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Detecting Stress at the Whole-Ecosystem Level: The Case of a Mountain Lake (Lake Santo, Italy)

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ABSTRACT

Detecting the early signs of stress is imperative for the conservation of natural ecosystems. They may, however, go unrecognized because ecosystems, when disturbed, may act as sinks that absorb the external impact without showing any evident changes. This seems to be the case for Lake Santo, a small water body located in a mountainous area of northern Italy. Tourism activity in this area began to develop in the early 1970s and grew continuously over the following 20 years. This activity caused a continually increasing nutrient load into the waters, but surprisingly the lake has remained oligo-mesotrophic, as it was before human pressure became a stressor to the lake. To anticipate possible severe damage to the ecosystem, we searched for early signs of stress by carrying out a retrospective analysis based on a whole-ecosystem approach using trophic flow networks. Ecosystem properties of the lake as calculated from network analysis for the disturbed (year 1991) and unimpacted (year 1973) configurations were compared, with the support of sensitivity analysis and statistical tests. We found evidence that in the period 1970–90 nutrient enrichment did change the course of normal development as the observed increase in system throughput was accompanied by a drop in the level of mutual organization of flows, which instead would be expected to increase during the natural progression of the ecosystem. The scenario that emerged from the comparison of system-level indices, cycling activity, trophic structure, and trophic efficiency indicates that the ecosystem has been subjected to stress. In particular, the type of disturbance corresponds to a quantitative definition of eutrophication.

Key words: ecosystem development; lake ecosystem; network analysis; nutrient cycling; stressed ecosystem; system-level trends; trophic analysis.

INTRODUCTION

Ecosystems are experiencing increased levels of stress due to climatic shifts (He and others 1999; Dale and others 2001), invasions of exotic species (Williamson 1999; Simberloff 2000), air pollution (Likens and others 1996; Walker 1999), land-use changes (Foster and others 1998; Fuller and others 1998), and many other causes. Moreover, in a world of ever more pervasive anthropogenic impacts, more serious ecological consequences are expected from compounded perturbations (Mack and D'Antonio 1998; Paine and others 1998). Stress may be acute or chronic (Odum 1985; Freedman 1995). In the former case, the effects are immediate and, when the mechanisms that maintain ecosystem resilience are not disrupted, recovery to the preceding unstressed state quickly follows. In the

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latter case, a prolonged pressure acts on the ecosystem, which may absorb the increased stress without showing any evident changes. In both cases, ecosystem breakdown may occur if some threshold of tolerance or resistance is exceeded (Holling 1973; Carpenter and Cottingham 1997).

Because the appearance of ecosystem dysfunction often reflects an advanced stage of stress, detecting the early signs of degradation is one of the greatest challenges that scientists and policy makers have to face (Rapport and others 1985; Schindler 1987). Early-warning detection is possible through long-term monitoring (Jope 2001) or by reconstructing an ecosystem's history (Whillans 1979; Hildén and Rapport 1993). The latter approach is used in this study, which focuses on a small mountain lake, Lake Santo, located in the northern Apennines region of Italy.

Lake Santo is an oligo-mesotrophic water body. Its trophic state has not changed since the 1970s, when an intensive campaign (from 1971 to 1975) (Ferrari and others 1975, 1977) enabled a preliminary characterization of the lake's limnology and ecology. In the following 3 decades, the area became a popular recreational site; tourism presence on the lake shore, being particularly intense during the summer months, brought about an increased nutrient load (Ferrari and others 1992). After 30 years of this continuous pressure, the lake has surprisingly maintained a good trophic condition, although this situation raises particular concern. In fact, water bodies that receive nutrient inputs for an extended period of time may suddenly shift to an irreversible eutrophic state (Jeppesen and others 1991). At some point, recovery becomes impossible, even by severe nutrient reduction, because of the breakdown of internal resilience mechanisms (Larsen and others 1979; Carpenter and Cottingham 1997; Carpenter and others 1999).

The conditions at Lake Santo are such that severe damage can still be avoided, but early signs of degradation must be searched for to convince the local authorities that action must be taken to prevent irreversible damage. Long-term research would be an appropriate means to this end, but, for several reasons, no intensive campaign has been conducted on the lake in recent years. A limited number of parameters, such as temperature, dissolved oxygen, dissolved inorganic nitrogen, and chlorophyll *a*, as well as the zooplankton communities, have been monitored but not systematically (G. Rossetti, unpublished); therefore, a complete database that could be used to delineate the present status of the ecosystem is not yet available.

Lake Santo was the subject of two intensive ecological campaigns, the first from 1971 to 1975 (Ferrari 1976; Ferrari and others 1977) and the second from 1990 to 1993 (Paris 1993; Paris and others 1993); during these cycles of investigation, a vast array of data were collected on the lake's limnology and ecology. We used these data to conduct a retrospective investigation of the lake under the hypothesis that the stressor has acted long enough to produce some modification.

According to the pertinent literature, there are several properties of ecological systems that change when the system is under stress (Odum 1985; Rapport and others 1985; Schindler 1990; Rapport and Whitford 1999), and these properties can be used to detect symptoms of the distress syndrome. The database at our disposal suggested that we focus on only a few attributes—namely, trophic efficiency, recycling function, and overall system activity, the latter being measured by ecosystem size and level of organization. Ulanowicz (1996) suggested that these are important features one should look at to unveil the effects of continuous acting stress.

To study the effect of continuous nutrient loading on the selected features, the Lake Santo ecosystem was portrayed as a suite of ecological flow networks (Patten 1985; Ulanowicz 1986; Higashi and Burns 1991) that described the lake in the two periods of investigation. These models were analyzed by techniques that are commonly referred to as ''network analysis'' (Ulanowicz 1986).

STUDY AREA

Lake Santo (10°00′38″ E, 44°24′06″N, 1507 m a.s.l) is a small dimictic lake of glacial origin located in the northern Apennines (province of Parma, Italy). The lake surface extends over about 8 ha, the average depth is 11.3 m, and the maximum depth is 22.5 m. The ratio between watershed and lake area is 11:3. The period of ice cover usually lasts from November to May. Although it is classified as a small lake, Lake Santo is the deepest and largest natural water body in the northern Apennines district. Its size is sufficient to guarantee stability with respect to meteorological events, whereas the biological community of other lakes in the same area, which are smaller in size, undergo significant changes from year to year (Rossetti 1994) due to climatic events. The capability to buffer external changes is witnessed by the persistence of the zooplankton biocoenosis; it has not changed in the last 50 years, and the seasonal succession is characterized by constant, definite patterns (Rossetti

1994; G. Rossetti, unpublished). In its natural states the lake is fishless, but it has undergone regular fish stocking since the beginning of the 20th century (Brian 1924). This fish stocking seems not to have affected the trophic state of the lake, which appeared to be oligo-mesotrophic in the early 1950s and so remained into the early 1990s. The zooplankton community shows stable seasonal patterns of abundance (Rossetti 1994; G. Rossetti, unpublished).

During summer thermal stratification, maximum surface temperature may reach 19–20°C; whereas in winter, when the lake is ice-covered, water temperature drops to lower values that range from 0°C to 4°C, from surface to bottom, respectively (Ferrari and others 1975). Because of its acidic watershed, ion concentrations remain below 1.0 meq L^{-1}, while conductivity lies between 20 and 50 $\mu S\ cm^{-1}$. The lake's buffering capacity is weak because its alkalinity is usually below the critical value (0.2 meq L^{-1}) for acidification risk (Viaroli and others 1994). Low temperature and reduced solar radiation have limited primary production (monthly weighted average chlorophyll *a* always below 5 $\mu g\ L^{-1}$), and the lake has been classified as oligo-mesotrophic (Ferrari and others 1992; Viaroli and others 1994).

A rich zooplankton community characterizes the lake. Its trophic structure comprises three basic components: macrofilter feeders, such as *Daphnia longispina* (Cladocera) and *Eudiaptomus intermedius* (Copepoda); smaller grazers, such as *Bosmina longirostris* (Cladocera), *Kellicottia longispina*, *Keratella cochlearis, Conochilus* sp., and *Synchaeta* sp. (Rotifera); and polyphagous species with predatory habits, such as *Asplanchna priodonta* and *Ploesoma hudsoni* (Rotifera) (Rossetti 1994).

As many as 27 taxa of algae have been identified (Paris 1993). Chlorophyceae, with its 12 taxa, is the most represented family, followed by Chrysophyceae (ten taxa), Diatomeae (six taxa), Cryptophyceae (four taxa), Dynophyceae (three taxa), and Cyanophyceae (one taxon). The main zoobenthos species are *Tubifex tubifex* (Tubificidae), *Parocaldius* sp., *Tanytarsus* gr. *gregarius*, *Zavrelimyia* sp. (Chyronomidae), *Betracobdella paludosa* (Hirudinea), *Pisidium* sp. (Bivalvia), *Gammarus lacustris* (Amphipoda), *Caenis horaria* (Ephemeroptera), and *Sialis lutaria* (Megaloptera) (Ferrari and Bellavere 1976). Fish species are represented by *Salmo trutta fario*, and *Oncorhynchus mykiss*. A small population of *Salvelinus alpinus* is also present in the lake, having been established after an introduction of 1,000 individuals in 1949. However, this population never adapted to conditions in Lake Santo and the initial stock rapidly declined, so that by the time of the first ecological campaign (1971) only a few individual persisted and most of the individuals that were caught showed signs of malformation and other pathologies (Maldini and others 2004).

