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## The two sides of man-induced changes in littoral marine communities: Eastern Mediterranean and the Red Sea as an example

L. Fishelson<sup>a,\*</sup>, V. Bresler<sup>b</sup>, A. Abelson<sup>b</sup>, L. Stone<sup>a</sup>, E. Gefen<sup>a</sup>, M. Rosenfeld<sup>a</sup>, O. Mokady<sup>b</sup>

<sup>a</sup>Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

<sup>b</sup>Institute of Nature Conservation Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

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### Abstract

Over the last two centuries, the marine life of the Eastern Mediterranean has been influenced by two major factors: one is beneficial, and concerns species migration, such as the opening of the Suez Canal, which enriched the impoverished eastern Mediterranean Sea with over 300 species of fish and invertebrates of Red Sea origin; while the other, a negative and possibly wider-reaching factor, is that of man-made pollution, which has induced unpredictable changes, destabilizing the biological world in both magnitude and duration. Initially cryptic, the effects caused by pollutants first occur at the biochemical and cellular levels of an organism, causing alterations and deviations from the normal, strongly mobilizing its defense systems. Conventional methodologies of ecological analyses, based on species and specimen numbers, cannot detect such alterations. Studying several mollusk populations from polluted and reference sites of the Red Sea and Mediterranean littoral of Israel, we used specific markers for *in vivo* and *in vitro* studies to expose the state of micronucleation; levels of defense transport systems such as membrane transport system of organic anions (SATO) and organic cations (OCT); the state of the multi-xenobiotic resistance-mediating transporter (MXRtr). Based on fluorescent microscopy and microfluorometry, these markers offer powerful tools to expose cryptic changes in the affected populations and provide data necessary for planning and management to protect animal communities and preserve their biological diversity. Comparative analysis of general gene-expression in polluted and reference sites indicates that stress factors have differentially affected the various biological taxa and separated phenotypic sub-populations, producing a novel type of punctuated selection. Such factors, although negative in their influences, in some instances altering the qualities of organisms, and establishing alterations in their hereditary information, pre-adapt them to survive and succeed in new situations.

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**Keywords:** Red Sea; Mediterranean Sea; Molluscs; Pollution; Phenotypic alterations

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\*Corresponding author. Tel.: +972-3-6408655; fax: +972-3-6409403.

E-mail address: fishelv@ccsg.tau.ac.il (L. Fishelson).

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## 1. Introduction

It is well recognized that the stability of most aquatic habitats is endangered, as exemplified in the severe eutrophication of lakes and rivers, and the worldwide and dramatic degradation of coastal marine ecosystems and coral reefs, biotopes of highest species richness and biological diversity (Suchanek, 1994; Nias, 2001). Coral bleaching, which was rare and insignificant approximately 20 years ago, has spread throughout the warm seas today, killing entire reef formations. This, moreover, is occurring at a time when millions of people are beginning to recognize the biological richness and natural beauty of these sites, and films and books extol their wonders. In the oceans, fishery is dramatically declining (FAO, 1999), and in some regions, the cost of fishing is often higher than the yield. As a present-day result of the industrial and technological revolutions in the consumer-oriented society, man has begun to show that, by acting in such a manner, he is actually cutting the very branch on which his existence depends. It is from here that modern ecology has developed in an attempt to understand ecosystems and answer questions related to their biology.

The anthropogenic impact on the littoral habitats also results from the discharge of effluents into the marine littorals, overuse of marine resources and increasing industrial pollution, involved in the provision of 'goods' to the growing human population. In recognizing the impact of such phenomena, today ecology stands at a crossroads in its analytic practices. Ecology is currently expected to provide scientific answers to practical problems connected with preserving animal communities. The challenge facing ecology is particularly critical in aquatic littoral ecosystems, which differ from terrestrial, where locally afflicted chemical or physical stresses disperse into wider regions, and often do not remain isolated. Such sites are frequently situated close to human industry and its by-products, and are also subject to increasing mechanical and other pressure from tourism (Bell et al., 1989; Kay and Liddle, 1989; Liddle, 1991; Rinkevich, 1995, 2000). It has also long been recognized that the opening of new waterways and an increase in marine transport routes, paralleled

by an increasing discard of ballast waters by ships, has enabled the active redistribution of various animal species from their original sites, often bringing a negative 'biological pollution' to the new ones.

