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Organisms' response in a chronically polluted lake supports hypothesized link between stress and size

Abstract—Most of the available support for the hypothesis that pollutants' effects depend on size comes from short-term experiments. We applied a size-based approach to the analysis of the fossil remains of Lago d'Orta in Northern Italy, a lake that has been polluted with copper, other metals, and acid for more than 50 yr. Once this pollution began, the size distribution of diatoms, thecamoebians, and cladocerans shifted to smaller individuals. These changes in size reflected shifts in the taxonomic composition of the assemblages, but we also observed reduction of body size within a single taxon (the diatom *Achnanthes minutissima*). These reductions in the average size across three communities from different kingdoms and trophic levels provide strong evidence that chronically stressed environments select for smaller organisms.

Body size is a fundamental and easily measured property of single organisms and whole communities (Peters 1983; Calder 1984). It has been proposed (Odum 1985; Rapport et al. 1986) that large organisms are more sensitive to stress than short-lived, fast-reproducing small organisms. This size-dependent sensitivity holds many implications for community functions: systems under stress would be dominated by smaller organisms with faster metabolism and flux rates (Peters 1983), but the length and efficiency of trophic food chains would decline (Ryther 1969; Stockner and Shortreed 1989). In practice, the analysis of community size structure could be an alternative to traditional taxonomy for biomonitoring present and, through the fossil records, past episodes of acute and chronic pollution (Kerr and Dickie 1984; Sprules and Munawar 1986; Cattaneo et al. 1995).

Most available evidence for the relation between size and stress is based on short-term experimental perturbations (O'Connors et al. 1978; Menzel 1980). Long-term community responses to anthropogenic stresses can be gleaned through studying sediment fossil remains (Charles and Smol 1990; Dixit et al. 1992). Although a size-based approach is rarely applied in paleolimnology (Lami et al. 1987), mostly to evaluate changes in predation (Kerfoot 1981; Leavitt et al. 1994), that approach would allow a long-term test of the link between size and stress. We attempted such a test in this study by considering the size distribution of fossil remains in the sediments of Lago d'Orta before and after the onset of chronic industrial pollution by copper and ammonium.

Lago d'Orta is a deep (maximum depth, 143 m), warm, oligomonomictic lake of subalpine Italy. In 1926, the lake was polluted by copper and ammonium sulphate when a rayon factory was established at the southern end of the lake, dramatically reducing phytoplankton, zooplankton, fish, and even bacteria populations (Monti 1930). This first pollution impact was mainly due to copper toxicity. The few analyses available between the 1930s and 1950s indicate high concentrations of copper (30–100 μg liter^{-1}), while biochemical oxidation of ammonium prevented its accumulation until the 1960s (Calderoni et al. 1992). Although copper discharge from the factory was significantly reduced in 1958, loading of Cu, Zn, Ni, and Cr from electroplating industries became significant. The rayon factory continued to release ammonium sulphate, so NH_4^+ remained at high concentrations until 1980, when a stripper system reduced ammonium sulphate load. The biochemical oxidation of ammonium acidified the lake (pH $\approx$ 4) from 1970 onwards (Bonacina et al. 1988). In 1989–1990, the lake was treated with lime to reduce acidity, toxic metals, and ammonium in the water (Calderoni et al. 1992) (Fig. 1).

A sediment core 37 cm long was collected from the lake's south basin in 1994 with a gravity corer. Immediately after collection, the core was sectioned at 1-cm intervals in the upper 20 cm and every other 1-cm interval in the lower 17 cm. This core was analyzed for diatom and cladoceran remains. A parallel core 89 cm long, collected in 1990 at the same station, was analyzed for thecamoebians and dated with ^{210}Pb (Constant Rate of Supply model; Appleby and Oldfield 1978) and ^{137}Cs (P. Appleby, pers. comm.). Dates before A.D. 1850 were extrapolated from ages in a uniform lithological unit (P. Guilizzoni, pers. comm.). The two cores correlated well, as indicated by similar depth distributions of percentage loss on ignition (Manca and Comoli 1995).