Methods

Parsing the Networks and Estimation Techniques

To describe the lake's status, we deemed the years 1973 and 1991 to be representative, respectively, of the nonimpacted and impacted situation. For those years, the data set at our disposal was the most complete. Each year was divided into three seasons. The timing of the seasons was established according to events in the lake rather than by adhering strictly to the calendar. Thus, the winter season coincides with the period of ice cover. The phase of open water (May–October) is characterized by two complete mixing events that take place at the beginning (May) and toward the end (September) of the open-water season. When complete mixing occurs, production peaks, affecting the entire biological community (Paris 1993; Paris and others 1993; Rossetti 1994). The importance of these events for the seasonal succession of zooplankton and phytoplankton community led us to build two distinct networks: one for the period May–July and the other for the period August–October.

The overall approach was first to assemble, for each year, the three seasonal steady-state networks of carbon flux, capturing as much detail in trophic structure as possible from empirical measurements. Data collected in the sampling campaigns enabled us to build a detailed network, down to species level, for the zooplankton community. Although the same level of detail could be applied to phytoplankton as well, primary producer compartments appear as taxonomic families instead of single species because it was impossible to ascertain the contribution of every single species to higher trophic levels.

Standing stocks of species or guilds were different for the two periods and from one season to the next in the same year. This variation yielded different flow values in the networks. The way we estimated standing stocks and flow values is described in detail in the online supplementary material (http://www.springerlink.com). The only qualitative difference between the two periods concerns the rotifer species *Ascomorpha ecaudis*. Although it was absent in the 1970s, it subsequently appeared in the lake and was detected during the second limnological campaign.

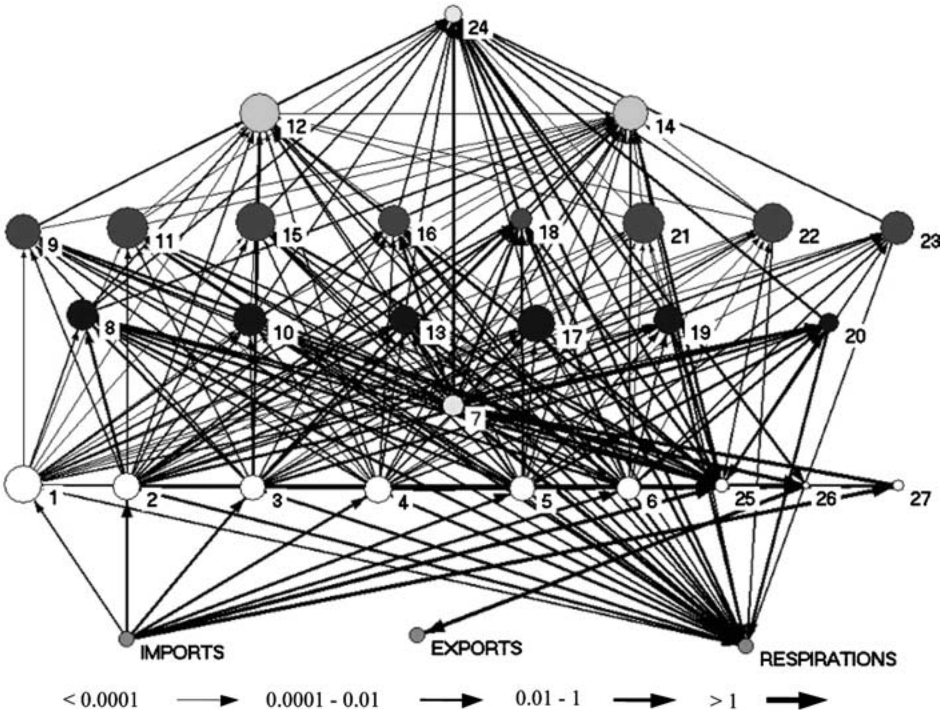


Figure 1. The 1991 summer network for Lake Santo. Each node's size is proportional to the logarithm of that compartment's standing stock. Arrow thickness is proportional to flow magnitude, as specified in the key below the graph. Values are gC m^{-2} y^{-1}. Keys for compartments are as follows: 1. Flagellatae, 2. Chlorophyceae, 3. Crysophyceae, 4. Dynophyceae, 5. Cryptophyceae, 6. Diatomeae-Cyanophyceae, 7. Living particulate organic mattrer, 8. *Keratella* gr. *quadrata*, 9. *Keratella cochlearis*; 10. *Kellicottia longispina*, 11. *Ascomorpha ecaudis*, 12. *Synchaeta* sp. + *Ploesoma hudsoni*, 13. *Polyarthra* spp. 14 *Asplanchna priodonta*, 15. *Filina* gr. *longiseta terminalis*, 16. *Conochilus* sp., 17. Other rotifera, 18. *Daphnia longispina*, 19. *Bosmina logirostris*, 20. *Eudiaptomus intermedius*, 21. Nauplii, 22. Copepodites, 23. Other copepoda, 24. Fish, 25. Water particulate organic carbon, 26. Benthic particulate organic carbon, 27.Dissolved organic carbon.

One major question involved how to treat functional groups, for which data at the level of individual species were not available. Heterotrophic microorganisms (bacterioplankton, protozoa) were assigned to a unique compartment, living particulate organic matter (living POC); the same was done for fish species. Although benthic fauna comprised several species, their contribution to benthic carbon was negligible in comparison with the amount of organic material that appeared as detritus. Therefore, we found it more appropriate to have only one benthic component, benthic particulate organic matter (BPOC), comprehensive of phytobenthos (Ferrari and Villani 1978). Finally, dissolved organic carbon (DOC) and water particulate organic carbon (WPOC) were added as other non living nodes.

Considering that trophic aggregation has been widely applied in ecosystem ecology (Johnson and others 1995; Hart and others 2000), parsing the network in this way seemed an appropriate way to encompass the essential features of ecosystem organization while keeping model complexity within the limits of methodological tractability. The final graphs consisted of a total of 27 (26 for 1973) separate components (see Figure 1).

Sampling activity provided data about phytoplankton taxa (biovolume) and zooplankton species (number of individuals per unit volume); information on fish abundance was gathered from local organizations that monitor recreational fishing. All these data were used to calculate standing stocks and quantify carbonaceous flows as usually done in network analysis (Johnson and others 1995; Wilson and Parkes 1998; Hart and others 2000). Details about estimation techniques are in the online supplemental material (http://www.springerlink.com). In what follows we provide only quick thumbnail sketches of the basic ideas behind the procedure.

The biomass of zooplankton species was calculated with reasonable precision from the number of individuals. Because the standard units in network analysis are grams of carbon per square meter, the available data had to be transformed to obtain dimensional consistency. To this end, information on the average dry weight (in grams) of animals and the percentage of carbon per gram of dry weight was gathered from technical manuals or from the literature. These parameters enable conversion from the number of individuals to the biomass in grams of carbon per square meter (See details in the online Supplementary material, http:www.springerlink.com). Data about fish species were treated in a similar way. For phytoplankton, available biovolume (cubic micrometers of algae cells per liter) was multiplied by the carbon content per unit volume (taken from technical manuals). The result was further multiplied by lake depth, and corrected by Secchi disk data (we considered only the productive part of the water column) to obtain phytoplankton biomass in the appropriate units.

Living POC comprises bacteria, ciliates, and flagellates. The logarithmic equations that relate bacterial biomass with chlorophyll *a* measures (Jorgensen and others 1991) were used to calculate bacterial standing stock. This relation yielded values that we translated into grams of carbon using the carbon content per cell. To estimate biomass for flagellates and ciliates, we used the biomass ratios bacteria/flagellate and bacteria/ciliate. The carbon content per unit weight typical of these two groups, as derived from the technical literature, yielded the final values in the appropriate units.

Laboratory determination on lake samples yielded the POC and DOC standing stocks. From POC we subtracted living POC, phytoplankton, and zooplankton standing stocks to obtain the amount of carbon in WPOC. Measures of organic matter performed on sediment samples (Ash Free Dry Weight (AFDW), ignition at 550°C in a muffle furnace) were necessary to quantify the carbon content in the sediment, used as an estimate of BPOC standing stock.

Once the biomass had been approximated, the quantitative characterization of the network required that carbon budgets be constructed for every compartment during each season, according to the general equation $C = P + R + E$. Consumption or total intake (C) is partitioned into production (P), respiration (R), and egestion (E), the latter being the portion of nonassimilated material that is released back into the environment and that, for primary producers, is commonly referred to as ''excretion'' (PujoPay and others 1997; Aota and Nakajima 2001). Information was sought on the consumption rate per unit of biomass per year for each species. For each compartment, we multiplied this factor by the biomass to obtain its total intake. Next, we apportioned this intake among the various prey items using dietary proportions. For many compartments, instead of these proportions, only a list of prey species was available. To set the magnitude of nutritional flows, we had no other option but to apportion the total input to the prey in proportion of the standing stocks of these prey. In this procedure, we did not take into account dietary differences that characterize different life stages of certain species, but considered the main dietary habits documented in various reports in the literature.

We then apportioned the compartmental throughput among the output processes. Respiration and excretion (egestion) rates per unit biomass were available from the literature for most species, so these outputs could be immediately established. Most of the losses to predation were estimated from the predator (input) side, as described above. Finally, the assumption that the networks balance over each season facilitated the estimation of several flows. However, uncertainties inherent in all these partially independent estimations kept many of the compartments from balancing exactly. The degree of imbalance was investigated by entering the existing flow estimates into a spreadsheet format. In particular, all the compartments could be balanced to within a few percent using literature values, and final balance was achieved using the program NET BALANCE (Allesina and Bondavalli 2003), which assumes linear, donor control.

To account for temperature variations (see Study Area) while parsing the networks, we corrected for temperature effects on the carbon content per unit weight of organisms and metabolic parameters. This information is detailed in the online supplemental material (http://www.springerlink.com).

