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Effects of marine pollution on benthic Foraminifera

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13.1 INTRODUCTION

13.1.1

Population growth and the resultant acceleration of domestic, municipal, industrial, agricultural, and recreational activity are the primary causes of anthropogenic pollution of the marine realm (Norse, 1993). Such pollution produces numerous obvious biological effects, including diseases in plant and animal species (e.g. Lamb *et al.*, 1991), local or complete extinction of some species (Vermeij, 1993), changes in community structure (Bresler and Fishelson, 1994; Suchanek, 1993), loss or modification of habitat (Nee and May, 1992), and human health complications. The marine environment, as the ultimate destination of virtually all terrestrial runoff, is especially affected by pollution, and the shallow nearshore marine environment is particularly subject to frequent and extensive industrial and municipal pollution.

With increasing worldwide awareness of environmental problems, ways to detect and monitor marine pollution over time are the subject of active research. Numerous studies have demonstrated the value of various animal species in detecting dangerous ecosystem contamination (James, and Evison, 1979; Organization for Economic Cooperation and Development, 1987). Species which occupy key positions in ecosystems are especially useful biomonitors. Thus, the continual global biogeochemical cycles of inorganic and organic compounds are regulated mainly by biological activity of benthic communities, especially bacteria and protozoa (Meyer-Reil, 1986; Santschi, 1988).

Marine protozoa, especially Foraminifera, play a significant role in global biogeochemical cycles of inorganic and organic compounds, making them one of the most important animal groups on earth (Anderson, 1988; Haynes, 1981; Lee and Anderson, 1991b). They are ubiquitous in marine environments. Their tremendous taxonomic diversity gives them the potential for diverse biological responses to various pollutants, which in turn adds to their potential as index species for monitoring pollution from diverse sources. Their tests are readily preserved, and can record evidence of environmental stresses through time, thus providing historical baseline data even in the absence of background

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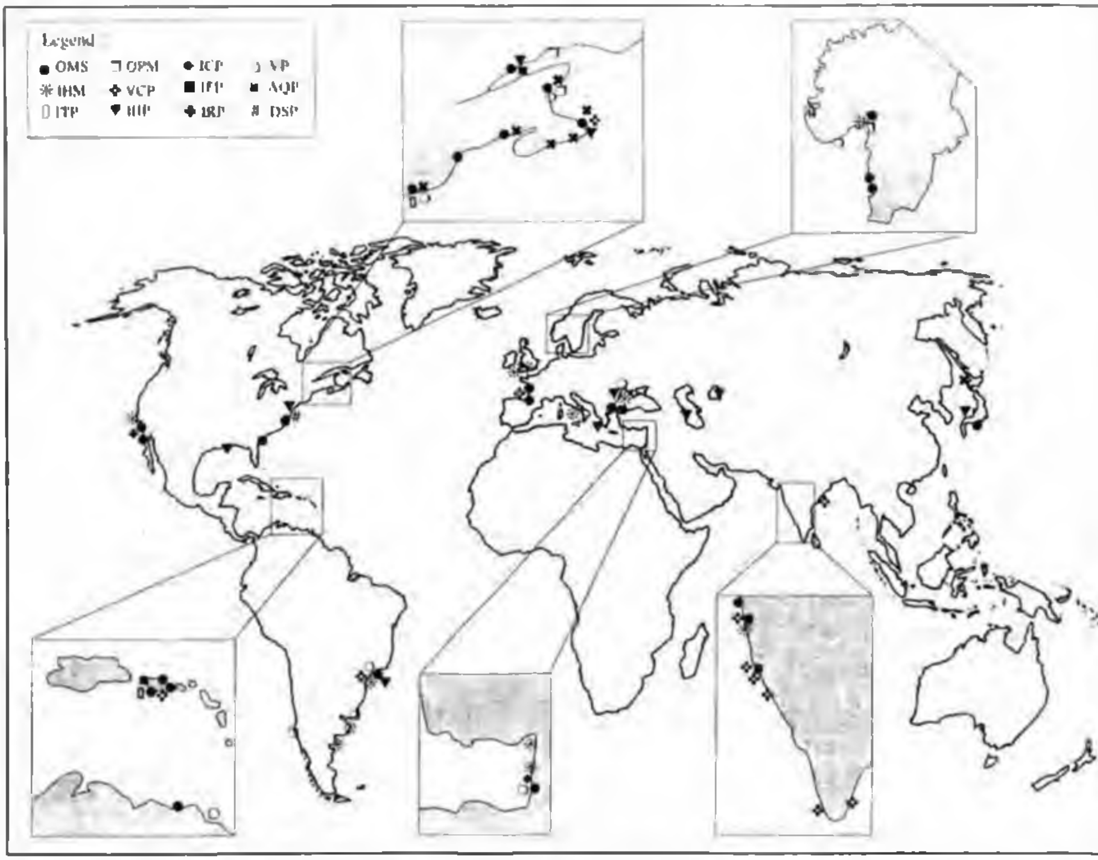


Figure 13.1 Global distribution of studies involving foraminiferal response to pollution. Symbols are as follows: OMS organic pollution by municipal sewage; OPM organic pollution by pulp and paper mills; ICP industrial pollution by coal and fuel ash; IHM industrial pollution by heavy metals; VP various unspecified pollutants; VCP various chemical pollutants, including pesticides; IFP industrial fertilizer pollutants; AQP aquaculture pollution; ITP industrial thermal pollution; IHP industrial pollution by hydrocarbons; IRP industrial pollution by radioactive wastes; DSP dredging and stream discharge pollution.

1989; Paasivarta, 1991; Belfroid *et al.*, 1994; Bresler and Yanko, 1995a,b).

Contaminated estuarine and coastal ecosystems, especially benthic ecosystems, may also contain a broad variety of pollutants. For example, the active components of oil pollution include, as a minimum, numerous polycyclic aromatic hydrocarbons (PAHs), linear hydrocarbons, phenolic compounds, and heavy metals. Active components of fuel ash include various PAHs and heavy metals (Jenner and Bowmer, 1990, 1992; Hamilton *et al.*, 1993). These pollutants may interact to influence toxicity in complex and nonlinear ways (e.g. Green *et al.*, 1993a). Additionally, some natural constituents in sea water and sediments (e.g. acid-volatile sulfide, dissolved organic carbon, or other organic matter) may also interact with pollutants to modify their toxicity (Versteeg and Shorter, 1992; Bresler and Yanko, 1995b). Sediment-dwelling benthic organisms can also affect the behavior of pollutants as well as the chemical properties of bottom sediments and water (e.g. Huttel, 1990). The biological effects of such complex mixtures can be more (or less) toxic than would be expected from simple additive effects of a single pollutant or several pollutants (Malins and Ostrander, 1993; Newmand and studies. They are small and abundant compared to other hard-shelled taxa (such as mollusks which are often used for pollution monitoring), which makes them particularly easy to recover in statistically significant numbers. They have short reproductive cycles (six months to one year; Boltovskoy, 1964), and rapid growth (Walton, 1964), making their community structure particularly responsive to environmental change. They often show species-specific responses to ecological conditions (Fursenko, 1978). They have biological defense mechanisms (Yanko *et al.*, 1994a) which protect them against unfavorable environmental factors, thus providing detectable biological evidence of the effects of pollution. These characteristics make them powerful tools for continuous *in situ* biological monitoring of marine environments.

Barely forty years have passed since Zalesny (1959) discovered the effects of pollution on foraminiferal distribution in Santa Monica Bay, California. Resig (1958) and Watkins (1961) subsequently suggested the use of benthic Foraminifera as proxy indicators of marine pollution. Since that time, the foraminiferal literature has seen an exponentially rising tide of papers on the behavior of Foraminifera in polluted areas. Brief reviews have been presented by Boltovskoy and Wright (1976), Alve (1991a,b), Culver and Buzas (1995), Yanko *et al.* (1994c) and Alve (1995a).