For diatom analysis, sediments were digested with H_2O_2 and mounted in Naphrax after microspheres had been added to calculate frustule concentration (Battarbee 1986). At least 300 frustules were identified, counted, and measured at $\times$1,000. For the cladoceran analysis, sediment was deflocculated in warm 10% KOH for 2 h and then resuspended in 5% formalin. Up to 120 remains were identified and counted per sample. The most abundant fragment was used as a measure of abundance (Frey 1986). For thecamoebians, the sediment was suspended in ethanol, washed through a 45-μm sieve and then resuspended in ethanol. At least 300 remains were counted.

Diatom and thecamoebian cell diameters were measured under the microscope. For cladocerans, where only fragments were available, body length was estimated from average values reported for these taxa in Italian lakes (Margaritora 1983). The effect of this approximation on our estimates of size patterns should be negligible because the differences in average length among the dominant taxa are much larger than the variation within each taxon. For *Sida crystallina,* however, we measured length and width of carapaces on old samples collected from Lago Maggiore, a deep lake very close to Lago d'Orta, where *S. crystallina* developed truly pelagic populations in the past (Manca and Comoli 1995). Volumes were calculated by approximation to geometrical solids.

The remains of diatoms, thecamoebians, and cladocerans that we examined in this study represent a period from about 300 yr before the onset of the pollution to 60 yr afterward.