Network Analysis

The systematic analysis of the ecosystem flow networks is comprised of several techniques. Trophic analysis, cycling analysis, and system-level index calculation were performed using WAND (Allesina and Bondavalli 2004), a Windows-compatible version of the original NETWRK program (Ulanowicz and Kay 1991).

Trophic analysis yields the effective trophic level occupied by each component in the ecosystem and employs input–output techniques to interpret the network in terms of a linear trophic chain (Levine 1980; Ulanowicz 1995). The procedure starts with a matrix of diet coefficients, [G], whose elements, g_{ij}, each designate the ratio between the flow from i to j and the sum of all direct flows to j. In other words, it is the fraction of the total input to compartment *j* that comes directly from *i*. A convenient property of [G] is that its algebraic powers provide quantitative information about indirect transfers in the system. For example, when we multiply the feeding coefficient matrix by itself, the result, $[G]^2$, is a matrix wherein the *ij*th component represents the fraction of the whole diet of *j* that comes from *i* over all pathways of exactly two trophic steps. Similarly, it must be shown by mathematical induction that the *ij*th entry of $[G]^m$ measures the fraction of total input to *j* that arrives from *i* flowing over all pathways of length *m*. Because the coefficients of [G] are less than one in magnitude, the sum of all the powers of this matrix converges toward $[I–G]^{-1}$, which is called the ''structure matrix'' (Leontief 1951). Levine (1980) pointed out that the *j*th column of this matrix describes how compartment *j* ultimately depends on all the other compartments of the system, so the sum of the *j*th column provides the average trophic position of species *j*.

To reduce a complex web to a linear trophic chain so that we can calculate trophic efficiency, we need to apportion each compartment's activity among a series of hypothetical integer trophic levels (Ulanowicz and Kemp 1979). If, for example, the [G] matrix is multiplied on the left by a row vector whose *j*th element represents the fraction of the total input to compartment *j* that comes from outside the system (the degree to which this compartment acts as a primary producer), the result is a row vector whose *j*th element represents the fraction of the total input of *j* that arrives after a single step; it measures the degree to which that compartment is acting as a primary consumer (herbivore). Multiplying this row vector successively by the integer powers of [G] yields a succession of row vectors, the *m*th of which estimates the activity of each compartment at trophic level *m*.

Cycling analysis considers the overall amount of currency involved in cycling, but it is also concerned with how the cycling is organized within the ecosystem. Finn (1976) used input–output analysis to calculate the fraction of whole-system activity that comprises recycling, and Ulanowicz (1983) devised a procedure to extract cycles from complex networks. In summary, by normalizing the matrix of direct transfers according to the total output of the compartment from which it originates, we obtain the matrix [F], a matrix whose *ij*th element represents the fraction of the throughput of *i* that flows directly to *j*. The sum of all the integer powers of matrix [F] gives the output structure matrix, $S = [I - F]^{-1}$. Any diagonal entry of this matrix, which exceeds unity, implies that the designated compartment engages in cycling. If we designate as S_{ii} the diagonal element of the output structure matrix, the fraction of the throughput of *i* devoted to cycling becomes $(S_{ii} - 1)/S_{ii}$. Multiplying each fraction by its corresponding throughput and summing over all components yields the Finn cycling index (FCI) — the portion of the total system throughput T that is attributable to cycling: $Tc = \{Ti \ (S_{ii} - 1)/S_{ii}\}$. An updated synopsis on cycling in trophic flow networks and a correction for Finn's calculations can be found in Allesina and Ulanowicz (2004).

System-level indices are used to quantify ecosystem growth and development (Ulanowicz 1986). Their calculation is based on information theoretical analysis (Hirata and Ulanowicz 1984). Ecosystem activity is measured by the total system throughput (TST), which is the total amount of medium flowing through the network. It is calculated by summing all flows in the network. In a network of exchanges, many configurations are compatible with the same activity level. More constrained topologies are those in which a restricted number of flows exist, so that the medium is forced to move along a few pathways. This occurs when compartments are more functionally specialized in the system. The average mutual information (AMI) measures this degree of specialization, or the amount of constraints on the medium. More constrained networks would take shape during ecosystem development, because positive feedback would select those pathways that are more efficient in passing the medium, pruning away less efficient connections. There is, however, an upper limit to development that is measured by an index called development capacity (C). It quantifies the entire potential for development of an ecosystem, and it depends on the amount of medium available and the number of components that share it.

A single measure of activity and organization is the product of TST and AMI; this index is called ascendency (A) and quantifies the fraction of the total activity that has been converted into organized complexity. What remains once A is subtracted from C is called system overhead, it is the fraction of development capacity that has not yet been organized. This residual disorder is made up of four different components. The first is the overhead on import (OI), which is higher when inputs are many and evenly distributed. It measures system inefficiency at getting medium from the outside environment. The second component is the overhead on export (OE), which quantifies the amount of medium that exits the system in a usable form; it is higher when exports are many and evenly distributed. The overhead on dissipation (OD) relates to the fraction of medium that is lost by internal processes (that is, metabolism in living systems). The fourth component of overhead is pathway redundancy (R) within the system. It is a contribution to disorder because sending medium over diverse routes costs more in terms of dissipation than channeling it all over a few efficient pathways. Overhead has conflicting interpretations. On the one hand, it represents the system's inefficiencies at processing material and energy; on the other hand it is ''strength-in-reserve'' of degrees of freedom that the system can call upon to adapt to a new threat (Ulanowicz 1997).

Sensitivity Analysis

Most of the flow values in the networks were calculated as a function of species' standing stocks. Because species' biomass in the lake varies from one season to another, this variability likely reflects ecosystem attributes as they are evaluated through network analysis. Thus, they may show variations from season to season within the same year and from year to year. In accordance, we thought that the variations in ecosystem properties that were observed between the 1970s and the 1990s networks should be tested for their significance, to ascertain whether they could be due to altered conditions in the lake were or simply due to interannual variability.

Because the two intensive ecological campaigns were not planned for whole-ecosystem studies, the data set they produced was not sufficient to build multiple-year networks for the two periods, and a complete suite of seasonal flow networks could be built for only 1 year in each period (1973 and 1991). This excluded the possibility of making a complete within- and between-periods comparison of the various indices. To overcome the problem of testing the observed differences in the ecosystem attributes, we performed a sensitivity analysis on flow matrices to obtain simulated distributions for some of the indices that quantify ecosystem activity. To this end, we imposed random variations in flow values in a range defined by observed changes in the standing stocks of the species.

In practice, we explored the data set produced by the two periods of investigation — from 1971 to 1975 and from 1990 to 1993 — in search of the species for which measures of abundance (biomass) were available for all years, season by season. We obtained this information for all zooplankton species and most of the phytoplankton groups. Next, for both periods, we considered the maximum observed variation, season by season, with respect to the standing stocks used to build the seasonal networks in 1973 and 1991. These magnitudes were used to define ranges of variability for the standing stocks of the species (reference value ± maximum observed variation, with the lower limit of this interval always greater than zero). Because standing stocks serve to estimate the magnitude of flows in the networks (see the preceding section, Parsing the Network and Estimation Techniques, and the online supplemental material at http://www.springerlink.com), intervals of variability of standing stocks as obtained from the data set were used to establish intervals of variation for the flow values.

This second step of the procedure required particular attention. In fact, for the same flow, we had two sources of variation: variability in the donor standing stock and variability in the consumer standing stock. The variation in the consumer biomass affects its overall intake, but apportioning this variation to the various prey items, following a procedure conceptually similar to the method we used to estimate the flows, was not easy. We also had variation in the prey items, and in almost all cases these prey items shared different consumers with their own variability. In this phase, we only had to estimate ranges of variation for flow coefficients, rather than calculating their precise values, so we considered an approach in which the range of variability for every flow was obtained as the sum of the percentage of variability due to variation in the donor standing stock and variation in the consumer standing stock. In this way, we could take into account all possible combinations of variability due to both the compartments involved in a given flow. However, by checking the database, we determined that no extreme situations characterized the patterns of variability. In other words, we never observed seasons in which, for any given donor–consumer couple, the two species changed their biomass in the opposite direction by an amount equal to the maximum observed variation in the whole period. In addition, the balancing procedure that places the networks in steady state would impose constraints on the variability of flows. We therefore decided that for every flow the interval of variability corresponded to the average variation between that imposed by the consumer and that imposed by the donor. In this way, we created two variability matrices for each period: one for the spring and one for the summer. Two of these matrices, one for the 1970s and the other for the 1990s, are presented in the online appendix (http://www.springerlink.com). As many as 1,000 networks were built for every season in both periods by changing the flow values randomly, according to the intervals of variability computed for each flow. The algorithm we used can be summarized by the following formula:

$$\mathrm{M}[i,j]' = \mathrm{M}[i,j] + (\mathrm{uniform}[-\mathrm{C},\mathrm{C}])\mathrm{M}[i,j]$$

in which M[*i,j*] is the original coefficient (flow value), and [−C,C] is its interval of variability (as a fraction of the original value), and uniform is a random-number generator that extracts values from an uniform distribution.

Network analysis was applied to the 1,000 different matrices created for each season in both periods and system-level indices were calculated. Because network analysis requires the system to be in steady- state, we balanced the matrices created for simulation using the Averaging input-output coefficients (AVG) algorithm proposed by Allesina and Bondavalli (2003). The distributions of the indices were tested for normality using the Shapiro–Wilks test and the Kolmogorov–Smirnov test (Royston 1982). Finally, the seasonal distributions of the indices were tested for a statistical difference between the two periods.

