Hydrobiologia **436:** 131–143, 2000.

An artificial neural network approach for studying phytoplankton succession

Julian D. Olden
Dept. of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 3G5, Canada
Present address: Dept. of Biology, Colorado State University, Fort Collins, CO 80523-1878, U.S.A.
Phone ++1-970-491-2414. Fax ++1-970-491-0649. E-mail olden@lamar.colostate.edu

Received 15 December 1998; in revised form 15 July 2000; accepted 20 July 2000

Key words: bottom-up, top-down, trophic, zooplankton, randomization

Abstract

Artificial neural networks are used to model phytoplankton succession and gain insight into the relative strengths of bottom-up and top-down forces shaping seasonal patterns in phytoplankton biomass and community composition. Model comparisons indicate that patterns in chlorophyll *a* concentrations response instantaneously to patterns in nutrient concentrations (phosphorous (P), nitrite and nitrate (NO_2/NO_3–N) and ammonium (NH_4–H) concentrations) and zooplankton biomass (daphnid cladocera and copepoda biomass); whereas lagged responses in an index of algal community composition are evident. A randomization approach to neural networks is employed to reveal individual and interacting contributions of nutrient concentrations and zooplankton biomass to predictions of phytoplankton biomass and community composition. The results show that patterns in chlorophyll *a* concentrations are directly associated with P, NO_2/NO_3–N and daphnid cladocera biomass, as well as related to interactions between daphnid cladocera biomass, and NO_2/NO_3–N and P. Similarly, patterns in phytoplankton community composition are associated with NO_2/NO_3–N and daphnid cladocera biomass; however show contrasting patterns in nutrient–zooplankton and zooplankton–zooplankton interactions. Together, the results provide correlative evidence for the importance of nutrient limitation, zooplankton grazing and nutrient regeneration in shaping phytoplankton community dynamics. This study shows that artificial neural networks can provide a powerful tool for studying phytoplankton succession by aiding in the quantification and interpretation of the individual and interacting contributions of nutrient limitation and zooplankton herbivory on phytoplankton biomass and community composition under natural conditions.

Introduction

Freshwater phytoplankton communities often undergo pronounced seasonal succession (Reynolds, 1984). The successional pattern in a lake is fairly repeatable among years, and patterns among lakes are somewhat predictable according to trophic status (Reynolds, 1984; Sommer et al., 1986). The study of the forces driving phytoplankton succession, however, remains a difficult task since the temporal dynamics of algal communities are influenced by a complex array of biotic and abiotic factors operating through both direct and indirect pathways (Sommer, 1989; Vanni & Temte, 1990; Sarnello, 1992; Carillo et al., 1995).

Although it is recognized that many factors are involved in shaping patterns in phytoplankton succession, it is generally acknowledged that a combination of resource limitation and predation play the primary roles (Reynolds, 1984; Sommer et al., 1986; Sommer, 1989). Limitation of available phosphorous and nitrogen have been identified in numerous experimental studies as the two essential nutrients regulating algal biomass and community composition (see Sommer (1989) for review). Competition among algae for these nutrients will result in hierarchically structured communities, dominated by competitively strong species.

The effects of zooplankton on phytoplankton communities, on the other hand, are more complex to understand (Sterner, 1986). The most obvious impact of zooplankton is through direct grazing of algae, resulting in a reduction in overall phytoplankton biomass (Lambert et al., 1986; Sterner, 1989). The potency of herbivorous grazing will vary depending on the size structure and species composition of the zooplankton community (Bergquist et al., 1985). In general, it is believed that mesozooplankters (e.g. daphnids) have greater influence on phytoplankton biomass compared to smaller species (e.g. copepods) because of greater feeding efficiencies (Brooks & Dobson, 1965; Hall et al., 1976; Lynch & Shapiro, 1981). Phytoplankton also exhibit differential resistance to grazing resulting from morphological differences in cell size, shape and possession of hard coverings or gelatinous sheaths (Porter, 1976; De Bernard & Guissan, 1990).

While direct grazing on algal cells is arguably the greatest effect zooplankton have on phytoplankton, secondary or indirect effects of zooplankton grazing have also been recognized as being important in regulating phytoplankton succession (e.g. Sterner, 1989; Carillo et al., 1995; Queimaliños et al., 1998; Wickham, 1998). By reducing algal populations, zooplankton may increase the *per capita* availability of nutrient resources for other algal species (Sterner, 1990; Urabe, 1993), and may contribute to the available pool of nutrients by regenerating nutrients via excretion, egestion and sloppy feeding (*sensu* Lambert, 1978). In some cases, the degree of phosphorous and nitrogen regeneration can constitute a substantial proportion of the nutrients required for phytoplankton growth (e.g. Lehman, 1980; Urabe et al., 1995). Similar to the effects of direct grazing, the magnitude and variation of nutrient regeneration is dependent on the composition of the zooplankton community since fecal characteristics differ between species (Sterner, 1989; Urabe, 1993).

Given the array of interactions between nutrient availability and grazing zooplankton, and their effects on algal communities, studies examining phytoplankton succession have predominantly involved micro- and meso-cosm experiments. These experiments are designed to tease apart the relative contributions of bottom-up and top-down forces influencing phytoplankton communities by performing manipulations and examining the characteristics of the resulting interactions (e.g. Sterner, 1986; Sommer, 1988; Vanni & Temte, 1990; Carrilo et al., 1995; Hansen et al., 1997). Despite the important insights that such experiments have provided, detailed examinations of empirical data are still lacking and consequently an understanding of the factors influencing phytoplankton seasonal succession under natural conditions has not been clearly established. Current emphasis on experiments most likely arises from the fact that until recently, there lacked appropriate statistical techniques for quantifying and interpreting complex, non-linear interactions among variables from empirical data. For instance, regression analysis remains the most frequently used technique for modeling ecological relationships, although our confidence in the results is often limited by the inability to meet a number of parametric assumptions. Artificial neural networks are a promising statistical approach in this regard, as they provide a powerful, flexible non-linear statistical modeling technique for uncovering patterns in ecological data (Colassanti, 1991; Edwards & Morse, 1995; Lek et al., 1996). Applications of neural networks are diverse within the scientific literature, ranging from social sciences to chemistry, and recently have received more attention in the biological sciences for solving pattern recognition problems (e.g. Lek et al., 1996; Lek & Guégan, 1999). As such, a neural network approach may be useful for gaining important insight into the potentially complex influence of nutrient limitation and zooplankton grazing on phytoplankton succession.

This study investigates the influence of bottom-up and top-down forces in shaping patterns of phytoplankton seasonal succession. This is accomplished by using artificial neural networks to understand the individual and interacting effects of available nutrients (i.e. phosphorous and nitrogen) and zooplankton grazing (i.e. daphnid cladocera and copepoda) on seasonal patterns of phytoplankton biomass and community composition. Detailed model comparisons are used to assess whether phytoplankton biomass and community composition exhibit time lagged responses to changing patterns in phosphorous, total nitrites and nitrates, total ammonium, daphnid cladocera biomass and copepoda biomass. Together, this study aims to illustrate the utility of artificial neural networks for gaining a greater understanding of the causal mechanisms controlling phytoplankton succession under natural conditions.