13.1.2 General locations of studies

The geographic locations of studies on marine pollution and Foraminifera are dictated by two main factors: the distribution of human activities that generate pollution, and the distribution of foraminiferologists with support and interest in the study of pollution. As can be seen from Fig. 13.1, the geographic localities of studies in this field are concentrated primarily in industrialized countries. Studies of the effects of coastal pollution on the Foraminifera are concentrated mainly in the northern Pacific Ocean, with additional studies in the south Pacific and Indian Oceans, and the eastern Mediterranean Sea, northwestern Black Sea and Caspian Sea. Only one study has been undertaken on the Atlantic coast of the Americas. Pollution in these areas tends to be dominated by municipal sewage, but heavy metals, hydrocarbons, fertilizers, coal and fuel ash, and various other chemical pollutants are also represented.

The study of pollution in bays and harbors (again, mainly by municipal sewage but also by aquaculture, paper mills, heavy metals, fertilizers and chemical pollution) have been undertaken in the North Pacific Ocean, North and South Atlantic Oceans, the Caribbean Sea, the Indian coast, and the Gulf of Mexico. The influence of pollution on Foraminifera in estuaries and lagoons has been studied mainly in the North Atlantic Ocean (Canadian), North Sea, South Atlantic Ocean, and along the Indian coast. Most of these studies focused on pollution from aquaculture, paper mills, fertilizers, heavy metals, domestic sewage, and various chemicals.

13.1.3 Research strategies

The use of Foraminifera as indicators of marine pollution may involve quantitative analysis of: (1) foraminiferal diversity, (2) population density/abundance, (3) assemblage structure, (4) test morphology, including size and proloculur morphology, (5) test ultrastructure, (6) test pyritization, (7) test chemistry, and (8) cytoplasmic/biological response.

These eight approaches can be complicated by two general factors:

(1) The first complication relates to *covariation* within and among natural and anthropogenic environmental factors. Most toxins enter the marine system from terrestrial sources, primarily through coastal or estuarine ecosystems where steep natural environmental gradients would exert control over foraminiferal distribution patterns even in the absence of anthropogenic effluents. Separating the effects of these two coincident influences can be problematic (e.g. Collins *et al.*, 1995). Moreover, environmental factors (e.g. dissolved organic carbon, especially humic acids, pH, temperature, salinity, ionic strength, rates of biotic pollutant metabolism, and degradation) can modify the toxicity of pollutants (Forstner and Wittmann, 1979; James,

Jagoe, 1996; Howard, 1997). Moreover, effluent sources – particularly from chemical processing plants – rarely produce only a single toxin, which tends to result in covariation among toxin concentrations; this in turn makes it difficult to determine which toxins are producing the observed effects on foraminiferal populations (e.g. Yanko *et al.*, 1994c). The solution to these covariation problems takes three forms: (i) statistical analysis of faunal distribution patterns and toxin/pollutant concentrations, (ii) laboratory experimentation, and (iii) historical analysis.

The statistical analysis is easier where strong point sources of effluent interrupt natural environmental gradients, but can be complicated by nonlinear causal relationships. For example, intermediate levels of some urban and agricultural organic wastes can cause a 'hypertrophic' zone (Alve, 1995a) of increased foraminiferal productivity, while very high concentrations can result in an 'azoic' (Schafer, 1970a) or 'abiotic' zone (Alve, 1995a). As a general rule, the statistical analysis of complex interactions between numerous species and a large number of environmental factors will require a multivariate approach such as factor analysis (see chapter 5). A particular application of factor analysis, the transfer function discussed in chapters 5 and 7, is a technique that would be particularly well suited to such situations; surprisingly, it has seen little use in the field of foraminferal ecotoxicology.

Laboratory experimentation may identify interactions between species and toxins by growing them under controlled conditions; this allows the researcher to experimentally resolve the problems presented by covariation, if attention is paid to the possibility of interaction among toxins as well as with varying natural conditions.

Historical analysis may be undertaken when there is sufficient stratigraphic accumulation of a pre-industrial foraminiferal record; in that situation it becomes possible to compare modern conditions with preceding unpolluted conditions even without a background study. This approach is complicated by taphonomic effects that themselves may covary with (or indeed be caused by) pollution induced changes in bottom-water chemistry.

(2) A second broad problematic area relates to the *measurement of pollutants*. Effluent concentrations can be measured by extraction from sediments, or by sampling at the sediment-water interface or in the overlying water column, but the biological relevance of such measurements is not well understood. Effluent concentrations can also show considerable temporal variation, depending on the schedules of human activity that produce and release effluents; thus, sampling dates and frequency may have a major effect on results. Additionally, it is not uncommon for effluent concentrations to be taken from published literature rather than measured at the time of sampling for Foraminifera. Further, when toxin concentrations are measured in sediments, the differing properties of sediments must be taken into account. For example, clean sands will interact with toxins in different ways than muds and clays. Toxin concentrations can thus covary with sediment type (e.g. Yanko *et al.*, 1998), which, in turn may also influence foraminiferal distributions.

13.2 FORAMINIFERAL RESPONSES TO DIFFERING SOURCES OF POLLUTION

13.2.1

Given the recent emergence of foraminiferal ecotoxicology as a discipline, it is possible to provide a relatively complete account of work on the subject to date. The discussion below is divided into twelve broad categories: municipal sewage, fertilizers, aquacultures, pulp/paper mills, hydrocarbons, heavy metals, fuel ash, chemical pollutants including pesticides, thermal, radioactive, dredging, and stream discharge. References in these areas are grouped accordingly, and are virtually complete through 1997.

13.2.2 Municipal sewage, fertilizers, and aquacultures

Marine ecosystems are sensitive to suspended or dissolved organic matter from municipal sewage, agricultural fertilizers, and aquacultures. These pollutants can affect marine ecosystems chemically (by depletion of oxygen through oxidation and bacterial decay), biologically (by the introduction of disease-bearing organisms such as viruses, bacteria, and parasitic worms), and physically (warming due to fermentation and reduced photic levels due to turbidity). Dissolved organic material as well as other compounds of phosphorus, silicon, and nitrogen, are usually abundant in such areas, creating artificially high nutrient levels. This organic material is readily metabolized by marine organisms, and can cause an increased foraminiferal abundance (e.g. Watkins, 1961; Bandy *et al.*, 1964a; Seiglie, 1971; Setty, 1976; Yanko *et al.*, 1994c) and diversity (e.g. Resig, 1958, 1960; Yanko *et al.*, 1994c; De Casamajor and Debenay, 1995). Bandy *et al.* (1964a) discussed a typical example of anomalously high living and dead foraminiferal abundances near a sewage outfall (see chapter 11). Additionally, planktonic Foraminifera were fifty times as abundant in sediment near the outfall as elsewhere at similar depths. Bandy *et al.* (1964a) also noted that agglutinated species were most abundant among dead tests in the outfall area, which is probably a taphonomic effect related to dissolution in sulfide-rich (Fe_2S) black sediments. Other workers have noted that in some areas polluted by domestic sewage, Foraminifera can have unusually large, well ornamented tests and relatively few deformed tests (e.g. Watkins, 1961; Yanko *et al.*, 1994c). These observations suggest that artificially high levels of nutrients may enhance the general viability of foraminiferal populations.

However, this is not always the case. Clark (1971) found a strong inverse relationship between the density of Foraminifera in the sediment and the production level of nearby aquaculture operations. No live Foraminifera were found by Schafer (1970a) in an 'azoic zone' ('abiotic zone' of Alve, 1995a). Similar observations have been made by others (Bandy *et al.*, 1964b; Schafer and Sen Gupta, 1969; LeFurgey and St Jean, 1975). The azoic zone is usually located in the immediate vicinity of the outfall where bacterial breakdown of extreme concentrations of organic material causes oxygen depletion, eutrophication, and high mortality of Foraminifera (e.g. Bandy *et al.*, 1965b; Schafer *et al.*, 1991).