RESULTS

For both years (1973 and 1991), three seasonal networks were constructed. The qualitative structure of the networks is basically the same for all seasons in both periods. To give an idea of this structure, Figure 1 depicts the network for the 1991 summer season (August–October). Flow values associated with all six networks are in the online supplemental material (http://www.springerlink.com). Table 1 shows the values of system-level indices.

The 1973 seasonal networks showed correspondingly lower values for the TST than their 1991 counterparts. In both periods, summer and winter values of this index were the highest and the lowest, respectively. This increase in throughput from 1973 to 1991 was reflected in greater development capacity and ascendency for the ecosystem. On average, the AMI was higher in 1973 than in 1991 (1.44 and 1.37, respectively). This difference is due to summer and winter networks, whereas for the spring networks the AMI was slightly higher in 1991. In the 1973 networks, AMI increased from spring to winter; whereas the opposite trend characterized the 1991 networks.

Table 1. Seasonal and Yearly Average Values for System-Level Indices

	TST	Capacity	Ascendency	A/C %	AMI	OI	OI/C %	OE	OE/C %	DO	DO/C %	Red	Red/C %	FCI %
1973														
M–J	1.94 E+03	6.33 E+03	2.68 E+03	42	1.38	0.49 E+03	7.7	0.07 E+03	1.1	0.91 E+03	14.4	2.15 E+03	34.0	12.7
A–O	3.58 E+03	11.88 E+03	5.17 E+03	43	1.44	1.14 E+03	9.6	0.12 E+03	1.0	2.23 E+03	18.8	3.13 E+03	26.4	8.8
N–A	0.74 E+03	2.56 E+03	1.10 E+03	43	1.48	0.24 E+03	9.4	0.03 E+03	1.2	0.36 E+03	14.1	0.85 E+03	33.2	8.9
Avg.	2.09 E+03	6.93 E+03	2.98 E+03	42.7	1.44	0.63 E+03	9.1	0.07 E+03	1.1	1.17 E+03	16.8	2.04 E+03	29.4	10.2
1991														
M–J	4.32 E+03	14.27 E+03	6.00 E+03	42	1.39	1.39 E+03	9.7	0.18 E+03	1.3	2.73 E+10	19.1	3.74 E+03	26.2	11.3
A–O	5.19 E+03	16.96 E+03	7.17 E+03	42	1.38	1.89 E+03	11.1	0.17 E+03	1.0	2.99 E+10	17.3	4.66 E+03	27.5	7.7
N–A	1.04 E+03	3.09 E+03	1.41 E+03	45	1.36	0.24 E+03	7.8	0.04 E+03	1.4	0.43 E+10	13.9	0.94 E+03	30.4	1.8
Avg.	3.51 E+03	11.44 E+03	4.86 E+03	43	1.37	1.17 E+03	10.2	0.15 E+03	1.3	2.05 E+10	17.9	3.11 E+03	27.2	6.9

TST, total system throughput; OI, overhead on imports; OE, overhead on exports; AMI, average mutual information; A, ascendency; C, capacity; DO, dissipative overhead; Red, Redundancy; FCI, Finn cycling index.
Seasons are from May to July (M–J), from August to October (A–O), and from November to April (N–A).
All indices but FCI are given as absolute values and fraction (%) of the development capacity. Absolute values have the unit of carbon flow (gC m^{-2} y^{-1}) times ''bits'' of information.

Table 2. Number of Cycles of Different Length in the Different Networks

	Total Number of Cycles					
Cycle Length	Spring '73	Spring '91	Summer '73	Summer '91	Winter '73	Winter '91
2	18	18	18	19	18	18
3	50	52	50	54	50	52
4	60	65	60	66	60	65
5	34	38	34	38	34	38
6	8	9	8	9	8	9

The increase in TST was more than enough to compensate for the reduction in AMI, and ascendency—that is, the product of TST and AMI—increased from 1973 to 1991. As a percentage of the development capacity, ascendency remained more or less the same in the two years. The 1991 networks showed higher overhead values than the 1973 counterparts. Considering relative yearly averages of the indices we observed that the OI increased from 9% (1973) to 10.2% (1991); the OE accounted for 1.0% of the development capacity in 1973 and increased to 1.3% in 1991. Also, the dissipative overhead increased from 16.8% to 17.9% of the development capacity.

The cycling analysis yielded an FCI (Table 1), the number of active cycles (Table 2), and the total amount of currency associated with all the cycles (Figure 2).

The FCI was higher in 1973 than in 1991 for all seasons. On average, this difference is reasonably high (10.2 and 6.9%, respectively). A closer look at the cycling features reveals that for every season the overall number of cycles was higher in 1991 than in 1973 (Table 2). Figure 2 shows how the magnitude of cycling is distributed among cycles of different length; most cyclic activity is concentrated on two-, three-, four- step cycles. In all seasons, the amount of matter that circulated through longer cycles (five or six steps) decreased from 1973 to 1991. Overall, with the exception of the winter season, more carbon circulated along shorter cycles in 1991 than in 1973. Based on these observations, one would deduce that longer cycles decreased in importance with respect to shorter ones from 1973 to 1991. Further, Figure 2 shows that in winter the overall amount of carbon that recirculated in the lake was higher in 1973 (the dark line is always above the gray line in the chart). In that season, the increase in TST did not compensate for the reduced FCI.

The 1,000 flow matrices created by randomly changing flow coefficients for the two open-water seasons in both periods (two seasons, two periods, 4,000 matrices overall) were analyzed via network analysis, producing seasonal distributions for FCI and AMI (a total of eight distributions, four for FCI and four for AMI). Figures 3 and 4 shows the distributions obtained for the AMI and the FCI, respectively.

All the distributions were tested for normality using the Shapiro-Wilks and the Kolmogorov–Smirnov tests. They were significantly different from a Gaussian distribution. Table 3 summarizes the statistics of the distributions.

We then analyzed the FCI and AMI distributions generated for the same season to test whether populations would be significantly different between periods (70 vs. 90). The Student *t*-test was used for this purpose. Although the distributions that we obtained were significantly different from a Gaussian distribution, we applied the Student *t*-test because the variances were homogeneous, a more severe condition for this statistical test. Distributions for the FCI for the spring seasons were significantly different (avg. $\mathrm{FCI}_{70} = 0.117246$, avg. $\mathrm{FCI}_{90} = 0.113733$; $t = 24.7387$, $df = 1998$, $P < 2.2e^{-16}$). When we compared the distributions for this index in the summer seasons the difference was still significant (avg. $\mathrm{FCI}_{70} = 0.10500793$, avg. $\mathrm{FCI}_{90} = 0.076048$; $t = -6.7764$, $df = 1998$, $P = 8.081e^{-12}$). Also, the AMI distributions showed statistically different results for the two periods. The simulation predicts a higher level of organization of flows in the 1970s for the summer season (avg. $\mathrm{AMI}_{70} = 1.452247$, avg. $\mathrm{AMI}_{90} = 1.389966$; $t = 41.9941$, $df = 1998$, $P < 2.2e^{-16}$) whereas the distributions obtained for the spring the higher organization in 1990s (avg. $\mathrm{AMI}_{70} = 1.388998$, avg. $\mathrm{AMI}_{90} = 1.396583$; $t = -4.1182$, $df = 1998$, $P = 1.987e^{-05}$). Thus, a clear trend between periods did not appear, although, on average, AMI seemed to decrease from 1973 to 1991.

Table 4 and Figure 5 summarize the results of trophic analysis. In both years, all species but pri-