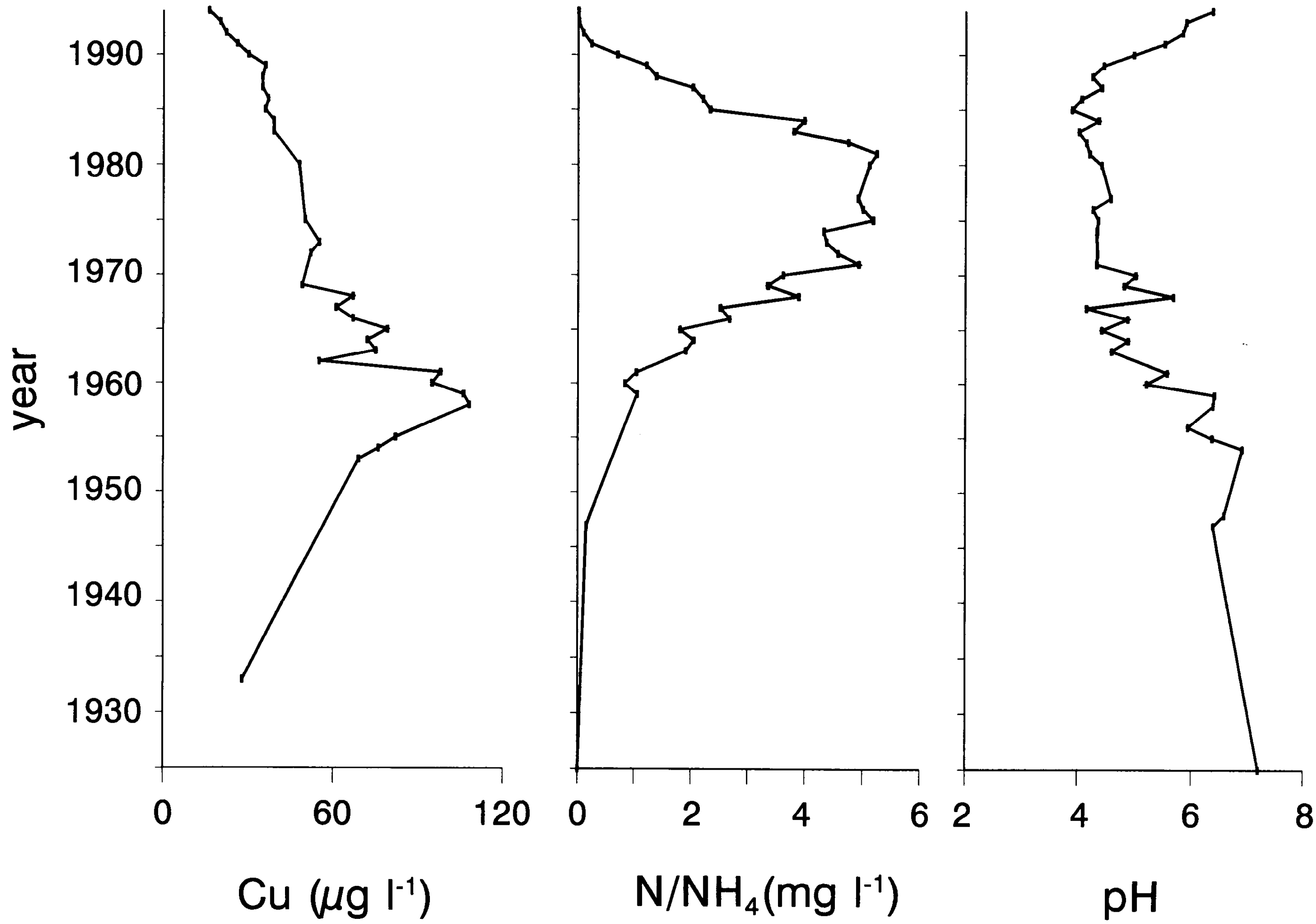

Fig. 1. Concentrations of copper, ammonium, and acid in Lago d'Orta at winter circulation. Copper concentrations before pollution are not available but are assumed to be negligible given the mineral composition of the drainage basin and of waters in nearby unpolluted lakes (A. Calderoni, pers. comm.). The figure is redrawn from Calderoni et al. (1992). Analytical methods changed throughout the years in accordance with those suggested by contemporary editions of Standard Methods (American Public Health Association).

To describe the changes in size structure of these three assemblages, we grouped the organisms in logarithmic size classes and calculated the percentage of the total biovolume in each stratum represented by the different classes. For diatoms and cladocerans, where remains were counted in a weighted sediment fraction, total biovolumes could be expressed per gram of dry sediment.

In all three groups of organisms, the smallest size classes clearly increased in importance in sections of the core that corresponded to the period of industrial pollution (Fig. 2). At least for diatoms and cladocerans, where we have quantitative data, the transition in size structure coincides with a sharp decrease in biovolume (Fig. 3). This trough should reflect the collapse of lake biota following the onset of pollution documented by Monti (1930).

For diatoms (Fig. 2A), the change in size structure corresponded to a drastic shift in taxonomic composition (Fig. 3A) from assemblages dominated by *Cyclotella comensis* Grunow (100–1,000 μm^3) and *C. bodanica* Eulenst. (1,000–10,000 μm^3) to those dominated by *Achnanthes minutissima* Kützning (<100 μm^3). The largest size classes (mainly represented by *Surirella* spp.) disappeared completely after pollution began (Ruggiu et al. 1998). *C. comensis* and *C. bodanica* are abundant in the fossil remains of other subalpine lakes (Candido et al. 1985; Lami et al. 1986; Marchetto and Bettinetti 1995). *A. minutissima* is cosmopolitan and often dominant in benthic assemblages in circumneutral waters (Lowe 1974). However, this alga can apparently withstand strong metal stress, since it is dominant in streams subject to long-term heavy metal contamination (Leland and Carter 1984; Deneiseger et al. 1986; Takamura et al. 1990).