This is exemplified in the invasion of the European gastropod *Littorina obtusata* to the New England littoral and subsequent displacement of the endemic *Illyonasa obsoleta*; the appearance in 1986 of the Far Eastern *Potamocorbula amurensis* in the San Francisco Bay, which 10 years later numbered 10 000/m<sup>2</sup>. Similarly, the invasion of North American waters by the Zebra mussel, *Dreissena polymorpha*, led to severe damage to various installations for water transport (Nalepa and Schloesser, 1994); the invading population of the gastropod *Repana repana* destroys the commercially important black mussel population along the Black Sea shores (personal information); the flourishing of the tropical medusa, *Rhopilema nomadica* along the East Mediterranean (Galil et al., 1989), endangers bathers on the Israeli shores; and the expanded distribution of the algae *Caulerpa taxifora*, along the West Mediterranean shores, is destroying marine formations. Analogous to this, the relatively new technology of fish mariculture in net-cages in the sea, moreover, especially species foreign to the region, as practiced in the Gulf of Aqaba, is not only increasing the organic load on the environment, but has also introduced new pathogens virulent to the natural fish-populations (Diamant, 1996). Parallel to this, thousands of new man-made chemicals and xenobiotics are being released into the environment, either directly eliminating many of the sensitive taxa, or acting cryptically on the organisms' vital defense systems, on their DNA-structure and membrane functions, inducing deviations that adversely affect the survival and stability of biota (Fishelson et al., 1994). Although such subtle changes of a pathological nature are not detectable at the organism level, they are able to generate unpredicted selective processes (Bresler et al., 1999). We can quote here from Cicero (*De finibus*): *Omnium rerum principia parva sunt* (all big things start from small).

In order to understand such cryptic, local anomalies, there is a need to gather temporal and spatial

Table 1

Selected parameters of animal health reflecting stress that can be studied in different taxa and their sub-populations

A.	Stress-protein levels
B.	Enzyme activity, especially: MFO, acetyl cholinesterase, peroxidases
C.	Membrane transport systems of organic anions (SATO) and organic cations (OCT)
D.	MXRtr—Multi-xenobiotic transport systems
E.	State of DNA (unwinding), RNA, level of cell micronucleation
F.	State of mitochondria
G.	Permeability of membranes to fluorescent markers

quantitative data on representative species and changes in marine biological communities, and to provide scientifically and practically sound suggestions for tackling them. This study reviews findings on the positive and negative effects of Man on marine shallow-water communities of species along the shores of the Gulf of Aqaba (Red Sea) and the littoral of the Mediterranean Sea of Israel. On the basis of this, we provide some suggestions related to understanding the problems of preservation of marine natural resources.

## 2. Materials and methods

The data for this study were based on a dataset accumulated over 40 years of observations and collections in the Red Sea and the Mediterranean (Fishelson, 1973a,b, 1977, 1999, 2000), and on results from our recent studies on selected species of mollusks from the Red Sea and Israeli Mediterranean littoral using specific biochemical and fluorescent methods (Bresler and Yanko, 1995; Bresler et al., 1999). At present, in order to ascertain health parameters and before death occurs, we can determine enzyme activities, membrane transport systems of organic anions (SATO) and organic cations (OCT), particularly the state of the multi-xenobiotic resistance-mediating transporter (MXRtr) (Table 1). By using specific fluorescent markers we can also, within a few hours and using minimal cell-samples, determine the state of nucleic acids, micronucleation, state of mitochondria, and permeability of membranes. Alteration of such parameters in living organisms is evidence of stress and can be detected at very early stages. Monitoring of these parameters also involves specific antibodies and fluores-