The existence and spatial distribution of the azoic zone is influenced by the sewage type (activated or treated vs. non-activated or untreated), its geographic location (estuary, bay, or open shelf), hydrodynamic regime (current strength, direction), salinity, temperature, and effluent outfall rates (Bates and Spencer, 1979; Alve, 1995a). For example, in the eastern Mediterranean approximately three miles off Palmahim, Israel, a pipe fitted with a diffuser at its end disposes of 1,400 m^3/day of domestic sewage in a water depth of 38 m. The activated sludge (after secondary treatment) is composed of 95% water and 5% solids. In addition to the stable biomass, it may contain pathogenic bacteria, inorganic nutrients, and synthetic organic compounds. The lowest foraminiferal abundances were found at the mouth of the distal end of the pipeline, but there was no azoic zone. The highest diversity and abundance were found at stations located to the north and northwest of the sewage outfall, in an open coastal area downcurrent of the outfall with salinities of 39‰ (Yanko *et al.*, 1994c). By contrast, an azoic zone was noted near the Beledune Fertilizer factory in Restigouche estuary, Atlantic Canada, where salinity varied between 21 and 28‰, and currents spread pollutants throughout the entire estuary (Schafer, 1970a). In this situation, the combined effects of water temperature and salinity caused a density-stratified water column that led to reduced overturn and local enhancement of oxygen deficiency (Schafer *et al.*, 1995). This may be an example of the kind of nonlinear interaction that complicates our understanding of the relationship between pollutants and natural environmental factors.

Additional work on organic pollution by municipal sewage includes Alve, 1991a,b; Alve and Nagy, 1986; Bandy *et al.*, 1965a,b; Bonetti *et al.*, 1996; Cato *et al.*, 1980; Collins *et al.*, 1995; Debenay *et al.*, 1997; Eichler *et al.*, 1995; Govindan *et al.*, 1983; Hirshfield, 1979; LeFurgey and St. Jean, 1973, 1976; Latimer *et al.*, 1997; Lidz, 1965, 1966; Matoba, 1970; McCrone and

Schafer, 1966; Nyholm and Olsson, 1973; Nyholm *et al.*, 1977; Resig, 1960; Schafer, 1970b, 1971a, 1973, 1982; Seiglie, 1964, 1968, 1975; Setty, 1982; Setty *et al.*, 1983; Setty and Nigam, 1982, 1984; Scott *et al.*, 1993, 1997; Yanko, 1993, 1994, 1995, 1996a,b; Zalesny, 1959. Additional work on pollution by fertilizers includes Schafer, 1970a, 1992; Seiglie, 1975; Setty, 1976; Yanko and Flexer, 1991. Work on pollution by aquaculture includes Grant *et al.*, 1995; Schafer, 1970b, 1973; Schafer *et al.*, 1993; Scott *et al.*, 1993, 1995, 1997; and Preobrazhenskaya *et al.*, 1991.

13.2.3 Pulp and paper mills

Effluent from pulp and paper mills consists mainly of resistant organic material such as cellulose and lignin, major constituents of wood fibers. The most toxic compounds associated with this effluent are those used to break down wood fibers, rather than the wood fibers themselves. Compounds of mercury are particularly toxic; additionally, sulfides will, under well-oxygenated conditions (usually found downcurrent from the point of discharge), oxidize to sulfates. The presence of sulfides (recognizable by the smell of rotten eggs) normally indicates oxygen-poor conditions, and Foraminifera tend to have low abundances in these areas. Where sulfate levels are high, the environment is usually oxygenated and nutrient-rich, and Foraminifera grow quickly with rapid generational turnover, high productivity, and high abundances. Under these conditions, adult specimens tend to be smaller, probably because of the rapid growth and high reproductive rates characteristic of opportunistic ecological response. However, where sulfate concentrations reach their maximum, foraminiferal abundance is often lower, probably due to coincident high sedimentation rates (Tapley, 1969). In areas surrounded by pulp mills, extreme concentrations of sulfides can create an azoic, reducing environment where coal tar accumulates and Foraminifera are excluded. This variable response to pulp and paper mill effluent is another example of nonlinear faunal response to pollutant concentration.

Additional work on organic pollution by pulp and paper mills includes Alve, 1994; Alve and Nagy, 1986, 1988; Bartlett, 1966; Buckley *et al.*, 1974; Latimer *et al.*, 1997; Nagy and Alve, 1987; Schafer, 1970a,b, 1971a, 1973, 1982; Schafer and Cole, 1974, 1995; and Schafer *et al.*, 1975, 1991.

13.2.4 Hydrocarbons

In addition to 5–15% alkanes, typical petroleum products contain aromatic hydrocarbons, including monoaromatic hydrocarbons such as benzene and toluene (the most frequently used light petroleum products). Some of these are proven to be carcinogenic (Kennish, 1992). Lethal doses of aromatic hydrocarbons for marine organisms are in the range of 0.00001 to 0.01% for adults, and at the low end of that range for juveniles; doses as low as 10^{-6}% can inhibit physiological activity, and 10^{-7}% can cause pathological reactions (Bokris, 1982; Rubinin, 1983). Depending on the fraction and molecular weight, the hydrocarbons may form a film on the surface of the water, or heavier oil suspensions and tar may sink to blanket the seafloor.

The small amount of work that has been done on the effect of hydrocarbons on Foraminifera has produced conflicting results. Lockin and Maddocks (1982) reported that petroleum operation at Louisiana offshore petroleum platforms had only a minor negative effect on benthic Foraminifera. Similar results were noted around Ekofisk and Forties petroleum platforms in the North Sea (Murray, 1985). Dermitzakis and Alafousou (1987) suggested that meteoric rainfall intensified the effect of terrestrial oil seeps on the offshore marine environment near Zakynthos Island (Greece), possibly due to changes in pH. Vénec-Peyré (1981, 1984) studied the consequences of the Amoco Cadiz incident (March 16–17, 1978) on benthic Foraminifera in Cale du Dourduff, south of Roscoff, northwestern France, and concluded that it did not affect relative abundances or diversity; however, the accident did cause morphological abnormalities in foraminiferal tests. In contrast, a pronounced negative effect of oil on benthic Foraminifera was noted by Mayer (1980) in the northern Caspian Sea and by Yanko and Flexer (1991) in Odessa Bay where foraminiferal abundance and diversity decreased dramatically in oil-polluted areas. Similar negative effects were noted by Witcomb (1977, 1978) in experimental work on calcareous (*Ammonia beccarii*) and agglutinated species (*Allogromia laticollaris*). He found that crude oil inhibited growth and reproduction in both species, and that petroleum products produced narcosis and death of Foraminifera, and speculated that petroleum products might also cause a decrease in nutrient supply (primarily diatoms). A full understanding of the biological causes of this result awaits further research. V. Yanko (unpublished data) noted that respiratory functions of Foraminifera were inhibited in Black Sea sediments with high concentrations of petroleum products. The breakdown of petroleum was accompanied by the release of H_2S, ammonia, and methane, and by pH as low as 6. (Note that Bradshaw, 1961, found *Ammonia tepida*, a common indicator species, to be very sensitive to pH.)

Work on hydrocarbons also includes papers by Akimoto *et al.*, 1997; Bonetti *et al.*, 1996; Buckley *et al.*, 1974; Casey *et al.*, 1980; Latimer *et al.*, 1997; Schafer, 1992; Schafer *et al.*, 1975; Venec-Peyré, 1981, 1984; and Yanko and Flexer, 1992.

13.2.5 Heavy metals

The biological relevance of temporal variation of heavy metal toxin concentrations, and of heavy metal concentrations in sediments versus in the water column is not fully understood and, although these factors are undoubtedly important, sampling programs have generally not been designed to assess their relative impact on the Foraminifera. Thus, it is prudent to discuss this research by separating three categories based on sampling methods. The first category includes papers concerning the distribution of living/fossil Foraminifera in sediment samples where the concentration of heavy metals was not measured directly but taken from literature reports. The second group includes information about Foraminifera based on correlation between their distribution and heavy metal concentrations measured in the same sediment samples. The third set of papers concerns ecotoxicological laboratory experiments on Foraminifera and heavy metals.