In the cladoceran assemblages (Fig. 2B), pollution was again accompanied by large changes in the size structure mediated by community shift. The largest animals (>1 mm^3; mainly *S. crystallina* O. F. Müller), which were previously dominant, disappear as does the fraction between 0.01–0.1 mm^3, composed of chydorids (mainly *Biapertura affinis* Leydig and *Alona quadrangularis* O. F. Müller) and *Bosmina* spp. At the same time, the smallest size fraction of the community increases, reflecting the dominance of *Chydorus sphaericus* O. F. Müller. The intermediate size fraction (between 0.1 and 1 mm^3) becomes more important after pollution. This increase reflects the

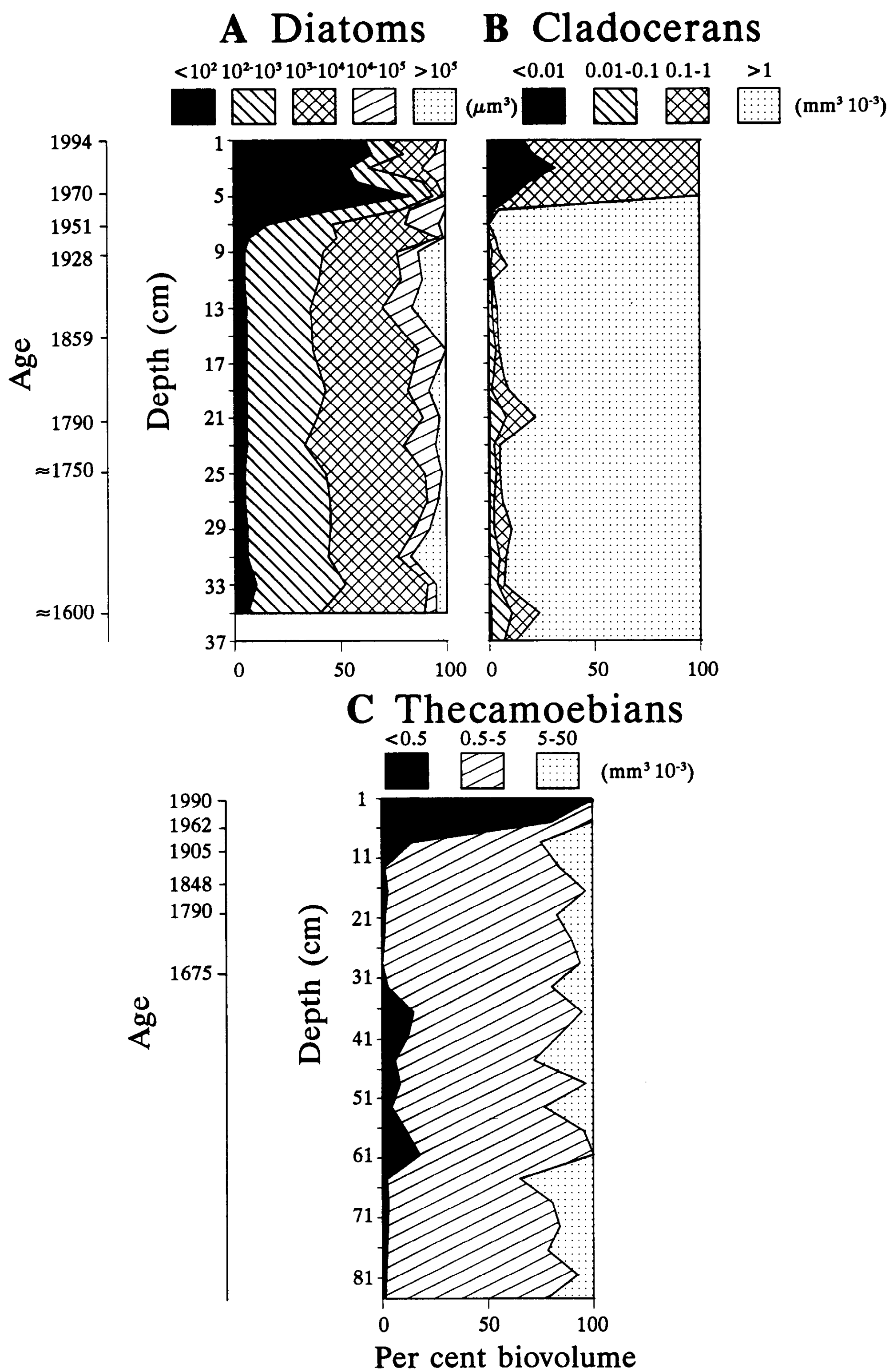

Fig. 2. Temporal variations in size structure of (A) fossil diatom, (B) cladoceran, and (C) thecamoebian assemblages in two sediment cores collected in Lago d'Orta. Shaded areas represent the relative contribution of logarithmically grouped size classes to the total biovolume. In all assemblages, there is a shift in size structure with the onset of pollution.