cent microscopy. The method utilizes in vivo and in vitro contact and epimicroscopy (Bresler and Fishelson, 1994; Bresler and Yanko, 1995). Specific fluorescent anionic and cationic markers, such as Rhodamine B (RhB) for the estimation of MXRtr-activity, its inhibitor Verapamil, fluorescein diacetate (FLU) to estimate the SATO levels, and acridine orange (AO) marker for DNA/RNA analysis, have proven to be equally valuable for unicellular organisms and multicellular, including mammals. These techniques examine not only what occurred in the past, but also what is actually happening now in the studied species-assemblages, providing instant tools and guidelines for possible management and protection. We recently revealed such alteration phenomena from comparisons of populations of Mediterranean and Red Sea bivalves and gastropod mollusks from polluted and reference sites that differ strongly in their phenotypic characters (Bresler et al., 1999). In addition, as shown by Mokady and Sultan (1998), RNA-level gene expression can also serve as an important tool to expose responses to environmental stress. Monitoring at this level, we can obtain the earliest warning signs from the studied organisms. For this purpose, we studied the bivalve *Donax trunculus*, collected from the polluted Haifa Bay, and compared it with samples from reference site, Ma'agan Mikha'el. Total RNA, pooled from 8–10 specimens, was extracted separately from the hepatopancreas, gills and mantle tissue. Differential display PCR (DD-PCR) analysis was performed according to established protocols, with the following modification: we used a lower degree of degeneration in the tailing primer at the DNA synthesis step (dT<sub>12</sub>VA), and two arbitrary primers instead of one. For technical details regarding amplification conditions, visualization of banding

Table 2

Fixation of species pairs of pomacentrid fishes on both sides of Bab-el-Mandab Straits

Red Sea	W. Indian Ocean
Red Sea Hambug— <i>Dascyllus marginatus</i>	Grey Hambug— <i>Dascyllus reticulatus</i>
Sergeant Major— <i>Abudefduf vaigensis</i> <i>Plectoglyphidodon leucozona</i>	Bengal Sergeant— <i>Abudefduf bengalensis</i> <i>Plectoglyphidodon cingula</i>
Black-Tail Demoiselle— <i>Stegastes nigricans</i>	Bluish Demoiselle— <i>Stegastes lividus</i>
Red Sea Clownfish— <i>Amphiprion bicinctus</i>	Alards Clownfish— <i>Amphiprion alardi</i>
White-tail Puller— <i>Chromis pelloura</i>	Aden Puller— <i>Chromis axillaris</i>

patterns and interpretation, see Mokady and Sultan (1998) and Sultan et al. (2000).

### 3. Results and discussion

#### 3.1. Example 1: the Suez Canal phenomenon

The Red Sea and Mediterranean have been separated for millions of years. During the Miocene, while the Red Sea, an extension of the Indian Ocean, swarmed with diverse life forms, particularly along the flourishing coral reefs, the Mediterranean was almost totally dry, enclosed behind the Rock of Gibraltar (Hsu et al., 1973). With the later rise in water levels and opening of the Gibraltar straits, the Mediterranean was flooded by eastern Atlantic waters carrying with them the life forms of this ocean (Por, 1978; Fishelson, 2000). During the Pliocene, a link was reestablished between the northern end of the Red Sea and the Mediterranean, interconnecting the Indo-Pacific and Atlantic regions. This enabled Red Sea animals and plants to penetrate into the Mediterranean, such as wrasse fishes of the genera *Thalassoma* and *Coris*, now found throughout the Mediterranean (Fishelson, 2000). At the end of the Pliocene, eustatic changes in plate levels and a drop in water levels finally and permanently separated the northern end of the Red Sea from the Mediterranean. During this period, the Red Sea, behind the southern straits of Bab el Mandab, was already teeming with life forms, and various processes of isolation initiated the development of endemism, so typical for this closed sea (Goren, 1987). For example, in a number of species-pairs currently found on both sides of the Bab el Mandab straits, the Red Sea species originally developed from the Indian Ocean stock (Tables 2 and 3).

