Modified Models.

[Plankton (4) + H_2O Column Bacteria (6) + H_2O Column Microprotozoans (7) + Zooplankton (10)]

Summary of Modifications
for Plankton Respiration

Phytoplankton

North, South, and West Ponds

- Decrease biomass 50%.

East Pond

- Decrease biomass 75%.

H_2O Column Microbes

North Pond

- Decrease biomass 40%.

East Pond

- Decrease biomass 75%.

Respiration
(g C m^{-2} d^{-1})
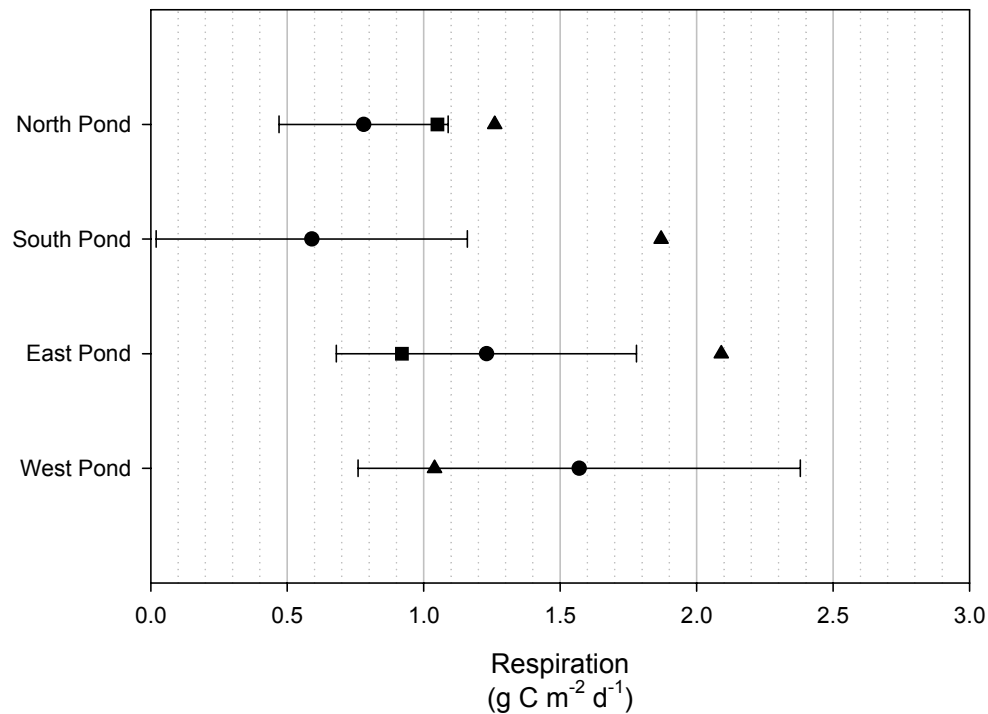
● Mean and 95% CI from Light/Dark Bottle Experiments
▲ Value from Ecopath Model with Modifications for Phytoplankton
■ Value from Ecopath Model with Modifications for Phytoplankton & Microbes

Figure 6-2. Ternary plots comparing carbon source estimates from unmodified and modified networks representing North Pond during late summer 2002: (a) detritivorous insects (compartment 14), (b) large herbivorous insects (compartment 16), (c) large predatory insects (compartment 18), (d) predatory fish that feed on large prey (compartment 25). Circles represent results from the total dependency matrix of the unmodified network. Inverted triangles represent the modified network. Polygons were derived from 95% confidence intervals obtained from dual isotope mixing models (Phillips and Gregg 2001): δ^{13}C vs. δ^{34}S and δ^{34}S vs. δ^{15}N (see Chapter 4, Table 4-3). Stable isotope data used in the mixing models were from individual taxa contained in each compartment (identified in parenthesis at the top of each plot). Compartment 16 (b) was not modified because results from the unmodified network agreed with the mixing models.