Table 1. Spearman rank correlations between the first axis of the correspondence analysis and the percent phytoplankton composition by class

Phytoplankton class	Phytoplankton edibility	Spearman correlation (r_S)	Probability $\|r_S\| > 0$
Chrysophytes	Highly edible	0.870	0.0011
Cryptophytes	Edible	0.853	0.0017
Dinophytes	Both	0.652	0.0410
Bacillariophytes	Edible	0.646	0.0440
Chlorophytes	Both	0.491	0.1490
Euglenophytes	Both	0.467	0.1740
Cyanophytes	Highly inedible	−0.985	0.0001

Methods

Study site

The study area was Grenadier Pond, a 18.9-hectare drainage lake located in south-central Ontario, Canada (43° 38′ N, 79° 28′ W). Total phosphorus (μg/l), total nitrites and nitrates (μg/l), total ammonium (μg/l), chlorophyll *a* concentration (μg/l), phytoplankton community composition, daphnid cladocera biomass (mg/m^3) and copepoda biomass (mg/m^3) were collected by the Ontario Ministry of Environment during 1995 using standardized protocols. Samples were taken at fortnight intervals during the period of thermal stratification from mid-May through mid-September, in the euphotic zone (3 m in depth) at the deepest point in the lake.

Phytoplankton community composition

Compositional patterns in phytoplankton community composition are summarized using correspondence analysis. Data reduction by means of correspondence analysis aids in the interpretation of phytoplankton community dynamics by producing a low-dimensional ordination space in which similar species and samples are close together and dissimilar entities are far apart (Gauch, 1982). The first dimension, by definition, captures most of the total variance in the original variables and is used as a single index characterizing the change in phytoplankton community structure in time, hereafter called the phytoplankton community index (PCI).

In addition to summarizing overall changes in phytoplankton composition, it would be optimal to have the ability to rank phytoplankton taxa in terms of their relative grazing susceptibility in order to examine in greater detail the effects of grazing by zooplankton. Unfortunately, such a task is not entirely feasible due to overwhelming variation in morphological and chemical characteristics of phytoplankton and feeding modes among freshwater zooplankton. However, although a definite ranking cannot be achieved, there are still some generalities can exist that may aid in studying the contribution of herbivory on the dynamics of phytoplankton community composition through time. I incorporated the relative susceptibilities of phytoplankton classes to grazing in two ways. First, potential differences among zooplankton grazers are assessed by examining the influence of two components of the zooplankton community on phytoplankton succession: daphnid cladocera biomass and copepoda biomass. Second, phytoplankton taxa are classified as ‘edible’ and ‘inedible’ types (*sensu* Porter, 1976) based on size and possession of hard coverings or gelatinous sheaths (Table 1). Here, I briefly describe the rationale behind these divisions. Chrysophytes are mostly non-gelatinous, unicellular algae lacking a rigid cell wall (bound only by a cytoplasmic membrane) causing this class to be considered highly edible (Wetzel, 1982; Vanni & Temte, 1990). Cryptophytes and bacillariophytes are also primarily non-gelatinous, unicellular algae, making them generally edible. Dinophytes contain both naked and armoured types of cells (Wetzel, 1982), while chlorophytes contain an array of colonial, gelatinous types and individual, non-gelatinous types, potentially making these classes both edible and inedible to zooplankton. Euglenophytes are flagellates that lack cell walls, and live most of the time as solitary cells, causing them to be considered edible. Lastly, the majority of the cyanophytes are enclosed in gelatinous sheaths either individually or in colonies, making them relatively inedible to grazing zooplankters. Although algal edibility was characterized at a coarse taxon scale, this classification is satisfactory for the purposes of this study.

Artificial neural networks

Artificial neural networks were used to model nutrient–phytoplankton–zooplankton relationships in time. One hidden-layer feedforward neural networks trained by the backpropagation algorithm (Rumelhart et al., 1986) were used since this family of networks is considered universal approximators of any continuous function (Hornick et al., 1989). Figure 1 illustrates the architecture of the neural networks used in this study. The networks were comprised of an input layer

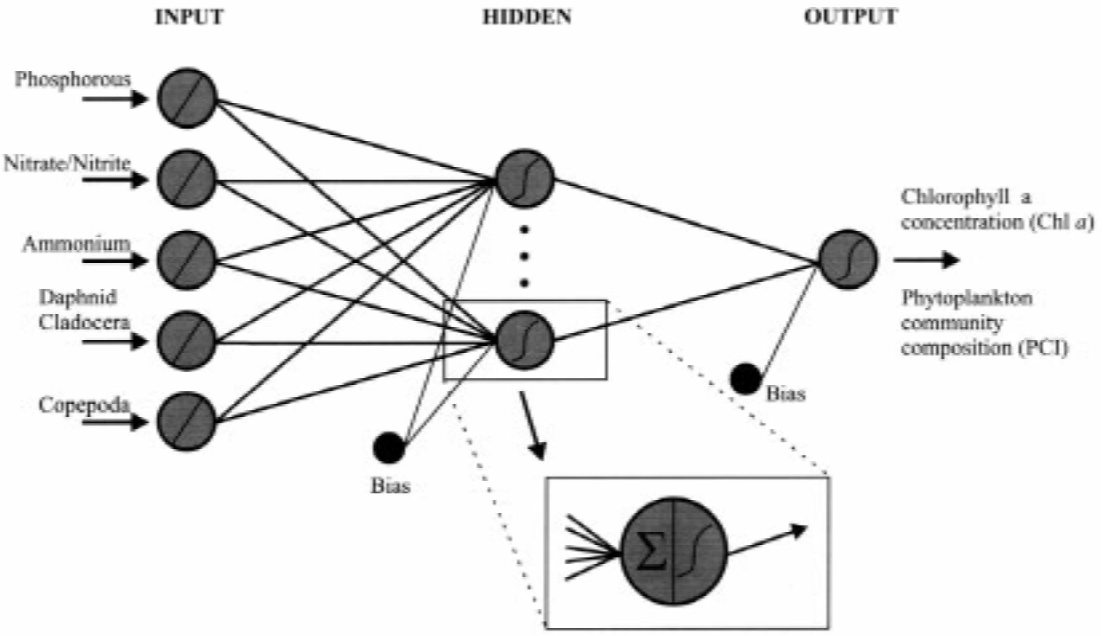

Figure 1. One-hidden layer, feedforward neural network design.

containing five neurons representing each of the predictor variables, i.e. concentrations of phosphorus, ammonium, nitrites and nitrates, and total biomass of daphnid cladocera and copepoda. A single hidden layer was chosen because it is generally satisfactory for statistical applications (Bishop, 1995), greatly reduces computational time, and often produces similar results compared to multiple hidden layers (Kurková, 1992). The optimal number of neurons in the hidden layer was determined empirically by comparing the performances of different networks, with 1 – 20 hidden neurons, and choosing the number that produced the greatest network performance. The output layer contained one neuron representing the predicted chlorophyll *a* concentration or PCI value. Additional bias neurons with a constant output, playing a similar role to that of the constant term in multiple regression, were added to the hidden and output layers.