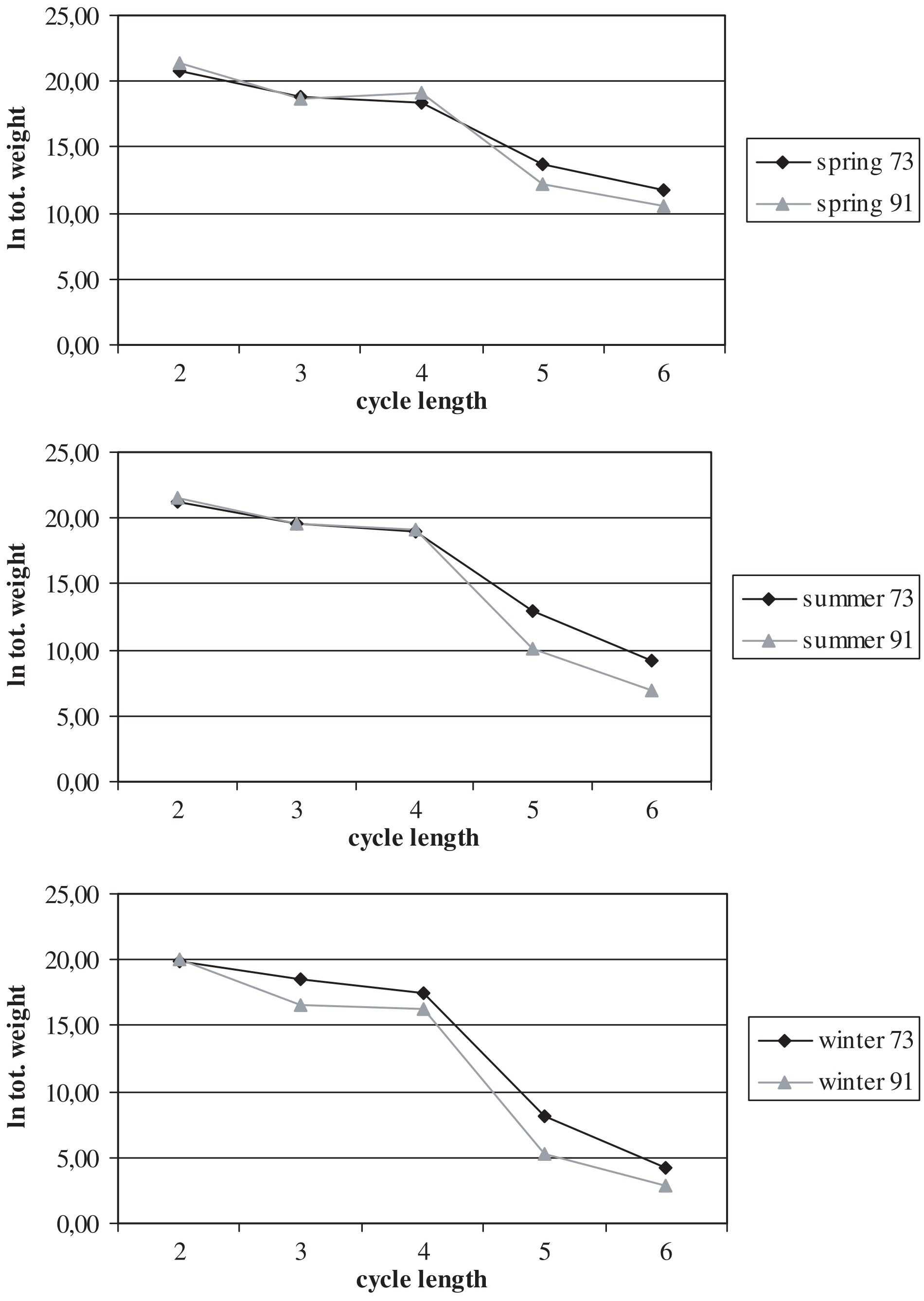


Figure 2. The overall recycling activity, expressed as natural logarithms, is distributed among cycles of different trophic length.

mary producers (compartments 1 – 6) were intermediate consumers between the second and third trophic level (Table 4). Species fed higher in 1973 than in 1991, and this pattern maintained a seasonal correspondence. Also, for every species, the average trophic position calculated over all seasons was higher in 1973 than in 1991. Within each year, species changed their trophic role from season to season. In 1991, all species but fish increased their trophic level from spring to winter. The 1973 network did not match this pattern, although all species but fish fed at their lowest levels during the period May–June.

Interpreting the networks according to the trophic concepts of (Lindeman 1942; Burns 1989) yielded the schemata presented in Figure 5. They translate the network of trophic transfers for each season of the two periods into linear food chains in which each box represents the tropic level as an abstract integer. These structures show the net amount of energy that each level receives from the preceding one, as well as the amount of energy

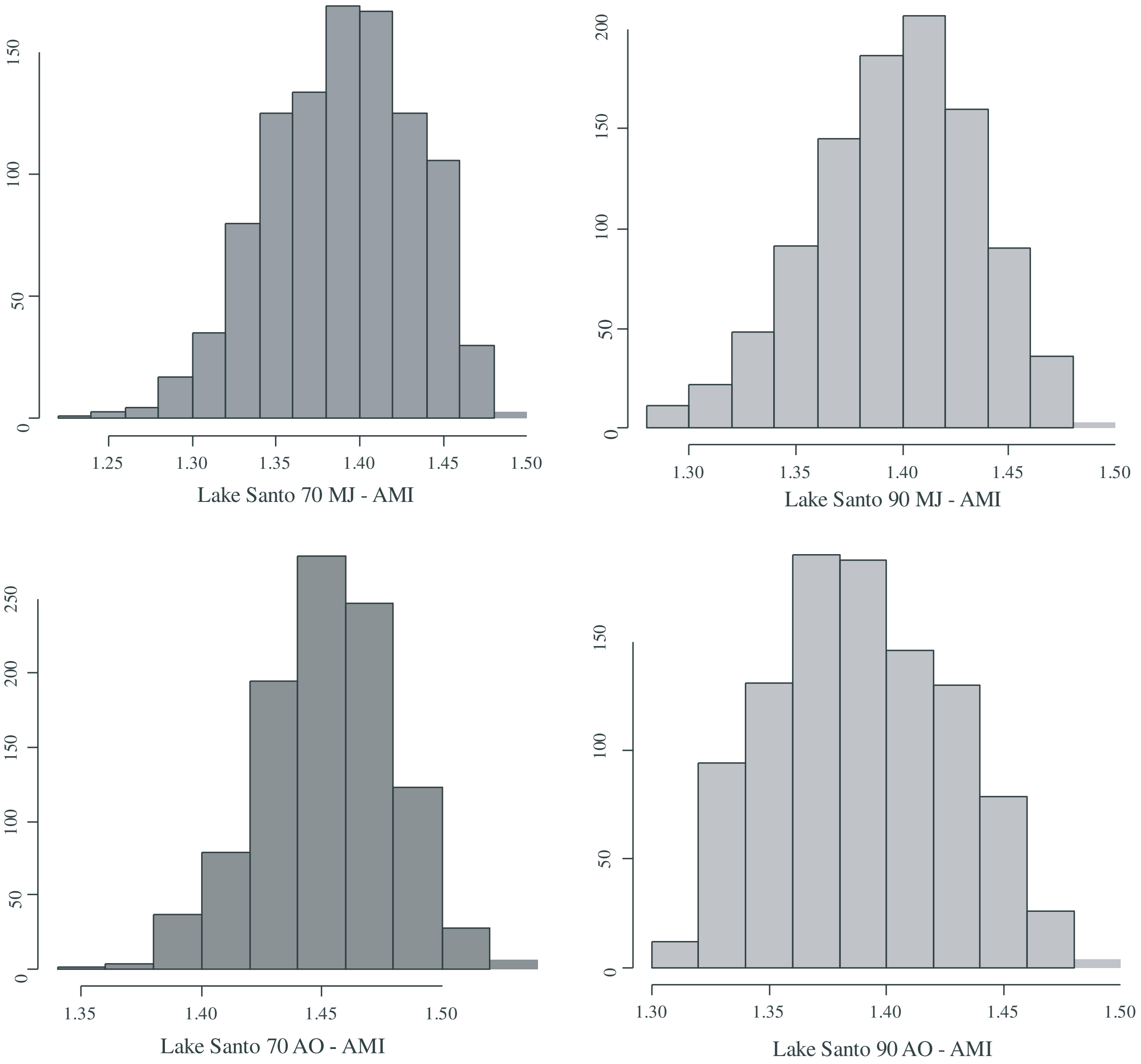


Figure 3. Average mutual information (AMI) distributions generated by simulation. The histograms refer to spring (*upper*) and summer (*lower*) seasons in the two investigated periods, the 1970s and 1990s. For the upper charts, the Shapiro-Wilks test for normality yielded: $W = 0.9871$, $P = 1.054\text{e}^{-07}$; $W = 0.9911$, $P = 9.115\text{e}^{-06}$. For the same graphs, the Kolmogorov–Smirnov test yielded: $D = 3.4907$, $P < 2.2\text{e}^{-16}$; $D = 4.3673$, $P < 2.2\text{e}^{-16}$. For the lower charts the same tests produced: $W = 0.996$, $P = 0.01096$; $W = 0.9891$, $P = 9.17\text{e}^{-07}$; $D = 3.8338$, $P < 2.2\text{e}^{-16}$; $D = 3.7517$, $P < 2.2\text{e}^{-16}$. All distributions, except for the A-O 70, were statistically different from a Gaussian distribution.

distributed among respiration, export, and detritus production. Trophic efficiency at any level is given as a percentage.

Lindeman's image of Lake Santo is a concatenated chain of transfers through six trophic levels, with differences in both the efficiency and the magnitude of energy transfers between seasons and periods. In 1991, the system imported much more carbon than in 1973, but a lower fraction of it reached the top of the food chain, except in the winter season. In both periods, the system showed a dramatic drop in efficiency between the first and second trophic level. Both primary producers and detrital components are part of this first trophic level (Ulanowicz 1995). In 1991, all the trophic levels, except for the third level in the spring season, were less efficient in passing medium to the next level.