(a) Detritivorous Insects, Compartment 14.
(Culicidae Larvae)
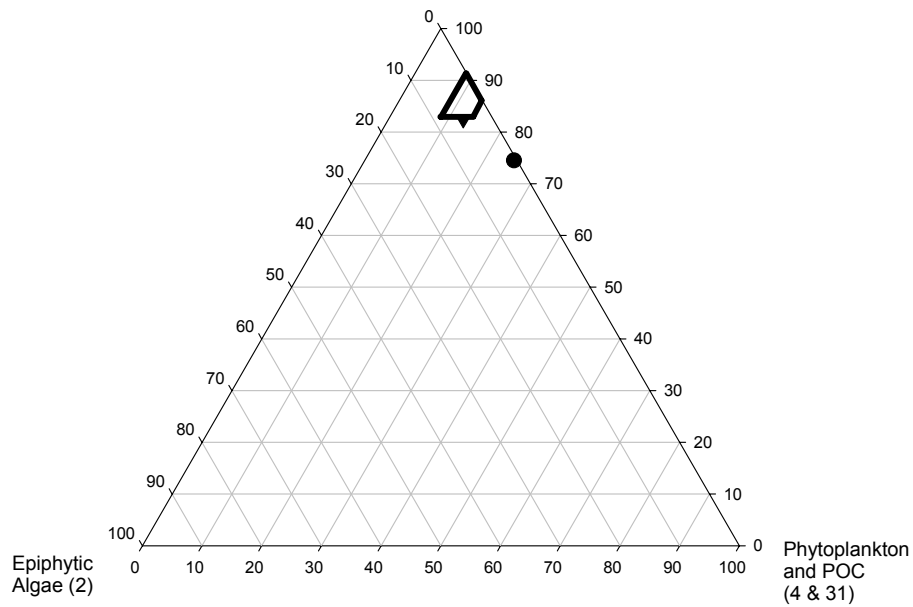
Benthic Microalgae (1)

Epiphytic
Algae (2)

Phytoplankton
and POC
(4 & 31)

(b) Large Herbivorous Insects, Compartment 16.
(Adult Hydrophilidae 1-2 cm)

Benthic Microalgae (1)

Epiphytic
Algae (2)

Phytoplankton
and POC
(4 & 31)

(c) Large Predatory Insects, Compartment 18.
(Odonata Larvae 1-2 cm)

Benthic Microalgae (1)

Epiphytic
Algae (2)

Phytoplankton
and POC
(4 & 31)

(d) Predatory Fish - Large Prey, Compartment 25.
(*Gambusia sp*. 2-3 cm)

Benthic Microalgae (1)

Epiphytic
Algae (2)