Each neuron (excluding the bias neurons) is connected to all neurons of adjacent layers with an axon. The 'state' or 'activity level' of each neuron is determined by the input received from the other neurons connected to it. The states of the input neurons are defined by the incoming signal (i.e. values) of the predictor variables at the entry of the network. The state of the other neurons is evaluated locally by calculating the weighted sum of the incoming signals from the neurons of the previous layer. The entire process can be written mathematically as:

$$y_k = \phi_o \left\{ \beta_k + \sum_j w_{jk} \phi_h \left(\beta_j + \sum_i w_{ij} x_i \right) \right\}, \quad (1)$$

where x_i are the input signals, y_k are the output signals, w_{ij} are the weights between input neuron i to hidden neuron j, w_{jk} are the weights between hidden neuron j and output neuron k, β_j and β_k are the bias associated with the hidden and output layers, and ϕ_h and ϕ_o are activation functions for the hidden and output layers. There are several activation functions (see Bishop, 1995) and this study used the logistic (or sigmoid) function.

The backpropagation algorithm trains the network by iteratively adjusting all the connection weights among neurons, with the goal of finding a set of connection weights that minimizes the error of the network, i.e. sum-of-the-squares between the actual and predicted output (least squares error function). Observations are sequentially presented to the network, and weights are adjusted after each output is calculated depending on the magnitude and direction of the error. This interative technique of minimizing the error is known as gradient descent, where weights are modified in the direction of greatest descent, traveling 'downhill' in the direction of the minimum. Learning rate (η) and momentum (α) parameters (varying as a function of network error) were included during network training to ensure a high probability of global network convergence (see Bishop, 1995 for details), and a maximum of 1000 iterations for the backpropagation algorithm to determine the optimal axon weights.

In the neural network, the connection weights between neurons are the links between the inputs and the outputs and, therefore, are the link between the problem and the solution. The weights contain all the information about the network. The relative contribution of the independent variables to the predictive output of the neural network depends primarily on the magnitude and direction of the connection weight between the neurons. In this study, Neural Interpretation Diagrams (*sensu* Özesmi & Özesmi, 1999) were constructed to visually interpret the magnitude and direction of the connections neurons of the networks. Using the connection weights, relationships between nutrient concentrations and zooplankton biomass, and chlorophyll *a* concentrations and PCI could be identified and quantified. Next, a randomization test for artificial neural networks was used to assess the statistical significance of connections weights and input variable contributions (Olden, 2000; Olden & Jackson, 2000). This approach randomizes the response variable, then constructs a neural network using the randomized data and records all input-hidden-output connection weights (product of the input-hidden and hidden-output weights). This process is repeated a large number of times to generate a null distribution for each input-hidden-output connection weight, which is then compared to the observed values to calculate the significance level. The randomization test provides a pruning technique for eliminating connection weights that have minimal influence on the network output and identifies independent variables that significantly contribute to the prediction process. By removing connection weights that do not contribute significantly to predicting chlorophyll *a* concentration or phytoplankton community composition, the individual and interacting effects of nutrient concentrations and the zooplankton community can be more readily identified and interpreted.

Neural network design for modeling phytoplankton succession

Prior to training the neural network, the data must be modified so that the dependent and independent variables exhibit particular distributional characteristics. The dependent variable must be converted in the range [0..1] so that it conforms to the demands of the transfer function used (sigmoid function) in the building of the neural network. The independent variables must be converted to *z*-scores to standardize the measurement scales of the inputs into the network, and thus to ensure that same percentage change in the weighted sum of the inputs causes a similar percentage change in the unit output.

A number of predictor variable combinations were considered for modeling temporal patterns in Chl *a* concentrations and phytoplankton community index (PCI). Four network designs were trained for predicting values of Chl *a* and PCI at time *t* as a function of phosphorous (P), nitrite and nitrate (NO_2/NO_3–N) and ammonium (NH_4–H) concentrations, and total daphnid cladocera (DC) and copepoda (C) biomass. The networks contained each of the following five input variables:

$$\begin{aligned}
&\text{network 1:}\quad P(t)\cdot NH_4-H(t)\cdot\\
&NO_2/NO_3-N(t)\cdot DC(t)\cdot C(t)\\
&\text{network 2:}\quad P(t-1)\cdot NH_4-H(t-1)\cdot\\
&NO_2/NO_3-N(t-1)\cdot DC(t)\cdot C(t)\\
&\text{network 3:}\quad P(t)\cdot NH_4-H(t)\cdot\\
&NO_2/NO_3-N(t)\cdot DC(t-1)\cdot C(t-1)\\
&\text{network 4:}\quad P(t-1)\cdot NH_4-H(t-1)\cdot\\
&NO_2/NO_3-N(t-1)\cdot DC(t-1)\cdot C(t-1)
\end{aligned}$$

Network 1 assumes that Chl *a* concentrations and values of PCI change instantaneously to fluctuations in nutrient concentrations and zooplankton biomass, whereas the other three designs account for the idea that phytoplankton communities may exhibit a time lagged response to either nutrient concentrations (network 2), to zooplankton biomass (network 3), or both (network 4). Performance of these networks were assessed using the Pearson product-moment correlation between predicted and actual Chl *a* and PCI values, and the root-mean-square-of-error (RMSE) of the predicted values. The Pearson correlation provides a measure of model accuracy, with better models represented by correlation coefficients approaching 1. RMSE measures model precision, with small values representing high precision and large values being indicative of poor precision.

Statistical analyses were performed using S-Plus software (version 4.5) and computer routines for training the neural networks and the randomization proto-