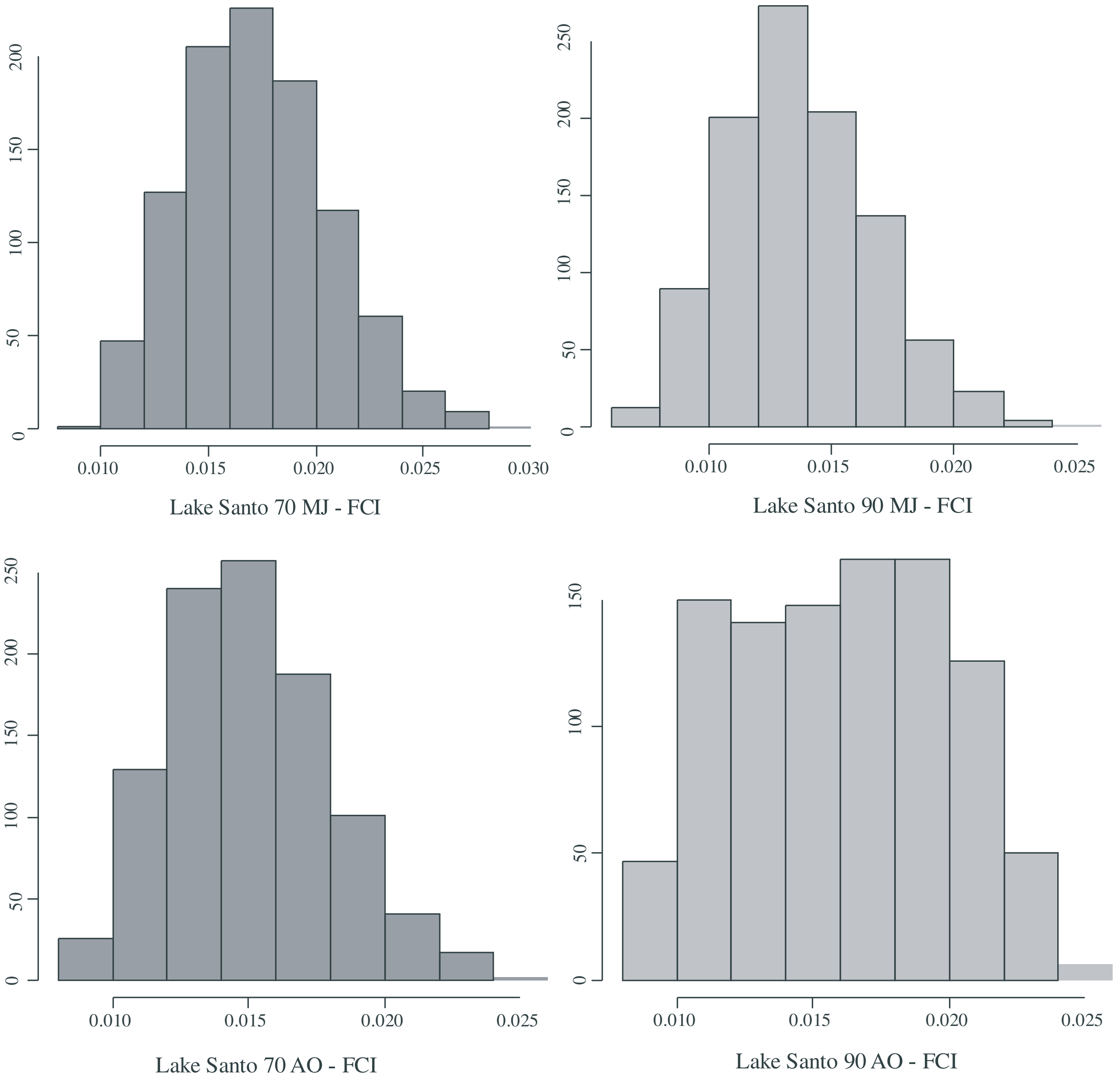


Figure 4. Finn cycling index (FCI) distributions generated by simulation. The histograms refer to spring (*upper*) and summer (*lower*) seasons in the two investigated periods, the 1970s and 1990s. For the upper charts, the Shapiro-Wilks test for normality yielded: $W = 0.9901$, $P = 2.756\mathrm{e}^{-06}$; $W = 0.9881$, $P = 2.990\mathrm{e}^{-07}$. For the same graphs, the Kolmogorov-Smirnov test yielded: $D = 3.6985$, $P < 2.2\mathrm{e}^{-16}$; $D = 4.2742$, $P < 2.2\mathrm{e}^{-16}$. For the lower charts, the same tests produced: $W = 0.9881$, $P = 2.990\mathrm{e}^{-07}$; $W = 0.9738$, $P = 1.875\mathrm{e}^{-12}$; $D = 4.2742$, $P < 2.2\mathrm{e}^{-16}$; $D = 4.1318$, $P < 2.2\mathrm{e}^{-16}$. All the distributions were thus statistically different from a Gaussian distribution.

DISCUSSION

Determining whether an ecosystem is changing under the pressure of stress is of critical importance for conservation purposes (Rapport and others 1998); however, it is not easy, for a number of reasons. First, the investigator must have the ability to contrast the unimpacted ecosystem with the ecosystem in the presence of stress, and data sets that enable such comparison are rare (Schindler 1990). Second, response to stress may vary from one ecosystem to another and relative to the type of stressor (Rapport and Whitford 1999). Also, in many cases, ecosystems may be capable of acting as sinks that absorb the effects of stress without showing measurable changes, so that when the

Table 3. Simulation Statistics

	1970s				1990s			
	M–J		A–O		M–J		A–O	
	AMI	FCI	AMI	FCI	AMI	FCI	AMI	FCI
Avg.	1.388998	0.117246	1.452247	0.105008	1.396583	0.113733	1.389966	0.076048
SD	0.037697	0.003348	0.027901	0.002948	0.037697	0.002993	0.037697	0.0038432

M–J, May to July; A–O, August to October; AMI, average mutual information; FCI, Finn cycling index.

Table 4. Species Trophic Position for Each Network in the Two Periods

	1973				1991			
Compartment	M–J	A–O	N–A	Avg.	M–J	A–O	N–A	Avg.
1. Flagellatae	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2. Chlorophyceae	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3. Chrysophyceae	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4. Dynophyceae	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5. Cryptophyceae	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6. Diatom. Cyano	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
7. Living POC	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
8. *Keratella* gr. *quadrata*	2.27	2.33	2.33	2.31	2.15	2.18	2.21	2.18
9. *Keratella cochlearis*	2.27	2.33	2.39	2.33	2.15	2.18	2.21	2.18
10. *Kellicottia longispina*	2.27	2.33	2.30	2.30	2.15	2.18	2.21	2.18
11. *Ascomorpha ecaudis*	—	—	—	—	2.15	2.18	2.21	2.18
12. *Synchaeta* sp.+*Ploesoma hudsoni*	2.30	2.35	2.36	2.34	2.15	2.19	2.21	2.18
13. *Polyarthra* sp.	2.27	2.33	2.36	2.32	2.15	2.18	2.21	2.18
14. *Asplanchna priodonta*	2.30	2.35	2.30	2.32	2.15	2.19	2.21	2.18
15. *Filina* gr. *longiseta terminalis*	2.27	2.33	2.30	2.30	2.15	2.18	2.21	2.18
16. *Conochilus* sp.	2.27	2.33	2.30	2.30	2.15	2.18	2.21	2.18
17. Other rotifera	2.27	2.33	2.30	2.30	2.15	2.18	2.21	2.18
18. *Daphnia longispina*	2.27	2.33	2.30	2.30	2.15	2.18	2.21	2.18
19. *Bosmina longirostris*	2.27	2.33	2.30	2.30	2.15	2.18	2.21	2.18
20. *Eudiaptomus intermedius*	2.27	2.27	2.30	2.28	2.15	2.18	2.21	2.18
21. Nauplii	2.27	2.33	2.40	2.33	2.15	2.18	2.21	2.18
22. Copepodites	2.27	2.33	2.40	2.33	2.15	2.18	2.21	2.18
23. Other copepoda	2.27	2.33	2.40	2.33	2.15	2.18	2.21	2.18
24. Fish	2.42	2.38	2.35	2.38	2.33	2.31	2.29	2.31

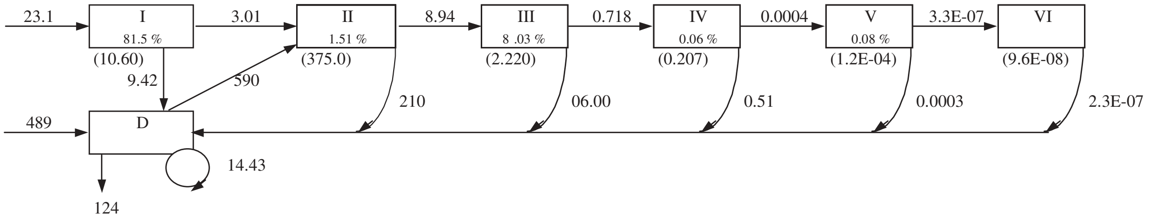
M–J, May to July; A–O, August to October; N–A, November to April; POC, particulate organic carbon.
An average trophic level is also calculated for each species in the two periods.

signs of degradation are finally detected, the thresholds of tolerance or resistance have already been exceeded and the integrity of the ecosystem has been disrupted (Holling 1986; Kay 1991).

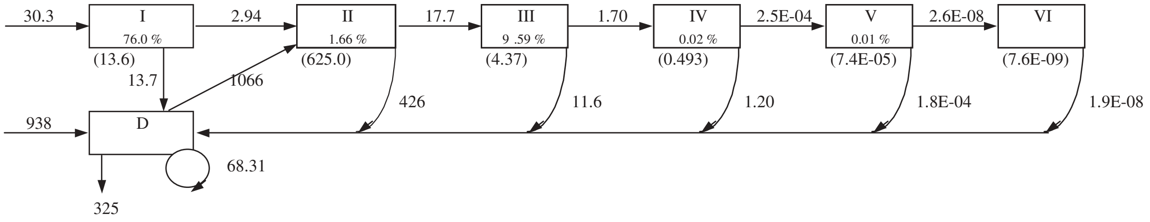
The human impact on Lake Santo was negligible in the 1970s but it increased progressively over the following 20 years. However, trophic conditions, as assessed using widely accepted trophic-state parameters, remained the same. The Organization for Economic Co-operation and Development (OECD) has devised a method to evaluate the trophic state of water bodies. To overcome the objective difficulties associated with attempts to classify the trophic state of a particular water body, the

Figure 5. Trophic aggregation of the six seasonal networks for the Lake Santo ecosystem. Each box represents a trophic level; recycling of nonliving material is through compartment D. All flows are in gC m^{-2} y^{-1}. Numbers in parentheses are dissipations; the percentage within each box is the trophic-level efficiency.