Phytoplankton
and POC
(4 & 31)
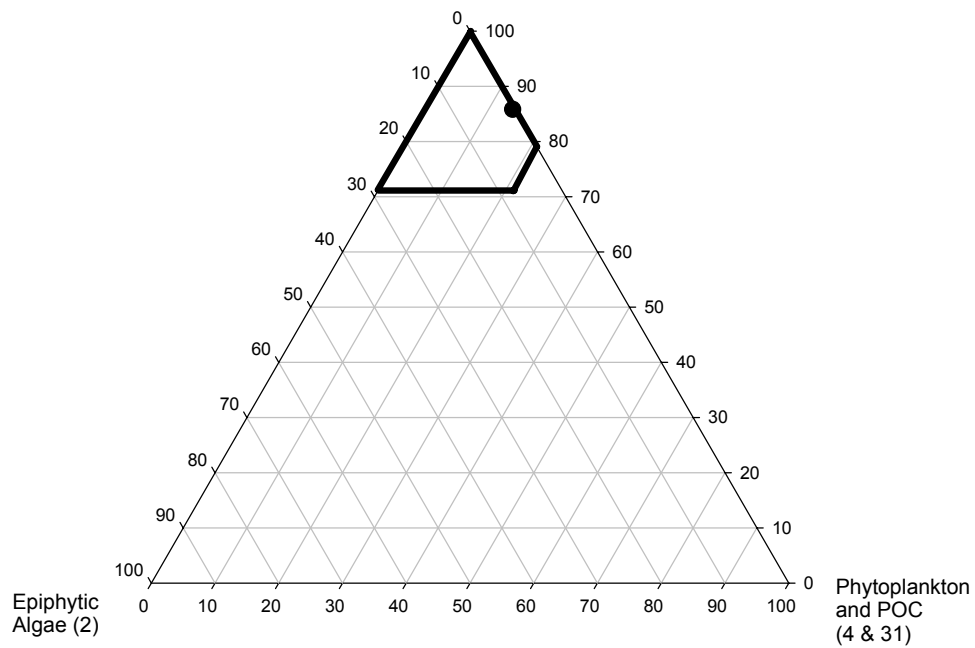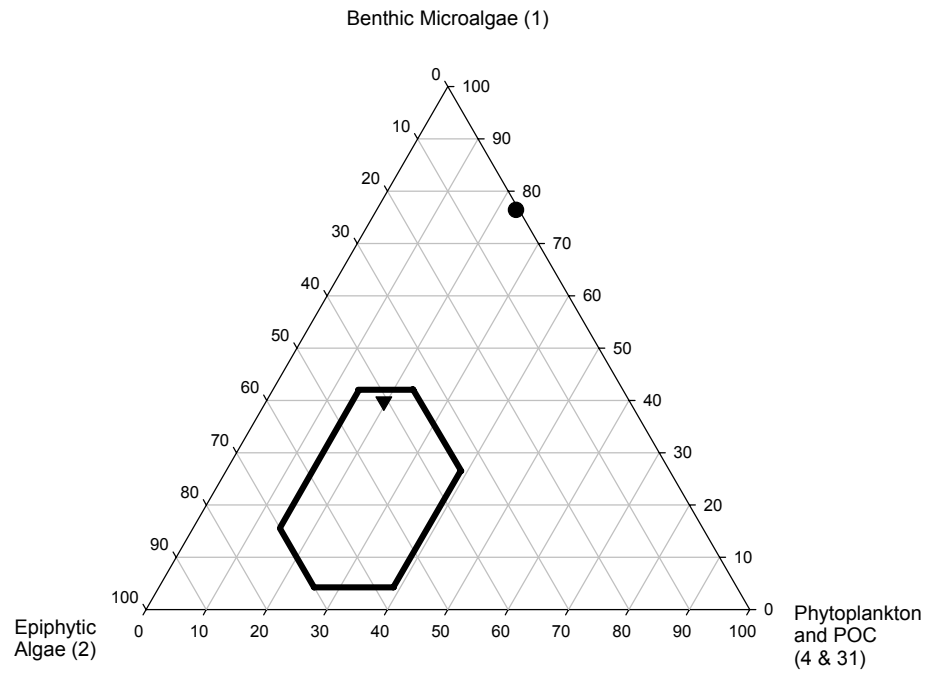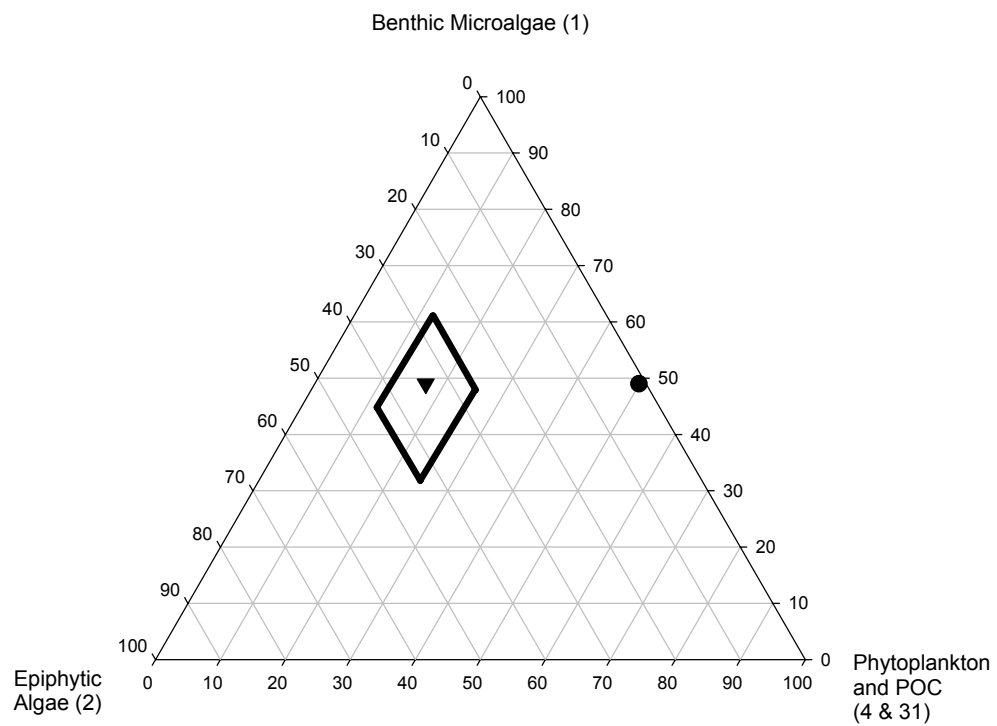
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