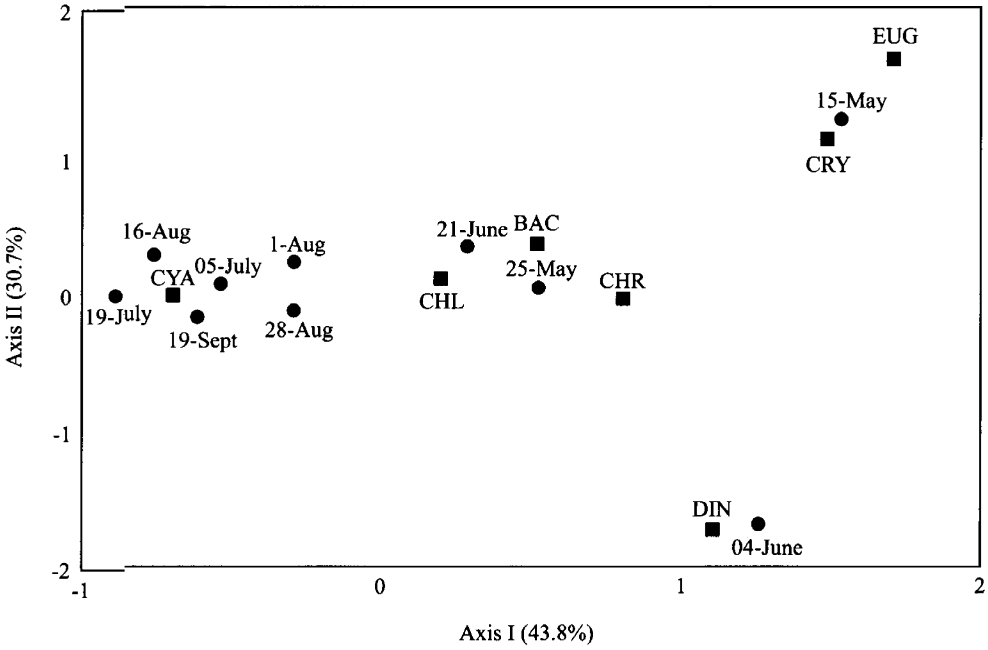

Figure 2. Joint correspondence plot of trends in phytoplankton community composition across sampling dates. Symbols differentiate sampling dates (circles) and phytoplankton classes (squares): BAC (bacillariophytes), CHL (chlorophytes), CHR (chrysophytes), CRY (cryptophytes), CYA (cyanophytes), DIN (dinophytes) and EUG (euglenophytes).

cols were written in MatLab programming language (version 5.3).

Results

Phytoplankton community composition

The CA of the phytoplankton community summarizes approximately 75% of the total variance in the first two axes, with the first axis explaining a significant proportion of the total variance in the original variables (43.8%) based on the broken-stick model (Jackson, 1993). Samples clustered close together in the joint species-sample plot (Figure 2) indicate sampling dates sharing similar phytoplankton community compositions, whereas samples positioned at opposite ends of the plot contain distinctly different fauna. Figure 2 separates sampling dates dominated by cyanophytes, positioned towards the negative end of the first axis, from sampling dates dominated by bacillariophytes, chrysophytes, cryptophytes and dinophytes, located towards the positive end on the first axis. The strength of this gradient is represented by the Spearman rank correlation coefficient for the relationship between the scores of the first principal component of the CA and the percent composition of the phytoplankton classes (Table 1). Phytoplankton diversity also increases with increasing CA axis I scores, with a community almost completely comprised of cyanophytes located in the left of Figure 2 and a highly diverse and homogeneous (in terms of class dominance) community located in the right. Furthermore, based on phytoplankton size, it is evident that a transition occurs from inedible to edible types of algae along the first axis (Table 1). Therefore, the scores of CA axis I (PCI) provide a summary of the dominant temporal patterns in phytoplankton community composition (i.e. relative dominance of particular phytoplankton classes and class diversity) and the edibility of algae present in the lake.

Neural networks for phytoplankton succession

The degree of predictability of chlorophyll *a* concentrations and PCI varies greatly among network designs depending on the type and state of the input variables used in the network. Models using either lagged nutrient concentrations or lagged zooplankton biomass (i.e. network 2 and 3) exhibit weak performance for both Chl *a* and PCI (Table 2). The poorest model performance is provided by lagged values of daphnid cladocera and copepoda biomass. In contrast, networks 1 and 4 predict Chl *a* and PCI with relatively high ac-

Table 2. Performance of neural networks for predicting chlorophyll *a* concentration (Chl *a*) and phytoplankton community composition (PCI) as a function of P, NH_4–H, NO_2/NO_3–N (Nutrients) and biomass of daphnid cladocera and copepoda (Zooplankton). 'Lagged' refers to predicting values of Chl *a* and PCI at time *t* based on values of the independent variables at time *t*-1. Reported values are the number of hidden neurons in the network (# HN), correlation coefficient between observed and predicted values (*r*) and root-mean-square-of-error (RMSE) of the predicted values

Network	Dependent	Independent	# HN	*r*	RMSE
1	Chl *a*	Nutrients – Zooplankton	2	0.884	29.74
2		Lagged Nutrients – Zooplankton	3	0.295	31.65
3		Nutrients – Lagged Zooplankton	1	0.031	41.30
4		Lagged Nutrients – Lagged Zooplankton	3	0.767	32.08
1	PCI	Nutrients – Zooplankton	2	0.807	0.633
2		Lagged Nutrients – Zooplankton	2	0.275	0.952
3		Nutrients – Lagged Zooplankton	1	0.081	1.345
4		Lagged Nutrients – Lagged Zooplankton	3	0.925	0.351

curacy and precision, respectively. The best network for explaining patterns in Chl *a* concentrations uses values of the input variables at time *t* (network 1), whereas PCI is best predicted using lagged values of all the input variables, i.e. time *t*-1 (network 4). The connection weights of the two best networks are examined in detail to quantify the individual and interacting contributions of nutrient concentrations and zooplankton biomass for explaining patterns in Chl *a* concentrations and PCI values.

Factors influencing chlorophyll a concentrations and phytoplankton community composition (PCI)

Here I first briefly describe the interpretation of connection weights in neural networks before discussing the nutrient–phytoplankton–zooplankton relationships observed in Grenadier Pond. Figure 3 illustrates the neural interpretation diagrams for Chl *a* concentration (network 1) and PCI (network 4). In these diagrams, the relative magnitude of the connection weights is represented by line thickness (i.e. thicker lines representing greater weights) and line shade represents the direction of the weights (i.e. black lines representing positive, excitator signals and gray lines representing negative, inhibitor signals). Input variables with larger connection weights represent greater intensities of signal transfer and, therefore, have a greater influence on the output (Chl *a* or PCI) compared to variables with smaller weights. Negative connection weights represent inhibitory effects on neurons (reducing the intensity of the incoming signal), while positive connection weights represent excitatory effects on neurons (increasing the intensity of the incoming signal). The relationship between the inputs and outputs is determined in two steps since there are first input-hidden layer connections and second hidden-output layer connections. Positive effects of input variables are depicted by positive input-hidden and positive hidden-output connection weights, or negative input-hidden and negative hidden-output connection weights. Negative effects of input variables are depicted by positive input-hidden and negative hidden-output connection weights, or negative input-hidden and positive hidden-output connection weights. Therefore, the multiplication of connection weight direction (i.e. positive or negative) delineates the effect each input variable has on the response variable. Interactions among predictor variables are identified as input variables with similar (i.e. same direction) or contrasting connection weights (i.e. opposite direction) entering the same hidden neuron. The total contribution of an input variable is calculated as the sum of the products of the input-hidden-output connection weights. Individual and interacting influences of nutrient concentrations and zooplankton biomass on predicted values of Chl *a* concentrations and PCI are interpreted when connection weights differed significantly from chance based on the randomization test (using α=0.05).