Studies based on literature reports of heavy metals. This body of research accounts for much of the early pioneering work in the area of foraminiferal ecotoxicology. Boltovskoy (1956) described a stunted fauna (small in size, lacking ornamentation, with a tendency to asymmetry and monstrosity) in some lead-polluted areas of the northern Argentinean shelf. McCrone and Schafer (1966) noted that trace elements may affect morphology of benthic Foraminifera in the Hudson estuary. Schafer (1973) found that foraminiferal diversity decreased in an area adjacent to a Pb–Zn smelter outfall. Setty and Nigam (1984) found depauperate (in both abundance and diversity) populations of benthic Foraminifera, with high percentages of abnormal individuals, in the area adjacent to a titanium processing plant (near Trivandrum, west coast of India) where a mixture of sulfuric acid, soluble iron, and other metallic salts were discharged. A similar pattern was discovered by Naidu *et al.* (1985) in the Visakhapatnam harbor area (east coast of India) where marine waters were polluted by domestic sewage and a variety of heavy metals (e.g. Cu, Fe, Pb, Zn, Ni, Cr, As). These papers served to draw attention to the fact that foraminiferal morphology – in addition to abundance and diversity – is affected by heavy metal pollution.

Studies based on heavy metal and foraminiferal analyses of the same samples. This body of research is generally more recent and planned with specific ecotoxicological analysis as the primary goal. Ellison *et al.* (1986) investigated foraminiferal response to trace metal (Zn, Cr, and V) contamination in the Patapsco River and Baltimore Harbor, Maryland. They discovered a very depauperate foraminiferal assemblage (fewer than 10 individuals per g^{-1} of sediment), composed of six species, and dominated by *Ammobaculites crassus*. Alve (1991a) analyzed fossil benthic Foraminifera in two short (170–190 cm length) sediment cores taken in

Sorfjo (Norway) at depths of 15–53 m, and found a historical record of foraminiferal populations that were characterized by reduced abundances, dominance of agglutinated species, frequent occurrence of abnormal tests (with seven different modes of deformation), and high concentrations of pyritized specimens in areas polluted by heavy metals. A comprehensive study of Foraminifera as indicators of heavy metal pollution was undertaken by Sharifi (1991) and Sharifi *et al.* (1991) in Southampton Water, southern England. Of the 67 species found in 250 grab samples, some were able to tolerate pollution, and their relative abundance increased at the discharge point, whereas other species developed test deformities. Banerji (1992) compared distributions of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn) in sediments and in foraminiferal tests. His results show that species diversity corresponds positively with Fe, Mn, and Zn concentrations in the sediments and negatively with Co, Ni, and Pb concentrations. He found that although *Ammonia* and *Elphidium* species occur together, the former prefers environments enriched with Fe, Mn and Zn, and that an increase in concentrations of Cd, Co, and Pb was accompanied by a decrease in the abundances of *Ammonia* and *Lagena* spp. Such elements as Cu, Zn, and Cr were more readily absorbed into foraminiferal tests than Ni and Pb. At present, comparative observation suggests that it may be possible to develop a scale of gradational species response to heavy metal contamination by using the Lagenida, various agglutinated species, species of *Spiroloculina*, *Triloculina*, *Quinqueloculina*, *Ammonia*, *Cibicides*, *Poroeponides*, and *Elphidium*, and possibly other indicator species. This body of work supports the conclusion that Foraminifera from areas with high concentrations of heavy metals develop stunted tests with numerous deformities. Coccioni *et al.* (1997) studied living and non-living benthic foraminiferal distributions at fifteen sites throughout the Goro Lagoon, Italy. The concentrations of eleven elements (V, Cr, Co, Ni, Cu, Zn, Ga, As, Pb, Th, and Cd) and total organic carbon (TOC) in the sediments were measured, and a total of 77 species from 43 genera were identified. Some sites displayed marked enrichment in Cr, Ni, Zn, Pb, and As, with Al-normalized values higher than the average values of nearby unpolluted alluvial plain pelites. These sites also exhibited an increase in TOC, a general reduction in foraminiferal test size, and a decrease of benthic foraminiferal species richness, with a concurrent increased abundance of tolerant and opportunistic species such as *Cribroelphidium translucens*. A similar trend was found by Stubbles (1993) and Stubbles *et al.* (1996a,b) who studied living and dead benthic Foraminifera in Restronguet Creek, Erme and Fowey estuaries, Cornwall, UK. Using semiquantitative microprobe analysis, they found higher concentrations of several heavy metals, including Cd, in the cytoplasm of deformed Foraminifera than in the cytoplasm of non-deformed individuals. The authors suggest that if heavy metals are responsible for deformation of foraminiferal tests, these metals should be dissolved in the water rather than stored in the sediments. This has opened a potentially important avenue of research that may increase our understanding of the biological relevance of heavy metals that are sequestered in sediments versus those dissolved in the water column. However, due to the semiquantitative nature of the instrumentation, further analysis is required.

Research reported in Yanko (1994, 1995, 1996a,b), Yanko *et al.* (1992, 1994a,b,c, 1995, 1998), and Yanko, ed. (1995, 1996) was directed at the study of benthic Foraminifera as indicators of heavy metal pollution along the eastern Mediterranean coast. Eighty-seven stations were sampled at one mile intervals on a rectangular grid covering the polluted Haifa Bay coastal area, with the relatively clean coast of nearby Atlit Bay as a control. The responses of benthic Foraminifera to ten heavy metals (Cd, Cr, Cu, Pb, Zn, As, Co, Ni, Ti, V) showed that reduced diversity and abundance, stunting of the tests, pyritization, and the presence of anomalous morphologies are closely related to trace metal contamination (Yanko *et al.*, 1998). According to the experimental results of Bresler and Yanko (1995b), there is significant biological influence of heavy metals on foraminiferal cytoplasm. Mercury ions that penetrate the cytoplasm readily interact with the sulfhydril groups of proteins by blocking them and inhibiting their function. Intracytoplasmic Cd especially interferes with sites that normally interact with calcium: particularly, Cd can penetrate mitochondria and interfere with respiratory functions. However, the Cd-sensitivity of different species, individuals, and even tissues, varies widely.

In general, it can be concluded that there is a negative correlation between concentrations of heavy metals in sediments and foraminiferal abundance and diversity, and a positive correlation between the abundance of deformed tests and heavy metals.

Work on heavy metal pollution also includes publications by Alve and Olsgard, 1997; Banerji, 1989; Bonetti *et al.*, 1996; Kravchuk *et al.*, 1997; Latimer *et al.*, 1997; Schafer and Cole, 1995; Van Geen *et al.*, 1993; Yanko and Flexer, 1991; Yanko and Kronfeld, 1992; and Yanko and Kravchuk, 1996.

13.2.6 Fuel ash

The influence of coal and fuel ash pollution on benthic Foraminifera has been studied along the coast of Hadera, Israel, near a coal-fired power station (Yanko, 1993, 1994, 1995; Yanko *et al.*, 1994c). Fuel ash particles, derived from the combustion of hydrocarbon fuels, dramatically decreases species richness and reproductive rate in Foraminifera, but does not seem to affect the test size. Agglutinated species are less affected by this kind of pollution than calcareous species. It has been speculated that coal and fuel ash particles decrease the food supply (bacteria and diatoms), and also affect foraminiferal metabolism directly (Yanko, 1994). Furthermore, some trace metals and PAHs, contained in fuel and coal ash in very low concentrations, interfere with defense systems and affect nutrient metabolism in Foraminifera (Yanko, 1994).

Additional papers on pollution by coal and fuel ash include work by Yanko, 1993, 1995, 1996b; and Yanko *et al.*, 1994b,c.

13.2.7 Miscellaneous chemical pollutants including pesticides

There are a number of chemical pollutants, including pesticides, that affect foraminiferal abundance and diversity in much the same ways as heavy metals. Some also may be responsible for erosion and corrosion of foraminiferal tests (e.g. in Thana Creek, Bombay Area, India; Setty, 1982). Following exposure to pesticides, foraminiferal biotas can exhibit biochemical and histological alteration in some specimens. Such exposure can also degrade immune systems and cause mutagenic changes (Komarovskiy *et al.*, 1993).

Additional research in this area includes work by Alzugaray *et al.*, 1979; Banerji, 1977; Bartlett, 1972; Bergsten *et al.*, 1992; Bhalla and Nigam, 1986; Boltovskoy and Boltovskoy, 1968; Bonetti *et al.*, 1997a,b; Bresler *et al.*, 1996a,b; Buckley *et al.*, 1974; Geslin *et al.*, 1997a,b, 1998; Jayaraju and Reddeppa Reddi, 1996b; Kameswara and Satyanarayana, 1979; Naidu *et al.*, 1985; Olsson *et al.*, 1973; Seiglie, 1973, 1975; Setty and Nigam, 1980, 1984; Schafer, 1968, 1970a; Schafer *et al.*, 1975; Varshney *et al.*, 1988; Wright, 1968; and Yanko and Kravchuk, 1992.