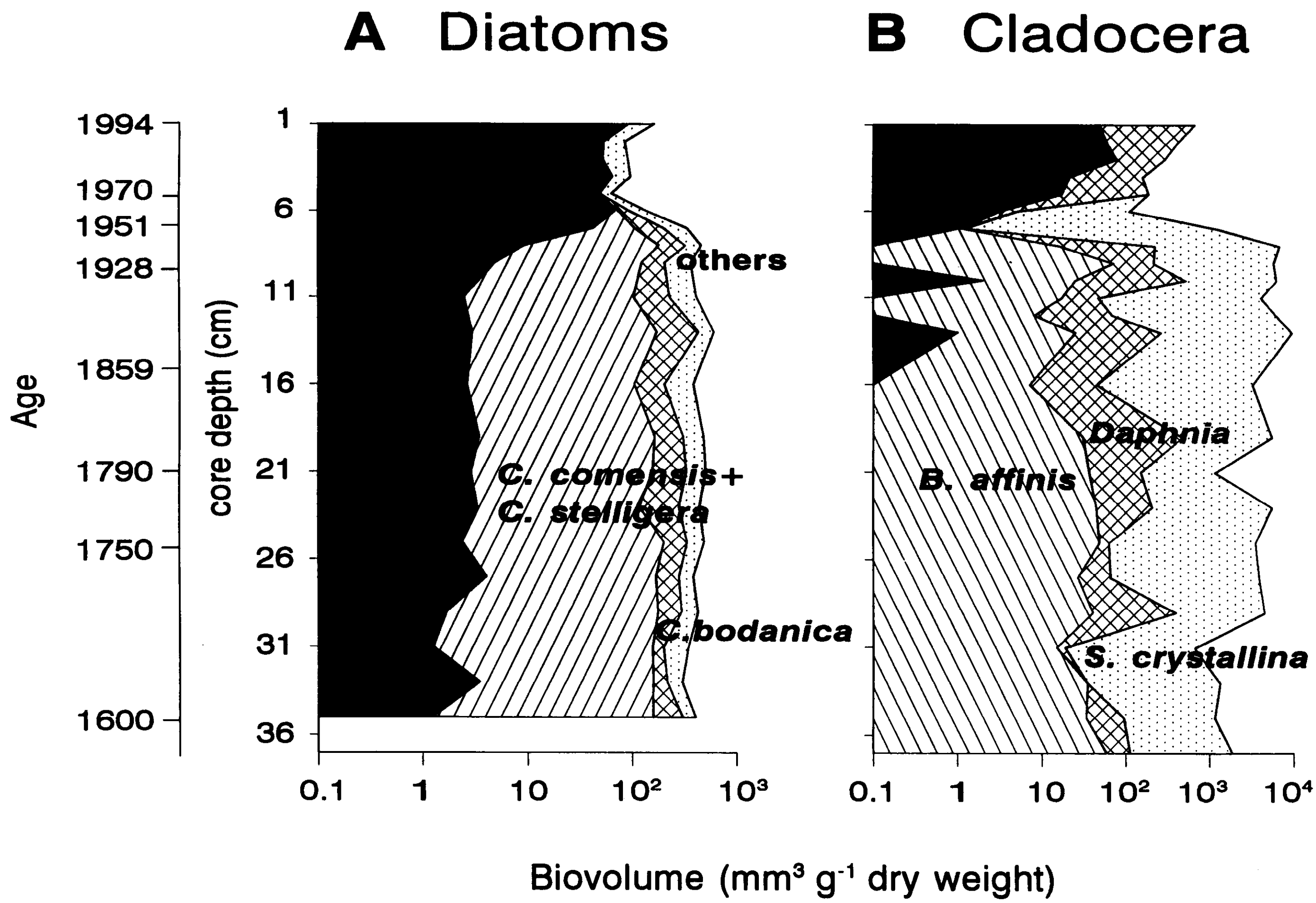

Fig. 3. Biovolume of diatoms (A) and cladocerans (B) along a sediment core collected in Lago d'Orta. The contribution to the total biovolume of the main taxa is also represented (note that the scale is logarithmic). Black shading represents *A. minutissima* in (A) and *C. sphaericus* in (B).

development of a *Daphnia* of the *pulex* group (*D. obtusa* Kurz emend. Scourfield) that replaces the taxa previously dominant in this fraction (*Eurycercus lamellatus* O. F. Müller and *Daphnia* of the *longispina* group) (Fig. 3B; for detailed taxonomic composition see Manca and Comoli 1995). This *Daphnia* is typical of temporary ponds; in the laboratory, juveniles grow rapidly and adults favor egg production over somatic growth (Manca and de Bernardi 1987). After the lake was polluted, it was repopulated by cladocerans (*C. sphaericus* and *D. obtusa*) and diatoms (*A. minutissima*) that are primarily littoral and only in particular conditions become pelagic. In acidified lakes in the Adirondack mountains, euplanktonic diatoms became very rare or absent (Charles 1985). Maybe littoral species living in a more variable environment are typically more resistant to stress.

As for diatoms and cladocera, thecamoebians also show a change in size structure along the core (Fig. 2C). After pollution began, the smaller species (<500,000 μm^3) *Difflugia proteiformis* Lamarck and *Difflugia viscidula* Penard dominate whereas the deeper sections are dominated by larger taxa (*Pontigulasia compressa* Carter, *Difflugia globulus* Ehrenberg, and *Difflugia oblonga* Ehrenberg) (Asioli et al. 1996).

These changes in size structure reflect shifts in the taxonomic composition of the assemblages. However, we also observed reduced size within a major taxon. The mean length of *A. minutissima* significantly decreased from about 14 μm before pollution to a minimum of 9 μm in the section corresponding to the years 1950–1970, when the copper pollution was greatest (Fig. 4). A partial increase in length is noted in the more superficial sediments, perhaps reflecting the pollution abatement programs. These changes reflect changes in the abundance of a variety or morph that is less slender than the typical *A. minutissima.* The addition of aluminum has been observed to reduce the size of the diatom *Asterionella ralfsii* in culture (Gensemer 1990).

Odum (1985) theorized that rapidly reproducing small organisms should dominate stressed ecosystems. Laboratory and microcosm experiments with metals and other contaminants have generally induced a decrease in average phytoplankton size among communities (O'Connors et al. 1978; Menzel 1980; Sanders and Cibik 1988) and a reduction of size within species (Gensemer 1990). In acidification, size response has not always followed this trend. Small cladocerans became dominant in acidified enclosures (Locke and Sprules 1993), but phytoplankton size increased in these enclosures (A. Locke, pers. comm.) and in experimentally acidified lakes (Schindler 1987). This