Concentrations of Chl *a* are positively correlated to P, and negatively correlated to NO_2/NO_3–N, and daphnid cladocera biomass through hidden neuron A (Figure 3a). Examining both hidden neurons it is evident that daphnid cladocera biomass positively interacts with available NO_2/NO_3–N, and negatively interacts

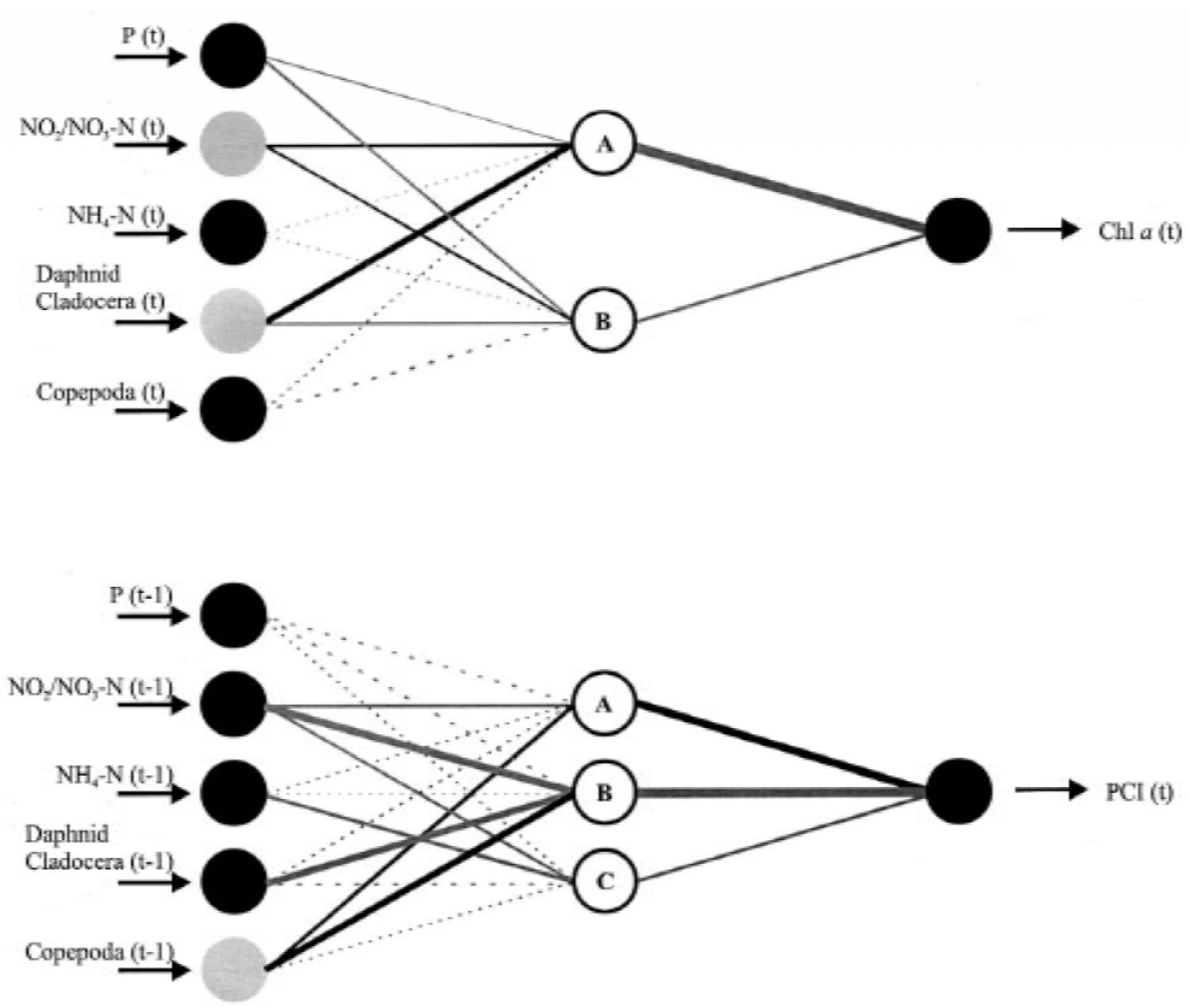

Figure 3. Neural interpretation diagram (NID) for predicting chlorophyll *a* concentration (A) and phytoplankton community index (B) as a function of nutrient and zooplankton variables. Best neural network design chosen from Table 2. The thickness of the lines joining neurons is proportional to the magnitude of the connection weight, and the shade of the line indicates the direction of the interaction between neurons; black connections are positive (excitator) and gray connections are negative (inhibitor). Solid lines represent connection weights statistically different from zero ($\alpha = 0.05$), whereas dashed lines represent non-significant connection weights. Black input neurons indicate variables that have an overall positive influence on the response variable, and gray input neurons indicate an overall negative influence on the response variable.

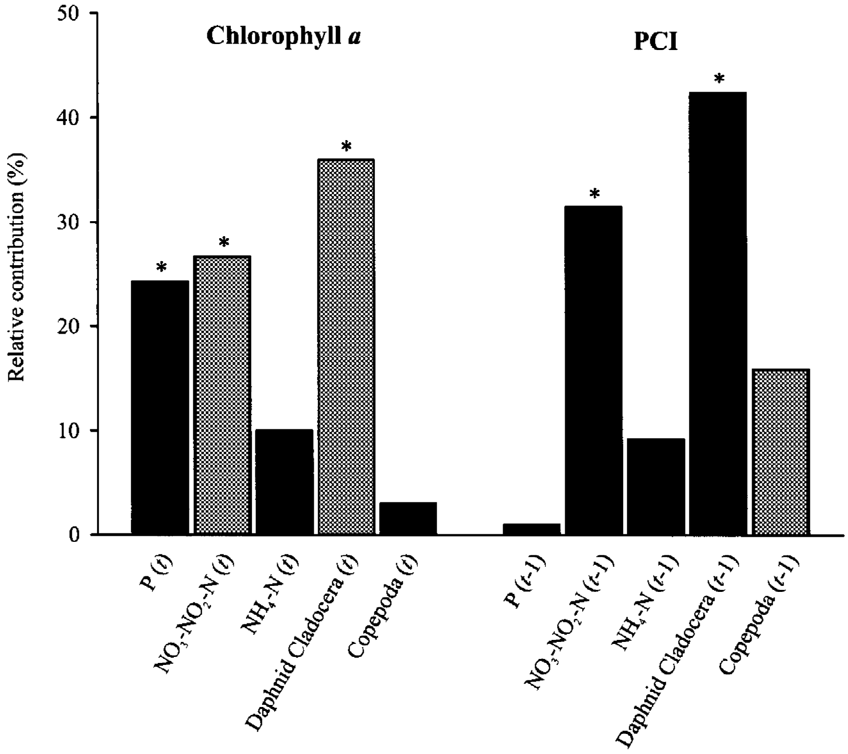

Figure 4. Relative importance (% of total contribution) of nutrient and zooplankton variables in predicting chlorophyll *a* concentration and phytoplankton community index (PCI) based on the sum of connection weights joining an input neuron and the output neuron. * notes sum of connection weights that statistically different from zero (α < 0.05).

with P. Figure 3a shows no evidence for variable interactions with copepoda biomass, since this input neuron does not exhibit significant connection weights with contrasting effects at any single hidden neuron with any of the other variables. Accounting for all connection weights between the input and output neurons, Chl *a* is positively correlated with P, NH_4–H and copepoda biomass, and negatively correlated with NO_2/NO_3–N and daphnid cladocera biomass (Figure 4). Based on the randomization test, P, NO_2/NO_3–N and daphnid cladocera biomass significantly contribute to predictions of Chl *a* concentrations.