13.2.8 Thermal and radioactive waste

Research on thermal and radioactive waste is not extensive. An increase in foraminiferal abundance and a decrease of diversity in the Long Island Sound have been related to a power-plant-induced increase of water temperature (Hechtel *et al.*, 1970). Attempts to relate ^{137}Cs contamination from the Chernobyl reactor accident to foraminiferal indices in Iskenderun Bay (Turkey) yielded inconclusive results (Yanko, ed., 1996).

Other works on industrial thermal pollution include Hechtel *et al.*, 1970, and Seiglie, 1975. Work on pollution by radioactive waste includes Reish, 1983.

13.2.9 Dredging and stream discharge

Although the ecological effects of dredging and stream discharge have not been extensively investigated, it is likely that they will affect turbidity (and therefore photic levels), and mobilize nutrients and pollutants that would otherwise remain sequestered in sediments. It has been suggested that these activities change the habitat and community structure of foraminiferal assemblages (Belanger, 1976), although the mechanisms are not well understood.

Additional work on pollution and Foraminifera that do not fall in the preceding categories include Alzugaray *et al.*, 1979; Banerji, 1977; Bartlett, 1972; Bergsten *et al.*, 1992; Boltovskoy and Boltovskoy, 1968; Boltovskoy *et al.*, 1991; Boltovskoy and Wright, 1976; Bonetti *et al.*, 1997a,b; Bresler *et al.*, 1996a,b; Culver and Buzas, 1995; Geslin and Devenay, 1998; Geslin *et al.*, 1997a,b; Olsson *et al.*, 1973; Schafer, 1968, 1971a, 1973, 1975; Wright, 1968; Yanko, 1997; and Yanko and Kravchuk, 1992.

13.3 SPECIAL PROBLEMS IN FORAMINIFERAL ECOTOXICOLOGY

13.3.1 Opportunistic (resistant) species

Species that are abundant in polluted areas are likely to be tolerant (resistant) to the pollutants found there. However, even in these species toxins may produce detectable effects in their cytoplasm and test (Bresler and Yanko, 1995b; Yanko *et al.*, 1998). Species that are sensitive to pollution often express that sensitivity through their absence.

Species identified as resistant in coastal zones, bays, harbors, estuaries and lagoons include approximately 11 agglutinated and 47 calcareous species (Table 13.1). The highest number of agglutinated species (6) was found in estuaries while the highest number of calcareous species (33) was noted in coastal zones (Table 13.1). This may be an example of covariation between a natural gradient (salinity) and foraminiferal distribution patterns in an area affected by pollution.

Most eurytopic species are geographically widespread, living within a broad range of latitudes, and many can tolerate a wide range of salinity and depth. For example, *Ammonia tepida* is distributed in the Black Sea from the Danube delta (depth 3–5 m, salinity 1–3‰) to the Bosphorus area (102 m, 26‰) (Yanko and Troitskaja, 1987; Yanko, 1990a,b). In the eastern Mediterranean, this species is widely distributed on the shelf at salinities in the range of 39‰ (Yanko *et al.*, 1994a). There are also several taxa (e.g. buliminids, bolivinids, uvigerinids) which can tolerate the oxygen-deficient conditions that often accompany pollution. The dominance of agglutinated Foraminifera in areas close to domestic outfalls (e.g. Bandy *et al.*, 1964a) may be more closely related to fresh-water input than to discharge of pollutants or dissolution of calcareous forms (Alve, 1995a).

Alve (1995a) concluded that, in general, those species that are most abundant and geographically widespread are most tolerant of environmental pollution. If Alve's conclusion is generally valid, then these eurytopic species offer the possibility of comparative studies in widely separated geographic areas – an aspect of foraminiferal ecotoxicology that has not yet been explored.

13.3.2 Morphological deformity of foraminiferal tests

13.3.2.1 Distribution and correlation

Morphological deformities in fossil foraminiferal tests have been noted by researchers since the last century (e.g. Carpenter, 1856; Rhumbler, 1911). In recent years, reports of deformities in modern Foraminifera have become increasingly common, and further documentation of deformed fossils has been made (e.g. Bogdanowich, 1952, 1960; Pflum and Frerichs, 1976). Most of the available data on deformities were summarized by Boltovskoy and Wright (1976), Haynes (1981), and Boltovskoy *et al.* (1991), who noted that deformities may be attributed to mechanical damage or environmental stress, but concluded that there is no consensus as to the underlying causes of most deformities. Deformed tests of Foraminifera have been reported from areas contaminated by heavy

Table 13.1 Pollution tolerant species and the environments in which they have been studied.

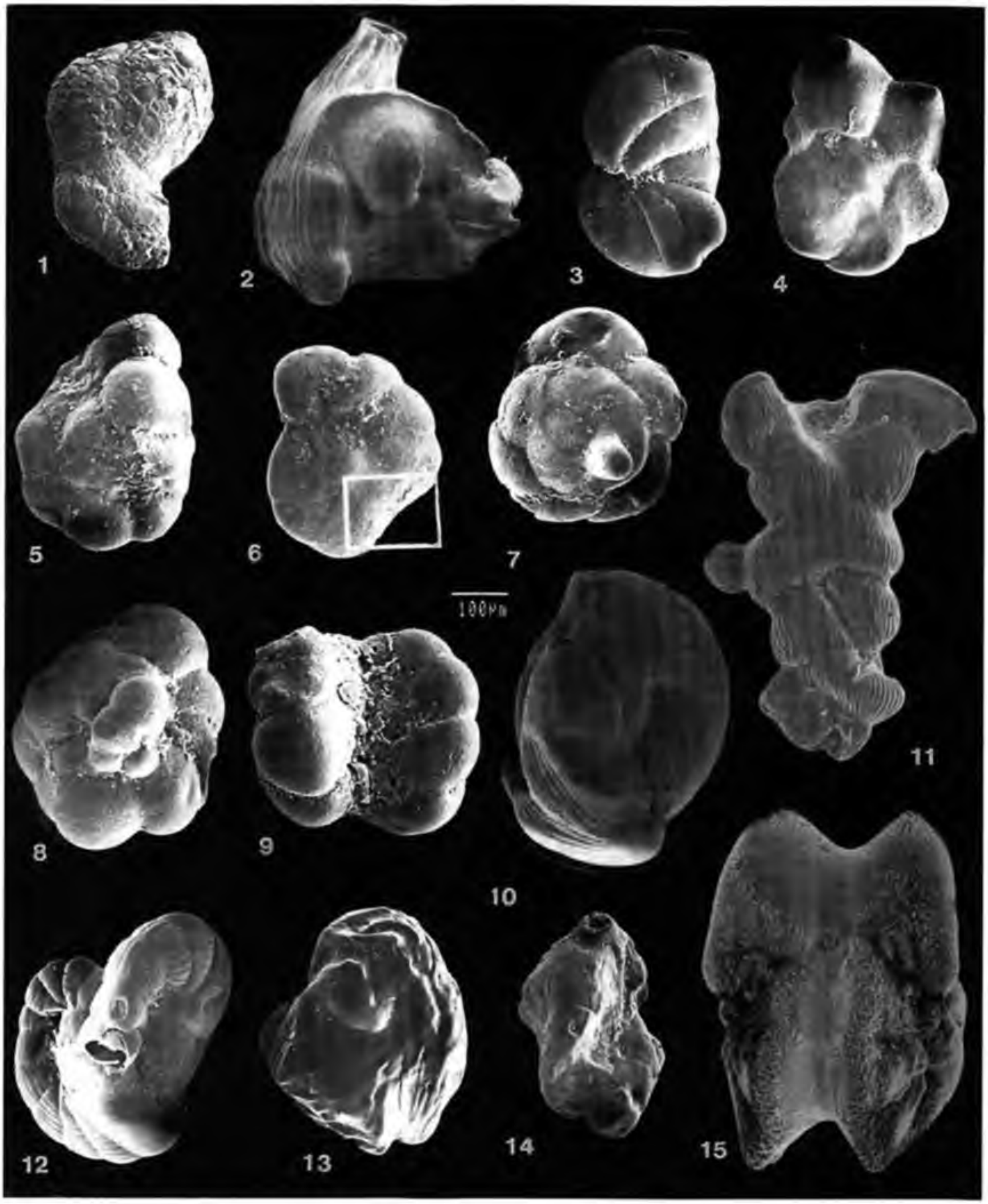
Species	Coastal zone	Bay and harbor	Estuary	Lagoon
Ammobaculites crassus	+			
A. salsus		+		+
Ammomarginulina fluvialis			+	
Eggerella advena	+	+	+	
Eggerelloides scabrus		+		
Miliammina earlandi?				+
M. fusca	+	+		
Reophax arctica	+			
Spiroplectammina biformis			+	
Trochammina inflata			+	
T. pacifica	+	+		
Ammonia annectens	+			
A. beccarii	+	+		
A. caspica	+			
A. dentata	+			
A. sobrina	+			
A. tepida	+	+		+
Ammonia sp.			+	
Amphistegina lobifera	+			
Bolivina vaughani	+			
Buccella frigida	+	+	+	
Bulimina marginata denudata	+			
B. striata	+			
B. subornata	+			
Buliminella elegantissima	+	+		
Cibicides aknerianus	+			
C. advenum	+			
Cribroelphidium translucens				+
Discorbis columbiensis	+			
Elphidium articulatum	+			
E. bartletti	+			
E. caspicum (= gunteri)	+			
E. clavatum/incertum group		+	+	
Elphidium excavatum	+	+		
E. lidoense	+			
E. margaritaceum			+	
E. norvangi	+			
E. orbiculare		+		
E. poeyanum	+			
E. translucens	+			
Florilus boueanum (= Nonion boueanum)	+			
F. grateloupi (= Nonion grateloupi)	+			
F. scaphus	+	+		
Fursenkoina pontoni	+	+		
Haynesina depressula			+	
Martinottiella communis	+			
Miliolinella subrotunda	+			
Protelphidium paralium (= Haynesina germanica)	+			
Pseudotriloculina subgranulata	+			
Quinqueloculina rhodiensis		+		+
Q. seminulum	+	+		
Rutherfordoides cornuta	+			
Spiroloculina excavata	+			
Stainforthia fusiformis			+	
Triloculina brevidentata		+		
T. marioni	+			
Uvigerina dirupta	+			
U. peregrina	+			