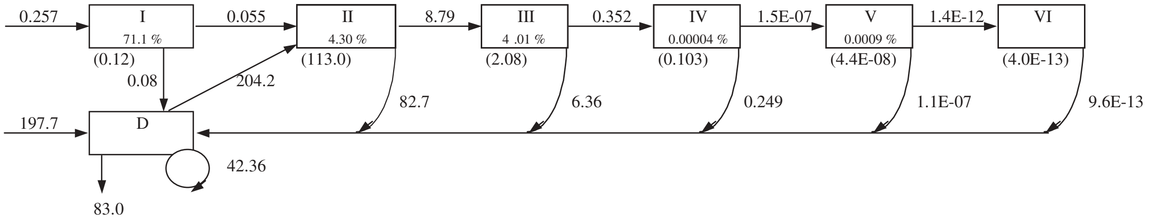
Season: May-July 73



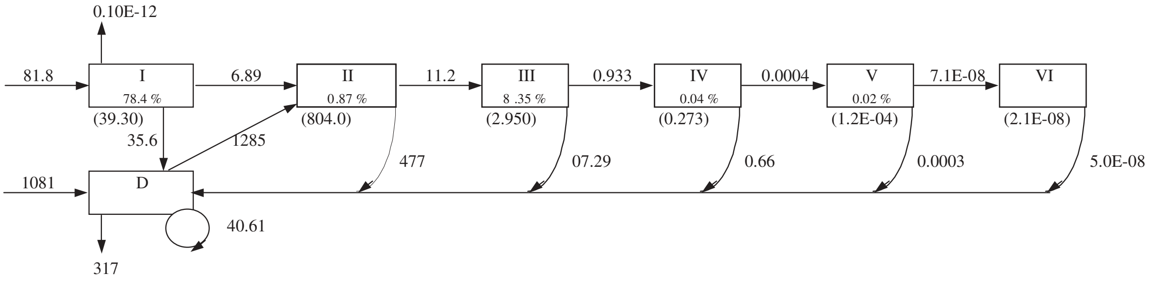
Season: August-October 73



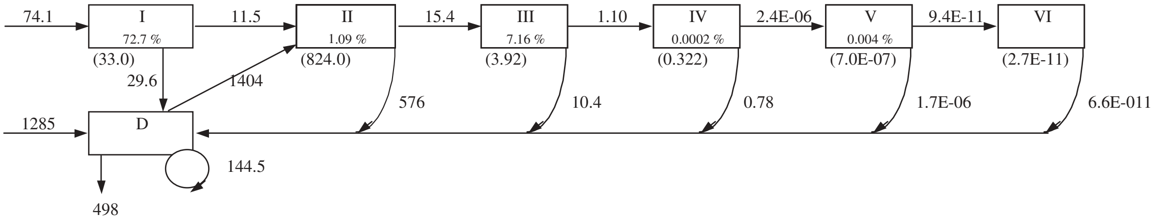
Season: November 73-April 74



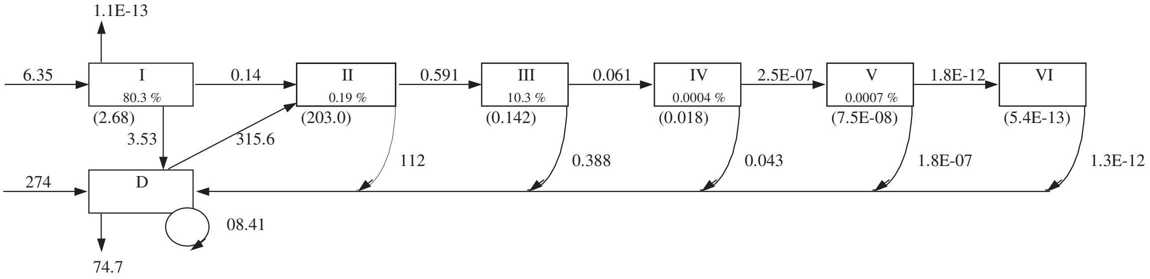
Season: May-July 91



Season: August-October 91



Season: November 91-April 92



OECD method proposes that the allocation of trophic category be done in a probabilistic way, using probability distribution curves built for critical parameters such as average lake phosphorus, average and peak chlorophyll *a* concentrations, and average yearly Secchi disk transparency (OECD 1982). According to calculations based on this method, in both 1973 to and 1991, Lake Santo had the following percentages in the different categories of trophic state: 52% oligotrophic, 32% mesotrophic, 14% ultraoligotrophic and 2% eutrophic (G. Rossetti, unpublished). These percentages indicate that the lake maintained its oligo-mesotrophic state over the entire 20-year period. Although increased extension of littoral algal beds was observed during the second campaign (G. Rossetti unpublished), evidence clearly signaling an ongoing process of deterioration was never detected (Ferrari and others 1992; Rossetti, unpublished).

To discover whether Lake Santo is changing, in spite of the apparent constancy of its trophic state, we considered a shift in focus: from measures of single ecological characteristics or parameters to a whole-ecosystem approach. Accordingly, we portrayed the ecosystem as a suite of energy flow networks. These models were analyzed by network analysis, with an emphasis on overall-system activity, cycling activity, community structure, and trophic efficiency. Ecosystems under stress are in fact expected to show qualitative trends similar to those reflected in these features (Odum 1985; Rapport and others 1985; Schindler 1990).

Values of TST (annual averages, Table 1) indicate that Lake Santo processed 60% more organic material in 1991 than in 1973. When single seasons were considered, the increase in activity ranged from 44% (spring) to 72% (summer). In parallel, the AMI fell from 1.44 bits (in 1973) to 1.37 bits (in 1991). Under conditions of relatively unimpeded growth, there would be a concomitant increase in activity and organization: ascendency is expected to increase in both its components, TST and AMI (Ulanowicz 1986). Because stress, in many instances, disrupts internal mechanisms, the tendency toward increasing activity and organization should reverse (Ulanowicz and Mann 1981); that is, both TST and AMI should diminish. This situation has been observed, for example, in two tidal marsh creeks of the Crystal River Florida (USA) (Ulanowicz 1996). One of them was impacted by a heated affluent; it showed a 20% drop in size (TST), and the AMI diminished from 1.27 to 1.24 bits. In the case of stress due to nutrient enrichment the trends expected for TST and AMI are different. Ulanowicz (1997) described eutrophic systems as systems in which ascendency increases due to a rise in TST that more than compensate for a concomitant fall in the AMI of the flow network. Thus, the changes observed in Lake Santo correspond to this quantitative definition of eutrophication.

The observed 60% increase in throughput did not lead to any enlargement of the biological community of Lake Santo; the sole difference in its make up—the additional presence of the zooplankton species *Ascomorpha ecaudis* in 1991—did not justify an increase in TST of that magnitude. Thus, more nutrition matter entered the lake in the period 1973–1991 than the amount needed to maintain the biological community. As more throughput becomes available to a system, the opportunity could arise for more species to appear. This situation is often observed in the early stages of ecosystem development. As the available flows of medium become distributed over more components, however, the average throughput per single compartment decreases, making the compartments more vulnerable to external perturbations. Thus, the rigors of the environment impose constraints on species appearance, whereas in the presence of more benign conditions tends to promote this phenomenon. Lake Santo is characterized by harsh abiotic conditions, low temperature, and a protracted period of ice and snow cover. For this reason, we would not expect the division into a high number of new elements to occur; however, we believe that the observed increase in TST is of a magnitude that would enable a community richer than that observed in 1991 to become established in the lake.

Using the Student *t*-test, we analyzed the AMI distribution for the spring and summer seasons to assess the significance of the different the levels of organization shown by Lake Santo in 1973 and 1991. By defining ranges of variations for flow coefficients to consider interannual variability, we were able to simulate 1,000 networks for the spring and summer seasons in both periods. We obtained the distributions that appear in Figure 3. Consider the spring season. The 1973 network appears to be less organized (AMI = 1.38) than its 1991 counterpart (AMI = 1.39), but this difference is quite small. Statistical analysis on system level indices has not been carried out often in the framework of network analysis; consequently, the statistical behavior of these quantities has yet to be fully explored. The problem with these indices is that they represent logarithms of change in actual probabilities, and they seem to be a much-attenuated measure of change (Hirata and Ulanowicz 1984). Accordingly, even small differences in AMI could account for major variations in flow values. Also, the result of the Student *t*-test could be an artifact due to the large size of the samples we used for statistical analysis (the 1,000 AMI values obtained from simulation). Although these are key issues that need further case studies to be resolved, we should trust the outcomes of the statistical analysis and base our conclusions on them. On the other hand, in the absence of better estimates based on a larger suite of networks built from field data (for which we would need a larger data set than was actually available), simulation was the only means we had to ascertain the effect of variability on AMI values.

The difference between the distributions for the summer was also significant. Two opposite trends seem to characterized Lake Santo's level of organization in the open water season: it increased slightly in the spring from 1973 to 1991, whereas it decreased in the summer (1973 AMI = 1.44, 1991 AMI = 1.38). Overall, the AMI dropped from the 1970s to the 1990s. The winter networks, for which we had insufficient data to run the simulations, confirm this drop in the level of AMI (1.48 for 1973, 1.36 for 1991). Therefore, the overall picture yielded by TST and AMI values corresponds with the quantitative definition of eutrophication given by Ulanowicz (1997): an increase in TST concomitant with a decrease in AMI.

The uncertainty that characterizes the results of the sensitivity analysis does not preclude the conclusion that the continuous addition of nutrient changed the natural course of ecosystem growth and development in Lake Santo. In fact, only in the presence of an increase in AMI could one speak of a ''normal'' course of development in the lake, given the 60% increase in TST. Thus, it is only the magnitude of this change that is in doubt. If the effect of sample size masked nonsignificant differences in the AMI values, then we could state that ecosystems growth occurred without any associated development; if so, it is possible that at the time of the second campaign the lake was initiating a reversal in its normal course of development under the pressure of enrichment—a hypothesis that we present with circumspection.