With the opening of the Suez Canal approximately 136 years ago, a process began of cross-canal migration termed ‘Lessepsian’ by Por (1978), in honor of the Canal’s engineer. However, numerous such tropical species also entered the Mediterranean passively, being transported by ships, such as, for example, the gastropod *Strombus decorus*, first found on the Turkish shores and only discovered several years later along the Israeli littoral (Fishelson, 2000). Most migrants were Red Sea species penetrating into the Mediterranean, with only a few species, such as the gobies, *Gobius paganellus* and *G. cobitis*, and the grouper, *Serranus cabrilla*, expanding in the opposite direction (Goren, 1987). Carried by the permanent along-shore anti-clockwise current of the eastern Mediterranean, the majority of the Red Sea migrants spread along the Israeli coast, and consequently, along the Levant littoral (Fishelson, 2000; Galil, 2000). Thus, ‘Red-Med’ communities of species became established along the Israeli Mediterranean continental shelf, with some species being remnants of the ancient Tethys Sea, some of the Atlantic fauna and some of the evolved endemics (Fishelson, 2000). Two vicarian communities of fish dwelling within rocky outcrops, for example, can be found at depths of 6–12 m in both the Red Sea and the Mediterranean (Table 2). This recolonization also includes corals, for example, the Red Sea gorgonian, *Acabaria erythraea* (personal observation) and the Atlantic stone coral, *Oculina patagonica* (Fine and Loya, 1995), has re-established small sections of the once flourishing Tethys fauna, and thereby increased the biological diversity.

This immigration greatly enriched the littoral of the eastern Mediterranean, which had been left

Table 3  
Vicarious fish communities in the Red Sea and Mediterranean Sea

N. Red Sea	E. Mediterranean Sea
Red Anthias— <i>Anthias squamipinnis</i>	Common Anthias— <i>Anthias anthias</i>
Bicolor Puller— <i>Chromis dimidiatus</i>	Brown Puller— <i>Chromis chromis</i>
Yellow Cardinalfish— <i>Apogon cyanosoma</i>	Imberb Cardinalfish— <i>Apogon imberbis</i>
Red Sea Wrasse— <i>Thalassoma klunzingeri</i>	Pecock Wrasse— <i>Thalassoma pavo</i>
Crowned Squirrelfish— <i>Adioryx diadema</i>	Red Squirrelfish— <i>Adioryx ruber</i>
Sweeper— <i>Pempheris vanicolensis</i>	<i>Pempheris vanicolensis</i>
Striped Grouper— <i>Epinephelus fasciatus</i>	Brown Grouper— <i>Epinephelus aeneus</i>

with a very impoverished fauna in the wake of its past desiccation (Por, 1978). An increasing number of species still continues to successfully establish themselves in the East Mediterranean. For example, only very recently, the Red Sea patellid gastropod, *Cellana rota*, was found to be forming populations on the intertidal rocks along the Israeli Mediterranean (personal observations), alongside its local sister-species, *Patella caerulea* and *P. radiata*. Over 300 animal species of Red Sea origin can currently be found along the Israeli littoral, including such economic important species as the shrimps *Penaeus sulcatus* and *P. japonicus*, and the fishes *Saurida undosquamis*, *Siganus rivulatus* and *S. luridus*. Some of these Red Sea fish, for example, the Indian mackerel, pike conger, Shrimpscad, Mollucian Goat Fish and Grunt, have become highly economically important for local populations along the Mediterranean. The importance of migratory fish for Israeli fishery was documented by Ben-Tuvia (1985). According to Spanier et al. (1989), 40.6% of fish biomass caught in the Israel Mediterranean waters is composed of Red Sea species, which comprised only 11.6% of the total species. The establishment of rocky piers along the shores, as well as sunken wrecks, have also become focal points for migrant fish-assemblages, in particular the crevice-dwelling sweepers, *Pempheris vanicolensis*, and the squirrelfish, *Holocentrus ruber*, as well as Mediterranean groupers and lobsters.

It would appear, therefore, that in this instance a man-made artifact, the Suez Canal and along-shore constructions have had until now a very positive effect, enriching the impoverished Levant marine fauna, and contributing economically