PCI is positively correlated to NO_2/NO_3–N (hidden neurons B and C), NH_4–H (hidden neuron C) and daphnid cladocera biomass (hidden neuron B) (Figure 3b). Copepoda biomass illustrates contrasting correlations with PCI through hidden neurons A and B. Positive interactions between daphnid cladocera biomass and NO_2/NO_3–N (hidden neuron B), and negative interactions between copepoda and NO_2/NO_3–N (hidden neuron B) and P (hidden neuron A) are also evident. Interestingly, unlike Chl *a*, daphnid cladocera and copepoda biomass appear to exhibit an interacting effect on PCI through hidden neuron B. Accounting for all connection weights, P, NO_2/NO_3–N, NH_4–H and daphnid cladocera biomass are positively associated with patterns in PCI, whereas copepoda biomass shows a negative association (Figure 4). Based on the randomization test, NO_2/NO_3–N and daphnid cladocera biomass exhibited significant positive relationships with PCI.

Discussion

Duarte (1990) has suggested that lagged algal growth responses are a general phenomenon in aquatic ecosystems. Time lags are believed to result from a combination of factors, including small proportions of viable algal cells, biochemical adjustments prior to rapid growth, and the need to establish threshold concentrations of required nutrients (Duarte, 1990). Comparison of the neural network models indicate that patterns in chlorophyll *a* concentrations (a surrogate of algal standing crop and, therefore, hereafter referred to as phytoplankton biomass) did not exhibit time lagged responses to patterns in nutrient concentrations and zooplankton biomass; whereas lagged responses in phytoplankton community composition were evident. These results suggest that although responses of algal biomass to bottom-up and top-down forces may be instantaneous; changes in the composition of the phytoplankton community are delayed. Admittedly, caution should be employed when interpreting these results since the temporal resolution of data examined this study was perhaps not entirely fine enough to detect all time lags. For instance, lagged responses of phytoplankton biomass may occur at much shorter frequency than the biweekly frequency of sampling (e.g. Sephton & Harris, 1984; Harris & Trimbee, 1986; Matveev, 1995). Regardless, given the strong empirical basis for the existence of time lags, investigators should recognize the importance of accounting for time lags prior to testing for association between variables, especially when studying phytoplankton community dynamics.

Influence of nutrient concentrations on phytoplankton succession

In most pelagic ecosystems, the availability of phosphorous and nitrogen are recognized as important variables limiting the growth rate of phytoplankton. This study suggests than phosphorous and nitrite/nitrate concentrations influence temporal patterns in phytoplankton biomass, whereas nitrite/nitrate concentrations influence patterns in algal community composition. Increasing concentrations of nitrite/nitrate may differentially influence algal species, resulting a shift from inedible (i.e. cyanophytes) to edible algal types, as well as greater homogeneity in terms of class dominance in the community. Cyanophytes are capable of fixing nitrogen, and are presumed to have a greater advantage when nitrogen levels are low. When the availability of nitrogen increases, other phytoplankton classes might differentially benefit, thus resulting in a decrease of cyanophytes and an increase in other classes.

Influence of zooplankton on phytoplankton succession

This study shows that the potency of herbivorous grazing may vary depending on the species composition of the zooplankton community. A strong negative correlation between daphnid cladocera biomass and phytoplankton biomass was observed, whereas copepoda exhibited no such relationship. These findings suggests that direct grazing by mesozooplankters, i.e. daphnid cladoceran, are a stronger force shaping algal standing biomass compared to smaller species, i.e. copepods, potentially a result of greater feeding efficiencies (Brooks & Dobson, 1965; Hall et al., 1976; Lynch & Shapiro, 1981).

In addition to effects on total phytoplankton biomass, the zooplankton community may impact the phytoplankton community in a class-specific manner. Daphnid cladocera biomass was found to correspond to decreasing cyanophytes and increasing bacillariophytes, chrysophytes, cryptophytes and dinophytes, whereas the opposite relationship was observed for copepoda biomass. This suggests that members of the zooplankton community represent contrasting forces shaping the composition of the phytoplankton community. For instance, daphnid cladocerans may be selectively feeding on larger algal classes (i.e. cyanophytes) since they are less gape limited compared to copepods (Burns, 1968), which in contrast may be actively discriminating between phytoplankton, perhaps using chemical characteristics (Butler et al., 1989).

Phytoplankton also exhibit differential resistance to grazing resulting from morphological differences in cell size, shape and possession of hard coverings or gelatinous sheaths (Porter, 1976). Variation in algal ‘edibility’ cause zooplankton to consume particular algal classes more readily, which consequently plays an important role in shaping community composition. It is generally believed that zooplankton consume larger, less digestible algae at slower rates than smaller, more delicate forms (Porter, 1977b), thus favouring ‘edible’ (i.e. small, naked) over ‘inedible’ (i.e. large, gelatinous) forms of algae during periods of zooplankton grazing (Porter, 1973, 1977a; DeMott, 1983). For example, cyanophytes (blue-green algae) are recognized as being unsuitable and inedible food for crustacean plankton (Brooks & Dobson, 1965; Shapiro et al., 1975). The difficulty that zooplankton may have in breaking blue-green colonies, as well as the clogging of their filtering apparatus have been identified as two of the main causes of this phenomenon (Burns, 1968). However, numerous studies have produced contrasting and inconclusive results regarding the relationships between blue-green algae and herbivorous zooplankton (see De Bernardi & Guissani (1990) for review). My results showed that increasing daphnid cladoceran biomass corresponded with a shift in phytoplankton community dominated by large, gelatinous cells to small, non-gelatinous cells. This suggests that daphnid cladocerans illustrate selective grazing on larger, gelatinous size fractions of algae, as compared to smaller, naked size fractions, which agrees with more recent studies that show that high biomass of large grazing zooplankton does not always favour inedible fractions of algae (e.g., Sarnelle, 1992; Carpenter et al., 1996; Kasprzak & Lathrop, 1997).