metals, domestic sewage, and various chemicals, including liquid hydrocarbons. See Boltovskoy *et al.* (1991), Alve (1995a), Yanko *et al.* (1998) for detailed reviews of deformities and their probable reasons. Examples of deformed tests from polluted environments can be seen in Fig. 13.2.

Measures of deformity rely on the kind, degree, frequency, and species-specificity of deformity, but the frequency is the easiest measure to quantify. The percentage of deformed Foraminifera can increase dramatically in polluted areas (e.g. Lidz, 1965) where Foraminifera display a wide variety of deformities, including extreme compression, double apertures, twisted coiling, aberrant chamber shape, and protuberances. Various other workers have catalogued similar lists of deformities, but the genesis of specific deformities is often an unresolved matter. Some deformities undoubtedly result from weak calcification or subsequent damage to areas that are weakly calcified. Other deformities (e.g. twisted keels, distortion of equatorial plane) may result from repair and overgrowth by subsequent whorls that cover such damage, resulting in distortion of the normal shell geometry (Röttger and Hallock, 1982; Wetmore, 1998). Thus, deformities that have quite different appearances may have the same underlying physiological cause. Other deformities (e.g. multiple apertures or twinning) are clearly different in nature and may be attributable to differing responses to different toxins. Yanko *et al.* (1995), using sulfaflavine fluorescence and chlortetracycline fluorescence, were able to distinguish morphological deformities caused by mechanical damage from those caused by pathological morphogenesis.

Compared with other pollutants, it appears that trace metals have the most conspicuous deleterious effect upon Foraminifera. Like many other kinds of pollutants, heavy metals often cause reduced population density and diversity, and stunting of tests, but increased frequency of deformed tests is a common aspect of virtually all studies of heavy metal toxicity. Yanko *et al.* (1998) report that at least 30% (65 species from 20 calcareous families and one agglutinated family) of all foraminiferal specimens living on the northern Israeli continental shelf exhibited one of 11 distinct types of morphological deformity in their tests. High proportions of deformed tests in living Foraminifera (up to 10% of the entire assemblage) were also found at all sites showing high metal concentrations in the Goro Lagoon, Italy. This contrasts with observations of 1% or fewer deformed specimens in natural populations. The types of deformity include: abnormal coiling, aberrant chamber shape and size, poor development of the last whorl, twisted chamber arrangement, supernumerary chambers, protuberances, multiple apertures, irregular keel, twinning, lateral asymmetry, and lack of ornamentation (Coccioni *et al.*, 1997). A similar pattern was found in Sorfjord, Western Norway (Alve, 1991a), and in Southampton Water, southern England (Sharifi *et al.*, 1991). Deformed Foraminifera are represented among the porcelaneous, hyaline, and agglutinated suborders, among various modes of life (epifaunal, infaunal, attached, epiphytic, symbiotic, mud-dwelling, sand-dwelling), and among different morphotypes (milioline, trochospiral, planispiral, flattened, etc.) (Setty and Nigam, 1980; Banerji, 1989; Vénec-Peyré, 1981). Thus, morphological deformities do not show differential

Figure 13.2 Examples of test deformities in live Foraminifera found in Haifa Bay region, Israeli shelf, eastern Mediterranean. 1, *Eggerella advena* (Cushman), specimen with twisted chamber arrangement; 2, *Adelosina pulchella* d'Orbigny, specimen with twisted chamber arrangement of the first whorl and aberrant chamber shape; 3, *Nonionella atlantica* Cushman, specimen with aberrant chamber shape and size, 4–6, *Ammonia compacta* Hofker, specimens with aberrant shape of the last three chambers (4), twisting of the test (5), and non-development of test (6); 7–9, *Ammonia tepida* (Cushman), anomalous protuberance on dorsal side (7), twisting of the entire side (8), and twinning of two specimens by ventral side (9); 10, *Adelosina intricata* (Terquem), specimen with wrong coiling, twisted chamber arrangement, aberrant chamber shape, and double aperture; 11, *Vertebralina striata* d'Orbigny, specimen with additional chamber, and aberrant shape of chambers and aperture; 12, *Peneroplis planatus* (Fichtel and Moll), twinning of two specimens; 13, *Triloculina marioni* Schlumberger, severely deformed specimen; 14, *Cycloforina villafranca* (J. and Y. Le Calvez), severely deformed specimen; 15, *Pararotalia spinigera* (Le Calvez), twinning of two specimens by dorsal side.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representation based on taxonomic affinity, feeding strategy, or test morphology.

There are indications, however, that some types of environmental stress may cause species-specific deformity. For example, *Adelosina cliarensis* exhibits increased frequency of deformation in response to low salinity, while *Amphistegina lobifera* appears to show an increased incidence of deformity in response to increased Cd concentrations, *Cibicides advenum* seems responsive to Cr, and *Pseudotriloculina subgranulata* to Ti.

High frequencies of test deformity of living benthic Foraminifera are generally regarded to be sensitive *in situ* indicators of marine pollution by heavy metals. However, the biochemical and crystallographic mechanisms of the development of such deformities remain to be studied by culture experiments under controlled conditions and known heavy metal concentrations.

13.3.2.2 Wall texture of deformed tests

The study of the wall texture of abnormal tests, as a possible explanation of deformation related to biomineralization processes, is a new research domain. Geslin *et al.* (1998) have studied ultrastructural deformation in the genus *Ammonia* using scanning electron micrographs. Abnormalities consistently observed in the walls of these deformed tests involve either disorganization of the wall texture due to changes in crystallite arrangement and orientation (Debenay *et al.*, 1996), or the presence of interlamellar cavities.