With a greater amount of energy at its disposal, the lake's development capacity increased noticeably, but this excess of potential was hijacked to overhead, which constitutes a sort of reservoir of plasticity that enables the system to face new threats (Ulanowicz 1997). In particular, the OI, OE, and OD all increased, on average, from 1973 to 1991. This increase was registered not only in absolute values but—more important—also as a percentage of the development capacity. The OI rose from 9 to 10.5% of C, OE increased from 1.05 to 1.31%, and, OD increased from 16.8 to 17.9%. By contrast, redundancy, as a fraction of the development capacity, decreased from 29.44 to 27.21%. Considering the change in magnitude observed for ascendancy (42.7 and 43%), these results suggest that the ecosystem basically underwent a redistribution in the components of the encumbered complexity.

However, the problem of the statistical significance of these changes remains. To explore this issue, we performed simulations and statistical analyses (using the Student *t*-test) first on the joint entropy, an index that groups together all the components of the overhead and scales this encumbered complexity by the TST (overhead/TST) (Ulanowicz 2004). Results of the *t*-test on the distributions obtained for this index were all significant (spring: avg. $JE_{70} = 3.26$, avg. $JE_{90} = 3.30$, $t = -6.9528$, $df = 1998$, $P = 2.41e^{-12}$; summer season: avg. $JE_{70} = 3.315$, avg. $JE_{90} = 3.236$, $t = 19.6265$, $df = 1998$, $P < 2.2e^{-16}$). Based on these results (very small differences that were all significant, as in the case for the AMI and FCI), we thought that changes in the values of single indices would be significant and therefore did not perform further simulations and statistical tests on the single components of the overhead.

Given that this quantity (and, thus the joint entropy) has been shown to be a proxy for ecosystem resilience (Mageau and others 1995) one could say that overall the ecosystem became slightly less resilient under conditions of continuous enrichment. This would contradict the idea that an impacted system becomes more resistant to further perturbation (a sort of ecological Le Chatelier-Braun principle) (Ulanowicz 1980, 1990). This conclusion is strengthened if, instead of overhead, redundancy alone is taken to assess resilience (Ulanowicz 2004), because the magnitude of this index's change is more pronounced than that of the total overhead. However, the concept of resilience is far more complicated than can be appreciated here, and our results do not embrace in full the multiple dimensions of that concept, which also include dynamic features such as recovery time (Mageau and others 1995). For this reason, we avoid drawing any further conclusion about resilience.

More important, our interest in the components of the overhead resides in the fact that they quantify the ecosystem attributes that Odum (1985); (see also Ulanowicz and Kemp 1979; Ulanowicz 1997) used to devise a scenario for ecosystem development. The OI, OE, and OD indicate that the ecosystem became less capable of processing energy and progressively more dependent on auxiliary energy from the outside environment. The augmented quota of exports, in particular, tells us that over the course of time the amount of energy that remained unused increased, although this component is only a small fraction of the total overhead. All these features figure in Odum's (1985) scenario (see also Barrett and others 1976) as typical attributes of ecosystems under stress. However, the results of Schindler's study of whole-lake experimental enrichment (Schindler 1987, 1990) do not mesh perfectly with Odum's point of view. In particular, Schindler found that exported and unused production increased in lakes that underwent enrichment, but whole-lake respiration did not change.

We searched for further indications of variation in the mutual organization of flows in the lake by comparing the quantity AMI/JE for the two periods. This index, which measures the ratio of realized development with respect to the fraction of development capacity that remains confined by the encumbered complexity, can give us a clue as to whether the lake underwent a retrogression in its level of organization. Results of the *t*-test on the distributions obtained for this index were all significant (spring : avg. $\mathrm{AMI/JE}_{70} = 0.4260297$, avg. $\mathrm{AMI/JE}_{90} = 0.4236525$, $t = 2.4124$, $df = 1998$, $P = 0.007969$; summer : avg. $\mathrm{AMI/JE}_{70} = 0.4383858$, avg. $\mathrm{AMI/JE}_{90} = 0.4299843$, $t = 10.3588$, $df = 1998$, $P < 2.2\mathrm{e}^{-16}$). The analysis showed that, despite the contrasting seasonal trends between the two periods, in both overhead and AMI, this index diminished from 1970 to 1990 in both spring and summer, providing additional evidence that the lake experienced a loss of efficiency and organization.

Cycling is a crucial feature in any assessment of ecosystem functioning (Odum 1969). Based on Odum's work one can infer that the cycling index should decrease with stress (Odum 1981, 1985; but see also Schindler 1987, 1990). In some cases, however, perturbed systems have shown larger cycle indices (Richey and others 1978; Ulanowicz 1996). Ulanowicz (1996) has suggested that we need to pay attention not only to the overall magnitude of cycling activity but also to the number and types of routes involved in this function. Thus, recycling may well increase under stress to compensate for diminished storage in the higher trophic compartments, because the disturbance should affect higher trophic levels disproportionately. In Lake Santo, although the overall number of cycles increased from 1973 to 1991 (Table 2), the index of cycling activity decreased, both between corresponding seasons and as yearly averages (Table 1). However, the magnitude of cycling activity devoted to recirculating carbon was greater in 1991 than in 1973; the winter season was an exception in this respect. As for the relative importance of shorter versus longer cycles, however, the pattern for the winter is the same as that observed in spring and summer. Less carbon used longer routes in 1991.

As for community structure, food chains would shorten in ecosystems under stress (Odum 1985; Ulanowicz 1997), although in Schindler's artificially enriched lakes (1987, 1990) this effect was not observed. In Lake Santo, the Lindeman profile, with its six trophic levels, remained the same for all seasons in both years (Figure 5). However, less energy reached the top of the food chain in 1991 than in 1973. This does not apply, however, to the winter season; more carbon is passed to the last trophic level and, as a consequence, more is recycled back through longer cycles. This result contrasts with the outcomes of the cycling analysis for the winter season. In fact, with respect to the total amount of currency involved in cycling, the fraction associated with longer cycles (five or six trophic steps) was lower in 1991 than in 1973. This contradiction may be explained by the fact that winter networks carry more uncertainty about the flow values. Sampling is difficult in winter because the lake is ice-covered and harsh climatic conditions prohibit frequent access to the area. Less frequent sampling might have hampered the construction of winter networks, causing them to be less accurate than those for the other seasons. Due to this sampling problem some uncertainity characterized flow value estimations, affecting some of the outcomes.

If we imagine food chain shortening as a gradual process that is brought to completion through a progressive reduction of the energy flow toward the top of the food chain, it is possible that the 1991 network portrays an intermediate stage in the course of this process. This hypothesis matches the idea that Lake Santo is an ecosystem in transition toward an ''unhealthy'' state, as suggested by the analysis of the AMI. An additional network built using more recent data would be essential to confirm this hypothesis.

The reduced activity at higher trophic levels is further witnessed by the reduction of the effective trophic level of all the consumers (Table 4). This is due to a change in the diet composition of species.

Most of the consumers—namely, zooplankton species—fed primarily on phytoplankton and living POC. From 1973 to 1991, phytoplankton biomass increased noticeably whereas living POC remained essentially unaltered; as a result, zooplankton feeding activity likely shifted toward phytoplankton. In 1991, these species ate a higher percentage of phytoplankton (trophic level 1) than Living POC (trophic level 2) in comparison to 1973. This shift translated higher up the food web to fish, which experienced a decreased trophic position. This result requires an explanation. Fish decreased their trophic level from spring to winter because during ecosystem evolution they shifted their alimentary habits and fed more on WPOC (trophic level 1) and relatively less on living POC (trophic level 2), while the contribution of zooplankton (which increased their trophic level from spring to winter) to the fish diet remained unchanged. At the same time, zooplankton species increased their trophic position from spring to winter, because in spring they fed more on phytoplankton (trophic level 1), their preferred diet item, which was abundant, and less on living POC (trophic level 2). The opposite occurred in winter: phytoplankton biomass diminished and zooplankton groups were forced to rely for a larger fraction of their diet on living POC. In this respect, other studies have produced contrasting results. Ulanowicz (1996) found that some species experienced a drop in their trophic position whereas others fed higher when the ecosystem was impacted. In other cases, this feature was not tested (Rapport and others 1985; Schindler 1990).

In summary, an intriguing picture has emerged from our study of the Lake Santo ecological flow networks with respect to the original question of whether the lake underwent pathological changes during the period 1970–1990 under the pressure of continuous nutrient loading. With reference to Odum's scenario, it appears that respiration and the export of medium increased, the ecosystem itself became more open (that is, input and output became more important), and bottom-up trophic efficiency diminished. Most species fed at lower trophic levels. In addition, although the amount of matter processed increased noticeably, the organization of flows diminished, suggesting that Lake Santo underwent eutrophication. However, given that the changes observed in the components of the overhead were not too large in magnitude (as a percentage of the development capacity), it seems that the transition to eutrophication has not yet been brought to completion. This hypothesis is confirmed by the results of the trophic analysis, in which the observed reduction in the amount of energy that reached the top of the concatenated chain of transfer described by Lindeman's schema could represent an intermediate stage in a shortening of the food chain. So it seems that a reversal in the ecosystem's developmental trend (Odum 1981; Connel and Noble 1987; Turner and others 1998) began to affect Lake Santo under the pressure of continuous nutrient additions. A definitive answer to our original question could be obtained by building networks using more recent data that cover a period of time longer than 1 year.

The construction of more extensive networks could also help us to overcome the difficulties associated with the need to perform meaningful statistical analyses to test the results of the network analyses. To accomplish this, a sufficient number of replicates for the same parameter or index must be produced, and this is notoriously difficult in whole-ecosystem studies. In this study, we performed statistical tests on index distributions built from randomly generated flow matrices based on within-period variations in species' standing stocks. Determining the extent to which changes in system-level indices can be considered significant must become a priority for ecosystem studies. Our ability to define whether an ecosystem is suffering from stress and, ultimately, to confirm qualitative or heuristic hypotheses about ecosystem growth, development, and health depends on the successful implementation of this approach.

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