important assets. Following this, the local shallow-water, benthic species assemblages, now present a unique mix of polychaetes (Fishelson and Rullier, 1969; Amoroux et al., 1978), mollusks (Barash and Danin, 1992) and other organisms (Fishelson, 2000; Galil, 2000). This enrichment of the Mediterranean Sea by larger Red Sea taxa is shown in Table 4. However, we should not ignore certain negative aspects of such immigration, such as the transport of parasites by their fish-hosts: the fish *Siganus rivulatus* and *S. luridus* have transported into the Mediterranean endo- and ectoparasites not formerly known from this sea (Diamant, 1996). The rhizocephalan, *Heterosaccus dolfusi*, parasite on the crab *Charybdis longicollis*, infests 1% or 2% of the host in the Red Sea, whereas in the Israeli Mediterranean the infestation can rise to over 70% (Galil, 2000) (Table 4). In some instances, immigrants over-compete the autochthon species: e.g. the Red Sea intertidal mussel, *Brachidontes pharaonis*, has displaced the local *B. minimus*, and the lizardfish, *Saurida undosquamis* has displaced the local *Saurida saurida*. It is possible that we are witnessing what Van Valen (1973) termed ‘The Red Queen’ phenomenon, in which the invading species are more fit to fluctuating environments than the local ones. Certain questions have remained unsolved in this and similar cases of faunal mixtures: How permanent are the migratory species assemblages? How profound are the developing differences between the root-populations and those of established immigrants? And how deep is the genetic asymmetry between the species in the Red Sea and its members now found in the Mediterranean? Or, as stated by Hewitt (2000), how far was ‘the genetic archi-

Table 4

Invertebrates of Red Sea origin dominant along the Israeli Mediterranean shore

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<b>Echinodermata</b>
<i>Synaptula reciprocans</i>
<i>Ophiactis parva</i>
<b>Crustacea</b>
<i>Peneus semisculatus</i>
<i>Alpheus rapacida</i>
<i>A. innopinatus</i>
<i>Charybdis helleri</i>
<i>C. longicollis</i>
<i>Portunus pelagicus</i>
<i>Atergatis roseus</i>
<b>Mollusca</b>
<i>Pinctada radiata</i>
<i>Brachidontes pharaonis</i>
<i>Spondylus spinosus</i>
<i>Strombus decorus</i>
<i>Cerithium kochi</i>
<i>C. scabridum</i>
<b>Polychaeta</b>
<i>Eurythoe complanata</i>
<i>Hermodice curunculata</i>
<i>Brachiomma lucullana</i>
<b>Scyphozoa</b>
<i>Rhopilema nomadica</i>

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texture remodeled by the dynamic of colonization' (p. 908)?

### 3.2. Example 2: anthropogenic stress

Environmental stress may generally be defined as a set of negative factors that adversely affect the organism, inducing activation of protecting systems, such as increased body cover impermeability, detoxification systems and exporting mechanisms. As demonstrated by Bresler and Yanko (1995), such defense systems are present from unicellular organisms to mammals. In fact, the formation of such systems against negative environmental forces of the habitat has shaped the organisms to a great extent during evolution. Responding to selective challenges, organ-systems evolve that form the front line of environmental defense. Today, new stressors that were not encountered during past evolution, act at various levels of biological organization, reduce reproduc-

tive success by inducing sex-change (Manelis et al., 1993), or trigger imposex phenomena, as is well known in the case of TBT-antifouling paints and recently described from the Mediterranean (Rilov et al., 2000). Such types of stress from alien chemicals, metal ions, synthetic substances imitating sex-steroids, as well as increased organic load, are introduced directly (as with TBT) or with effluents into the shallow waters. Additional stress is imposed by the huge tourism and associated industries that bring scores of bathers, divers and spear-fishers to even the most remote littoral regions, directly and negatively influencing the natural species assemblages. Some habitats, such as the delicately balanced coral reefs in the tropical and sub-tropical seas, are especially vulnerable to this kind of stampeding and related stress. Data on reef-destruction worldwide are rapidly accumulating, including information on species disappearance. For example, following prolonged pollution from oil tankers and phosphate dust from a nearby harbor (Fishelson, 1973a), the northern, Israeli part of the Gulf of Aqaba has seen a drastic decline in species richness and diversity, especially the disappearance or extreme rarity of once common littoral crustaceans, such as *Ibla cumingi*, *Lybia tessellata*, *Hymenocera picta*, of the irregular sea-urchins (*Clypeaster* spp.), the sea-stars of the genus *Astropecten* and various mollusks (Fishelson, 1973a,b, 1995). Censuses taken above the coral reef tables in the protected part of the Israeli Red Sea shore show an immense decline in numbers of the small bottom-dwelling fish there. In 1985, in a count taken along 10 transects of 10 m each, 85 ( $\pm 6$ ) gobies, blennies and clinids of various species, were counted per transect. A census at the same site in 1994 showed only 24 ( $\pm 4$ ) individuals/transect (Fishelson, 1995). A similar decline has been observed in the shallow-water schools of *Pseudanthias squamipinnis* and the cave-dwelling nocturnal *Pempheris vanicolensis (oulaensis)* (Fishelson et al., 1974). Recent studies demonstrated that below the net-made fish-cages situated in the north of the Gulf, the bottom is covered by black, almost anaerobic sediment. The former wide-spread lawns of *Halophila stipulacea* grasses have dramatically retreated, and the mollusks found nearby show deep-pathological