In addition to the direct influence of zooplankton on phytoplankton succession, secondary effects of grazing have also shown be important (e.g. Sterner, 1986, 1989, 1990; Vanni, 1987; Carillo et al., 1995; Queimaliños et al., 1998; Wickham, 1998). For instance, elements released by zooplankton (i.e. metabolites, feces, food breakage at feeding) are in a soluble form which are easily and rapidly available for all algae in small, highly concentrated patches, as well as being available in high concentrations in the gut of these zooplankton (Sterner, 1990; Hessen & Anderson, 1992). Algae that survive the gut passage might lose some sheath material and cells to the grazers, but are more than compensated by gaining access to a nutrient rich environment where they can take up nutrients from both algal remains and from zooplankton metabolites. The fact that daphnid cladocera biomass was shown to positively interact with NO_2/NO_3–N and negatively interact with phosphorous concentrations indicates that some form of nutrient regeneration may exist. For instance, the bottom-up control of algal biomass may weaken with increasing daphnid cladocera biomass due to increased additions of NO_2/NO_3–N. In this case, daphnid cladoceran effects could follow an indirect pathway where patterns in phytoplankton biomass and community structure reflect class-specific responses to NO_2/NO_3–N enrichment. Interestingly, interactions between daphnid cladocera biomass and NH_4–H were not observed, which is somewhat surprising given that ammonium is rapidly dissolved and is considered the greater biologically available form of nitrogen (Lehman, 1980; Sterner, 1989). The lack of interaction between copepoda biomass and available nutrients supports the notion that nutrient regeneration is limited in copepoda since they enclose their fecal materials in membrane-covered pellets which quick sink out of the water column. This is compared to cladocerans that commonly release fecal matter in a soluble state (Lambert, 1978; Sterner, 1989). Moreover, this finding also supports the idea that the magnitude of nutrient regeneration by zooplankters is inversely related to body size (Burns, 1968).

Together, differential digestion and access to additional nutrients by phytoplankton may create a dynamic relationship between zooplankton nutrient regeneration (a function of zooplankton community composition) and phytoplankton species still present to utilize the available nutrients (a function of zooplankton grazing and phytoplankton susceptibility to grazing) (Porter, 1973, 1976, 1977b). For example, the net effect of increasing daphnid cladocera biomass on phytoplankton succession may in fact be a combination of increased grazing and increased nutrient regeneration, which could help explain the corresponding increase in homogeneity of phytoplankton dominance observed with increasing daphnid cladocera biomass.

Conclusions

Understanding the relative contributions of zooplankton herbivory and nutrient limitation on phytoplankton biomass and community composition and interpreting the myriad of pathways in which these factors operate will result in significant insights into the dynamics of ecological processes occurring in aquatic ecosystems. Knowledge regarding nutrient–phytoplankton–zooplankton relationships is not only important for understanding the dynamics of phytoplankton succession, but also for the study of zooplankton communities and aquatic management. For instance, recent experimental studies indicate that production and composition of particular zooplankton are influenced by the relative elemental contents of consumed food (Hessen, 1992; Urabe & Watanbe, 1992; Sommer, 1992; Sterner, 1993). Therefore, if zooplankton can affect changes in nutrient availability through fecal deposition of soluble nitrogen and phosphorous, such changes may in fact facilitate the growth of zooplankton indirectly through promoted growth of phytoplankton. Furthermore, an accurate understanding of the factors shaping phytoplankton communities and the specific responses of different algal classes to top-down forces is required by lake managers prior to the development of biomanipulation protocols for any particular the waterbody since water transparency is directly related to the type of algal classes present (Porter, 1977b; Berquist et al., 1985; Carpenter et al., 1987).

Although the mechanisms driving phytoplankton succession cannot be inferred from correlative studies, artificial neural networks provide a powerful tool for studying relationships between biotic and abiotic factors and the dynamics of phytoplankton communities under natural conditions. Neural networks have an important advantage over approaches such as regression or path analysis in that they can accommodate interactions among independent variables without any *a priori* specification. Thus neural networks are particularly beneficial in studies of phytoplankton succession given the array of possible variable interactions between nutrient concentrations and zooplankton biomass, and their subsequent effects on algal biomass and community structure.

Acknowledgements

I would like to thank Andy Gemza, Lake Specialist from the Ontario Ministry of Environment, Lake Partner Program for providing the Grenadier Pond data. I greatly appreciated discussions with Sovan Lek and his insightful comments regarding the finer details of neural networks. Furthermore, I would like to acknowledge Don Jackson, Bryan Neff, Leslie Warren, Ann Zimmerman and two anonymous reviewers for their comments on early versions of this manuscript. Funding was provided by a Natural Sciences and Engineering Research Council (NSERC) Graduate Scholarship to J. D. Olden and an NSERC Research grant to D. A. Jackson.

References

Bergquist, A. M., S. R. Carpenter & J. C. Latino, 1985. Shifts in phytoplankton size and structure and community composition during grazing by contrasting zooplankton assemblages. Limnol. Oceanogr. 30: 1037–1045.

Bishop, C. M., 1995. Neural Networks for Pattern Recognition. Clarendon Press, Oxford.

Brooks, J. H. & S. I. Dodson, 1965. Predation, body size and composition of plankton. Science 150: 28–35.

Burns, C. V., 1968. Direct observation of mechanisms regulating feeding behaviour of Daphnia in lake water. Int. Rev. ges. Hydrobiol. 53: 83–100.

Carpenter, S. R., J. F. Kitchell, J. R. Hodgson, P. A. Cochran, J. J. Elser, M. M. Elser, D. M. Lodge & X. He, 1987. Regulation of lake primary production by food web structure. Ecology 68: 1863–1876.

Carpenter, S. R., J. F. Kitchell, K. L. Cottingham, D. E. Schindler, D. L. Christensen, D. M. Post & N. Voichick, 1996. Chlorophyll variability, nutrient input and grazing: evidence from whole-lake experiments. Ecology 77: 725–735.

Carrillo, P., I. Reche, P. Sánchez Castillo & L. Cruz-Pizarro, 1995. Direct and indirect effects of grazing on the phytoplankton seasonal succession in an oligotrophic lake. J. Plankton Res. 17: 1363–1379.

Colasanti, R. L., 1991. Discussions of the possible use of neural network algorithms in ecological modelling. Binary 3: 13–15.

De Bernardi, R. & G. Guissani, 1990. Are blue-green algae a suitable food for zooplankton? An overview. Hydrobiologia 200/201 (Dev. Hydrobiol. 61): 29–41.

DeMott, W. R., 1983. Seasonal succession in a natural Daphnia assemblage. Ecol. Monogr. 53: 321–340.

Duarte, C. M., 1990. Time lags in algal growth: generality, causes and consequences. J. Plankton Res. 12: 873–883.

Edwards, M. & D. R. Morse, 1995. The potential for computer-aided identification in biodiversity research. TREE 10: 153–158.

Gauch, H. G., 1982. Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge.

Hall, D. J., S. T. Threlkeld, C. W. Burns & P. H. Crowley, 1976. The size-efficiency hypothesis and the size structure of zooplankton communities. Ann. Rev. Ecol. Syst. 7: 177–208.