Normal tests of *Ammonia tepida* are made of crystallites arranged in elongate calcitic elements normal to the wall. These elements are roughly continuous from one lamella to the other. In places, large cleaved crystals may form, with cleavages regularly oblique to the test surface. In deformed tests, calcitic elements may be oriented irregularly. This 'crystalline disorganization' may result from introduction of alien elements in the crystalline framework during calcification; Sharifi *et al.* (1991) showed that the deformed tests contain a high proportion of metals such as Cu and Zn, and Yanko and Kronfeld (1993) found that the deformed tests exhibit an increased Mg/Ca ratio. Interlamellar empty cavities were also observed in some deformed specimens, even when crystalline organization was normal. Such cavities were reported by Venec-Peyré (1981). On a different scale, these cavities may be compared with the interlamellar cavities formed in the abnormal shells of some bivalves, particularly of the oyster *Crassostrea gigas* by the hypersecretion of a jelly (Héral *et al.*, 1981, *in* Alzieu, 1991). The organic process leading to these secretions may result from a metabolic perturbation.

Early exploratory research has led to several hypotheses regarding anthropogenic causes of morphological deformity in Foraminifera. Four general explanations are suggested. Test deformity may be caused by (1) direct physiological or metabolic interference of heavy metal pollutants; (2) pollution-induced changes in physical and chemical environmental parameters indirectly resulting in pathological morphogenesis; (3) poor nutritional state in Foraminifera, perhaps due to the effect of habitat damage on other organisms in the food web; and (4) anthropogenic intensification of the normal (low-level) background causes of test deformity seen in natural populations. It is clear that in the near future, controlled laboratory experimentation will play a central role in assessing these hypotheses before a full understanding of the relationship between foraminiferal test deformity and pollution is developed.

13.3.2.3 Morphometry and cytology

Morphometric and cytological studies of normal and abnormal *Amphistegina lobifera* tests from the north Israeli coast have been undertaken to distinguish and describe tests with varying morphologies and to understand the pathogenesis of abnormalities (Yanko, ed., 1995; Yanko *et al.*, 1995). Although only a single index of deformity was used, the deformed and undeformed populations of specimens were statistically distinct. A normal test of *A. lobifera* may be described as a bi-convex lens. Several categories of shell deformation were identified that caused distortion of that natural shape. These deformities include dorso-ventral asymmetry, loss of a segment of the peripheral margin, lateral asymmetry, irregular keel, abnormal aperture, and various other miscellaneous deformations.

Of these types of deformation, only one (loss of a segment of the periphery) was associated with an increase of sulfaflavine fluorescence (which measures protein content in the shell), and only in the immediate area of the defect. These data indicate that segment loss may have a different pathogenesis than other abnormalities; namely, segment loss is likely to be a result of shell damage and regeneration. During regeneration, especially in the early stages of this process, the protein/Ca ratio increases in the new part of the shell, probably due to intensification of protein synthesis. This suggests that the other remaining test deformities may be the result of pathological morphogenesis rather than of damage and subsequent repair. While the cytoskeleton may play an important role in normal and pathological morphogenesis in metazoan and protozoan cells (Albrecht-Buehler, 1977; Anderheide *et al.*, 1977; Brandle and Gabbiani, 1983), the synthesis of shell-building proteins and their calcification is not apparently involved in pathological morphogenesis of the shell.

It has been proposed that trace metal pollutants weaken the Foraminifera and enable bacteria to attack their cytoplasm (Alve, 1991a). This is readily apparent from stunting of the test and morphological deformities (Alve, 1991a; Yanko *et al.*, 1994c), and is probably related to the fact that heavy metals (e.g. Cd) dramatically increase membrane permeability and mortality of Foraminifera (Bresler and Yanko, 1995b).

The extent to which Foraminifera are protected from pollutants is also a factor deserving some attention, since it will increase our understanding of the factors controlling distribution patterns. Research in this area suggests several potential biological defense systems; they include (1) a mucopolysaccharide coat that forms an additional diffusion barrier and binds some cationic xenobiotics; (2) a membrane carrier-mediated transport system for elimination of anionic xenobiotics from the cytoplasm; (3) active intralysosomal accumulation and isolation of some cationic xenobiotics; and (4) haloperoxidases that transform xenobiotics to their haloderivatives (Bresler and Yanko, 1995a).

13.3.3 Pyritization

Pyritization has been found in living Foraminifera as well as in fossils, though the percentage of pyritizated tests is significantly higher in empty tests than in tests with protoplasm. Two taxa, *Ammonia tepida* and *Porosononion martkobi ponticus*, are regularly pyritizated in the Black Sea (Yanko and Kravchuk, 1992). The degree of pyritization of living individual tests ranged from 2% to 50%, and sometimes higher. The reasons for pyritization in marine sediments and foraminiferal tests are not completely clear. Some have suggested that it is connected to chemical processes that result in metabolization of organic matter under anaerobic conditions by sulfate-reducing bacteria, diffusion of sulfide into sediments, or concentration and reactivity of the iron minerals. The process is clearly connected to the redox conditions and the concentration of H_2S. High percentages of pyritized foraminiferal tests have also been found in oxygen-deficient polluted areas. The frequency of pyritized foraminiferal tests may therefore show promise as a parameter to indicate certain types of polluted environments.

13.3.4 Asexual reproduction

Foraminifera can reproduce by both sexual and asexual modes (see chapter 3). Fursenko (1978) has suggested that under stressful conditions Foraminifera preferentially revert to the asexual part of their life cycle. The causes of stress can include, for example, hyposalinity (Yanko, 1989, 1990a) and decreased nutrient levels (Fursenko, 1978). Trace metal pollution may also stress the Foraminifera by causing cellular injury and subsequently lead to a decrease in the efficiency of metabolism and protein synthesis (Ganote and Vander Heide, 1987). Life-supporting metabolic systems then function at a reduced level (Baserga, 1985) and the energetic cost of meiosis may be too great compared to mitosis (Effrussi and Farber, 1975). This hypothesis is supported by the observation that megalospheric forms of *Ammonia tepida* are strongly dominant where toxic trace metal pollution is prevalent (Yanko *et al.*, 1994c). If a high percentage of megalospheric forms is characteristic of stressed environments, then this characteristic, in conjunction with other indices, may prove a useful tool in monitoring marine pollution.

13.3.5 Chemistry of deformed tests

Several studies have been undertaken to determine whether elevated concentrations of heavy metals in sediments affect test chemistry and morphology (Sharili, 1991; Sharifi *et al.*, 1991; Yanko and Kronfeld, 1993). The primary result of these studies is that deformed Foraminifera show elevated Mg/Ca ratios when compared to non-deformed Foraminifera, especially in severely polluted areas. Deformed tests of all studied species are characterized by increased Mg concentration in their tests, so it is unlikely that this is a species-specific effect. Several hypotheses might be suggested to explain this observation: One is that heavy metals directly affect the crystal structure of the calcite shell, or foraminiferal cytoskeleton, or both. Another is that heavy-metal toxicity affects foraminiferal metabolism in ways that influence Mg/Ca ratios indirectly during calcification. A third is that other pollution-associated environmental effects mediate Mg/Ca ratios. The process of shell calcification includes the development of a glycoprotein organic matrix, *anlagen*, followed by mineralization by Ca^{++} and bicarbonate in the glycoprotein centers (Fursenko, 1978; Hemleben *et al.*, 1977). Other cations (e.g. Ba and Cd) can also be included in the crystal structure of the test (Fritz *et al.*, 1992; Lea and Boyle, 1989). It is likely that the biochemical transport systems and sites for the Ca^{++} binding cannot easily distinguish between these ions. Trace metals may also affect the ratio of the major element (Ca and Mg) uptake (Yanko and Kronfeld, 1992, 1993). Any of these factors may affect the crystal structure and cause strong morphological deformities in foraminiferal tests. The cytoskeleton, which defines the shape of the organism, may also be affected by heavy metals as has already been shown for the ciliates (Anderheide *et al.*, 1977).