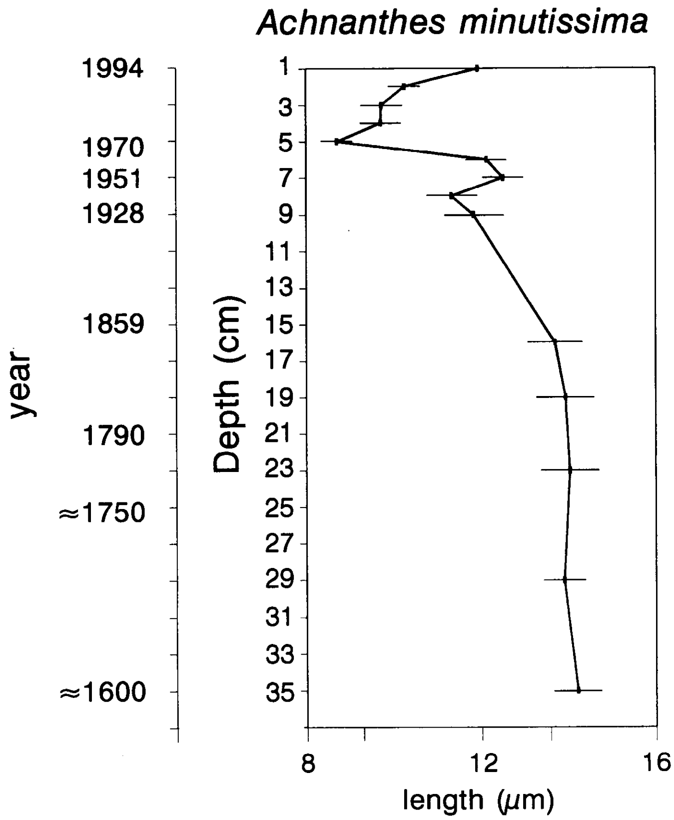

Fig. 4. Changes in average valve length of the diatom *Achnanthes minutissima* measured along a core collected in Lago d'Orta. Points represent the mean and bars represent the standard error; $n = 40–50$.

uncertainty could arise because the effects of acidity on size are mediated by changes in other trophic levels.

After pollution began in Lago d'Orta, planktivorous and piscivorous fish were practically absent (Bonacina et al. 1988). Biomanipulation experiments (Lynch and Shapiro 1981, Proulx et al. 1993) suggest that this lack of predation should result in larger zooplankton and phytoplankton, and the effect of this release from predation might extend to the benthic thecamoebians. Without selective predation, the success of smaller organisms in polluted water is even more striking. The rationale for this selective pressure toward smaller size is likely to depend on the increased resistance of smaller organisms to contaminants. The toxic doses of many contaminants decline with increasing size (Chappell 1992), and perhaps the longer generation times and lower clearance rates of larger organisms (Fenchel 1974) expose such organisms to effectively higher levels of pollutants, resulting in greater mortality.

Unlike previous short-term tests of the link between size and stress, Lago d'Orta demonstrates a response to chronic pollution that lasted over 50 yr. Lago d'Orta was first chronically polluted by copper whereas in lakes contaminated by acid rain metal increase is a secondary effect. Biochemical nitrification retarded the accumulation of ammonia in the water and later led to lake acidification. The massive changes in species composition and size distribution were thus likely initiated by copper contamination. Short cores collected in the 1950s, before acidification and ammonia accumulation, showed a shift in diatom composition similar to the one observed here, corresponding with increased copper content in the sediments (Corbella et al. 1958). In the later history of the lake, the effects of the different pollutants cannot be separated. Whatever the source of stress, it reduced average size in each of three communities at different phyletic and trophic levels. Within a taxon, pollution favored smaller morphotypes. Since our analysis addressed only fossil remains, we cannot exclude the possibility that other components of Lago d'Orta plankton whose remains were not preserved responded differently. Some available data seem to indicate the dominance of small green algae, rotifers, and *Cyclops strenuus* Fisher (Bonacina et al. 1988). However, large filamentous green algae developed in the littoral (Cattaneo 1992). The available fossil assemblages suggest strongly that organisms responded to pollutants by changes in size that could be detected at both community and population levels.

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