Hansen, A. M., F. O. Anderson & H. S. Jensen, 1997. Seasonal patterns in nutrient limitation and grazing control of the phytoplankton community in a non-stratified lake. Freshwat. Biol. 37: 523–534.

Harris, G. P. & A. M. Trimbee, 1986. Phytoplankton population dynamics of a small reservoir: physical/biological coupling and the time scales of community change. J. Plankton Res. 8: 1011–1025.

Hessen, D. O., 1992. Nutrient element limitation of zooplankton production. Am. Nat. 140: 799–814.

Hessen, D. O. & T. Andersen, 1992. The algae–grazer interface: feedback mechanisms linked to elemental ratios and nutrient cycling. Archiv für Hydrobiologie, Ergebnisse der Limnologie 35: 111–120.

Hornick, K., M. Stinchcombe & H. White, 1989. Multilayer feedforward networks are universal approximators. Neural Networks 2: 359–366.

Jackson, D. A., 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. Ecology 74: 2204–2214.

Kasprzak, P. H. & R. C. Lathrop, 1997. Influence of two Daphnia species on summer phytoplankton assemblages from eutrophic lakes. J. Plankton Res. 19: 1025–1044.

Kurková, V., 1992. Kolmogorov's theorem and multilayer neural networks. Neural Networks 5: 501–506.

Lambert, W., 1978. Release of dissolved organic matter by grazing zooplankton. Limnol. Oceanogr. 23: 831–834.

Lambert, W., W. Fleckner, W. Rai & B. E. Taylor, 1986. Phytoplankton control by grazing zooplankton: a study on the spring clear-water phase. Limnol. Oceanogr. 31: 478–490.

Lek, S., M. Delacoste, P. Baran, I. Dimopoulos, J. Lauga & S. Aulagnier, 1996. Application of neural networks to modelling nonlinear relationships in ecology. Ecol. Model. 90: 39–52.

Lek, S. & J. F. Guégan, 1999. Artificial neural networks as a tool in ecological modelling, an introduction. Ecol. Model. 120: 65–73.

Lynch, M. & J. Shapiro, 1981 Predation, enrichment and phytoplankton community structure. Limnol. Oceanogr. 24: 243–252.

Matveev, V., 1995. The dynamics and relative strength of bottom-up vs. top-down impacts in a community of subtropical lake plankton. Oikos 73: 104–108.

Olden, J. D., 2000. Predictive models for freshwater fish community composition. MSc. Thesis. University of Toronto, Toronto, Canada.

Olden, J. D. & D. A. Jackson, 2000. Illuminating the 'black box': a randomization approach for understanding variable contributions in artificial neural networks. Ecol. Model. (submitted).

Özesmi, S. L. & U. Özesmi, 1999. An artificial neural network approach to spatial habitat modelling with interspecific interaction. Ecol. Model. 116: 15–31.

Porter, K. G., 1973. Selective grazing and differential digestion of algae by zooplankton. Nature 244: 179–180.

Porter, K. G., 1976. Enhancement of algal growth and productivity by grazing zooplankton. Science 192: 1332–1334.

Porter, K. G., 1977a. The plant–animal interface in freshwater ecosystems. Am. Sci. 65: 159–179.

Porter, K. G., 1977b. Enhancement of algal growth and productivity by grazing zooplankton. Science 192: 1332–1334.

Queimaliños, C. P., B. E. Modenutti & E. G. Balseiro, 1998. Phytoplankton responses to experimental enhancement of grazing pressure and nutrient recycling in a small Andean lake. Freshwat. Biol. 40: 41–49.

Reynolds, C. S., 1984. The Ecology of Freshwater Phytoplankton. Cambridge University Press, London.

Rumelhart, D. E., G. E. Hinton & R. J. Williams, 1986. Learning representations by back-propagation errors. Nature 323: 533–536.

Sarnelle, O., 1992. Nutrient enrichment and grazer effects on phytoplankton in lakes. Ecology 73: 551–560.

Sephton, D. H. & G. P. Harris, 1984. Physical variability and phytoplankton communities: VI. Day to day changes in primary productivity and species abundance. Arch. Hydrobiol. 102: 155–175.

Shapiro, J., Lamarra, V. & M. Lynch, 1975. Biomanipulation: an ecosystem approach to lake restoration. In Brezonic, P. L & J. L. Fox (eds), Proc. Symp. on Water Quality Management through Biological Control, January 23-30, 1975. Gainesville, University of Florida: 85–96.

Sommer, U., 1988. Phytoplankton succession in microcosm experiments under simultaneous grazing pressure and resource limitation. Limnol. Oceanogr. 33: 1037–1054.

Sommer, U., 1989. The role of competition for resources in phytoplankton succession. In Sommer, U. (ed.), Plankton Ecology: Succession in Plankton Communities. Springer-Verlag, Berlin: 57–106.

Sommer, U., 1992. Phosphorous-limited Daphnia: Intraspecific facilitation instead of competition. Limnol. Oceanogr. 37: 966–973.

Sommer, U., Z. M. Gliwicz, W. Lambert & A. Duncan, 1986. The PEG-model of seasonal succession of plankton in fresh waters. Arch. Hydrobiol. 106: 433–471.

Sterner, R. W., 1986. Herbivores' direct and indirect effects on algal populations. Science 231: 605–606.

Sterner, R. W., 1989. The role of grazers in phytoplankton succession In Sommer, U. (ed.), Plankton Ecology: Succession in Plankton Communities. Springer-Verlag, Berlin: 109-172.

Sterner, R. W., 1990. The ratio of nitrogen to phosphorous resupplied by herbivores: zooplankton and the algal competitive arena. Am. Nat. 136: 209–229.

Sterner, R. W., 1993. Daphnia growth on varying quality of Scenedesmus: mineral limitation of zooplankton. Ecology 74: 2351–2360.

Urabe, J., 1993. N and P cycling by grazers' activities: food quality and nutrient release by zooplankton. Ecology 74: 2337–2350.

Urabe, J. & Y. Watanabe, 1992. Possibility of N- or P-limitation for planktonic cladocerans: an experimental test. Limnol. Oceanogr. 37: 244–251.

Urabe, J., M. Nakanishi & K. Kawabata, 1995. Contribution of metazoan plankton to the cycling of N and P in Lake Biwa. Limnol. Oceanogr. 40: 232–241.

Vanni, M. J., 1987. Effects of nutrients and zooplankton size on the structure of a phytoplankton community. Ecology 68: 624–635.

Vanni, M. J. & J. Temte, 1990. Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. Limnol. Oceanogr. 35: 697–709.

Wetzel, R. G., 1983. Limnology. 2nd edn. Saunders College Publishing, New York.

Wickham, S. A., 1998. The direct and indirect impacts of *Daphnia* and *Cyclops* on a freshwater microbial food web. J. Plankton. Res. 20: 739–755.