13.3.6 Cytology

Organisms have numerous adaptive biological defense mechanisms that protect them against foreign chemicals in their environment (xenobiotics). If these anti-xenobiotic defense mechanisms fail to protect the organisms, the results will be expressed as a pathological disruption of biological structures on various molecular to ecosystemic levels of hierarchical organization. Even if the biological effect of a single toxic compound is simple and well-understood in isolation, the behavior of the same pollutant in complex natural ecosystems inevitably becomes complicated and nonlinear. Understanding this complexity will require the application of cross-disciplinary new methodologies. An example is the use of fluorescent microscopy and fluorometry to study the cytoplasmic response of Foraminifera to pollution (Bresler and Yanko 1994b, 1995a,b; Bresler *et al.*, 1995, 1996a, 1997). If the 'health' of foraminiferal species can be objectively characterized by biological parameters (e.g. biophysical, morphophysiological, histopathological, cytogenetic, physiological, and biochemical bioindicators) and measured using vital/supravital microfluorometry, then changes in these parameters can be used as early response indicators of exposure to environmental pollutants. Fluorescent probes allow the scientist to visualize and examine quantitatively the structural and chemical organization of cells and their organelles, including specific functions, such as genetic activity or carrier-mediated transport and metabolism, with great precision and sensitivity. Such techniques would enable us to: (1) assess the interaction of pollutant and antixenobiotic biological defense mechanisms; (2) determine the effect of pollutants on structures and metabolic reactions; and (3) predict the future behavior of a given species under polluted conditions. Accordingly, the following objectives can be set: (1) to examine the health of pollution-tolerant species (e.g. *Ammonia tepida*) under natural conditions; (2) to examine the response of the these species to a spectrum of natural conditions (varying temperature, salinity, concentrations of natural xenobiotics, and dissolved organic matter); and (3) then to examine how varying levels of a contaminant can modify these natural responses to yield pathological and pre-pathological changes that could serve as indicators of the degree of pollution. As with other organisms, Foraminifera have a number of defense mechanisms that can protect them against xenobiotics (Bresler and Yanko, 1995a; Yanko *et al.*, 1994a). For example, carrier-mediated System of Active Transport of Organic Anionic xenobiotics (SATOA) eliminates many endo- and xenobiotics from the cytoplasm. Therefore, the quantitative study of SATOA in Foraminifera can be used for early ecological monitoring and prediction of the impact of anthropogenic pollution on ecosystems, as well as for retrospective analysis of paleoecosystems.

All these parameters can be examined by the use of biophysical, cytophysiological, biochemical, and morphological vital microfluorometrical techniques, including fluorescent analysis at the microscopic level (Bresler and Nikifirov, 1981). New devices for vital biophysical studies, especially contact fluorescent microscopes and microspectrofluorometers, can be used to examine, *in situ*, various biological parameters at the molecular and cellular levels and their distribution at higher levels of biological organization. The main parameters (and methods) of interest are the following:

(1) Monitoring of respiration and metabolic state of mitochondria by measurement of inherent blue fluorescence of reduced nicotinamide adenine dinucleotide (NAD) and green fluorescence of oxidized flavine adenine dinucleotide (FAD) in living cells and tissues.

(2) Detection of conformation changes of tryptophan-containing proteins by inherent ultraviolet (UV) fluorescence. For example, inherent UV fluorescence of some tryptophan-containing proteins has been used to study their binding with copper ions (Engel and Breawer, 1987; Bresler and Yanko, 1995b).

(3) Determination of enzyme activity *in situ* by using corresponding fluorogenic substrates and microfluorometry. The activity of some intracellular enzymes also may be sensitive indicators of cell viability (Bresler and Yanko, 1994).

(4) The study of activity and kinetics of transport systems used for the elimination of xenobiotics, such as the system of active transport of organic anions (SATOA) and multixenobiotic resistance (MXR) transporter, by using marker fluorescent substrates of SATOA or MXR, specific inhibitors, and microfluorometry (Bresler *et al.*, 1975, 1979, 1983, 1985, 1990; Bresler and Fishelson, 1994; Bresler and Yanko, 1995a,b; Fishelson *et al.*, 1996).

(5) The examination of cell cycle phases, functional state of nuclear chromatin, DNA concentration and aneuploidy (DNA variability in individual nuclei), and DNA damage by using versatile probe acridine orange (AO) and two-wavelength microfluorometry (Darzynkiewicz 1990; Fishelson *et al.*, 1996).

(6) Cell chemistry, i.e. content of DNA, RNA, proteins, mucopolysaccharides and carbohydrates, lipids, thiol groups, and intra- and extracellular ions (especially calcium), can be studied with special fluorescent probes and microfluorometry.

(7) Permeability of plasma membranes, epithelial layers, or histohematic barriers can be examined by using various fluorescent markers, especially fluorescein, and microfluorometry (Bresler and Fishelson, 1994; Bresler and Yanko 1995).

(8) Cell viability can be studied by using vital staining with AO or neutral red, fluorescent microscopy of their intralysomal, and microfluorometry (Bresler and Fishelson 1994; Bresler and Yanko, 1995b). This approach is often used in ecotoxicology to distinguish living and dead cells and tissues.

(9) Cell and tissue morphology and pathomorphology can be examined by using contact fluorescent microscopy and epi-microscopy to detect early signs of pathology, to identify the

type of pathology, and to identify specific target organelles, cells, and organs. In the field of ecology and ecotoxicology of aquatic animals, especially invertebrates, environmental pathology is clearly underdeveloped as compared with mammalian ecology and ecotoxicology (Hinton *et al.*, 1992; Cotran *et al.*, 1989; Wester and Vos, 1994). The field of foraminiferal pathology lags even farther behind. Contact epi-microscopy in incident light and contact fluorescent microscopy are promising tools for aqueous ecotoxicology (Bresler and Fishelson, 1994; Bresler and Yanko, 1994a,b, 1995a,b; Fishelson *et al.*, 1996).

(10) Cytological indications of genotoxicity and clastogenicity, especially micronucleus tests employing contact fluorescent microscopy, contact epi-microscopy, or conventional microscopy are the most universally useful and sensitive methods for detecting environmental genotoxicity produced by chemical or physical factors (Fishelson *et al.*, 1996).

13.4 CONCLUSIONS

An environmental event could be characteristically transient, resulting in varying rates of foraminiferal recovery that may range from rapid species-specific ecological opportunism to more delayed recovery and even to permanent local extinction. Even when an environmental insult is continuous rather than transient, some species may respond by showing a positive preference for the new conditions, others by showing varying levels of tolerance or intolerance. These kinds of behavior are reflected in shifting patterns of foraminiferal dominance and abundance that are not well understood – partly because the time scale of environmental insult is rarely well documented, partly because foraminiferal responses are often complex and nonlinear, and partly because biotic patterns often covary with natural and anthropogenic environmental gradients that can be difficult to distinguish. Although these questions are posed here in the specific context of foraminiferal response to pollutants, it is unavoidable that they will touch on more fundamental issues relating to the nature of opportunism, and the meaning of ecological specialization or generalization in other taxa. This is one area in which basic scientific research on Foraminifera is inseparable from the otherwise pragmatic issues of detection, monitoring, and remediation of pollution.

At the organismal level of analysis, we find that ecophenotypic responses among Foraminifera can range from test deformity to subtle changes in size and age distribution. Some of these responses are almost certainly pathological and species-specific, and some may be toxin specific as well. Some biological causes of morphological test deformity may be fundamentally genetic, or due to disruption of normal metabolic processes such as calcification, leading to weak, damage-prone test walls. Controlled laboratory experimentation and detailed cytological studies will certainly play a major role in resolving some of these questions.

Coal miners once used canaries to warn them of toxic atmospheric conditions; they did so without any understanding of the cellular biology of canaries. One might suppose that, as a purely practical expedient, we could take similar advantage of the Foraminifera as environmental indicators without understanding the underlying reasons for their ecological responses, but this is not the case. Foraminifera not only behave differently in different circumstances, but indicator species are rarely so obliging as to conveniently and consistently manifest themselves wherever they are needed to assist in our efforts at monitoring and detection of pollution. Thus, not only are species unique, but virtually every locality is unique as well, and generalization becomes difficult under the best of circumstances. For this reason, we are unlikely to identify species that have the simple, straightforward utility of the canary; instead, we will have to rely on a deeper understanding of biological and ecological processes if the Foraminifera are to achieve their full potential as environmental indicators.

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