alterations in their defense mechanisms and cytology (Bresler et al., 1999). With local currents, the sediment of this site is gradually carried toward the southwest, also sinking onto the coral reefs and possibly contributing to the death of young coral colonies. It cannot be precluded that the widely occurring bleaching of corals denuding coral reefs in the Indo–Pacific realm is also partly induced by anthropogenic factors, such as chemical pollution.

Various anthropogenic stresses in the seas have also caused a decline in commercial fish fecundity, and fisheries are collapsing in various regions of the world, such as the North Sea and the Baltic. This is the consequence of land-generated effluents bearing large amounts of fertilizers, which cause eutrophication; as well as synthetic antibiotics, steroid-mimicking chemicals and various heavy metals.

All these alien substances accumulating in the natural habitats generate specific and non-specific changes, whose long-term outcome is unpredictable. Numerous marine animal communities have changed today from stable to unstable, termed ‘Techno-Ecosystems’ by Odum (2001). Such ecosystems, once regulated by biological phenomena, are now being regulated by unpredictable stresses and xenobiotics (Fishelson, 1977, 1995). As a rule, the changes induced by these stressors depend on their magnitude and frequency of occurrence, as seen in the ability of corals to regenerate following denudation (Fishelson, 1973b). During such changes, the more sensitive organisms are eliminated and replaced by opportunistic ones, able to withstand such instability. Concomitantly, the disastrous anthropogenic impact causes a long-term accumulation of chemical and organic waste products in the environment. In some situations, for example heavy storms, such products are frequently redistributed in the water column, thus preventing rehabilitation of the species assemblages. Experiments have been made to re-establish coral reefs by planting small coral fragments (Rinkevich, 1995; Shekry et al., 2000). However, it is naive to perceive reefs as assemblages of single species and isolated colonies, and not as a synecological outcome of very complicated, co-evolutionary processes over thousands of years

(Fishelson, 1995). Establishment of barrier zones around sites to be protected, as suggested by Fishelson (1999) and by Epstein et al. (1999), can only help if the more general and ‘clean environment measures’ are also implemented.

The examples provided above underline the immense influence of man-induced alterations, both the apparently positive and the obviously negative, in the large-scale ecological stability and biological richness and diversity of our natural world. From this arises the important role of scientists involved in nature studies in revealing this role to the wider public and the decision makers. The environmental ecologists are the best equipped with the necessary theories, practice and methodological tools to reveal stress-induced phenomena in biological communities and provide reasonable argumentation for protection. However, most ecologists continue to use quantitative methods and models developed for ecosystems regulated by stable, biological and physical phenomena. For example, multivariate, deterministic models are used to monitor changes observed in various ecosystems, as if the regulating environmental factors observed in the past are inherent in the ecosystem and will continue to dominate them in the future too (see also Lewin and Lewontine, 1980). The basis for such thinking began with the simplest population index of Verhulst. Various equations, such as Sorenson’s similarity index, Shannon’s diversity index, Pielou’s evenness index and Simpson’s dominance index, which currently form the bases of ecological theory, were formulated in the first half of the 20th century. Assuming a continuous environmental stability, these statistical equations are based on species or individual per species counts, within quadrants or along transects, as the basis for population analyses and community studies. In essence, such studies, using what can be termed a ‘post-mortem’ technique, count organisms that have perished during past events and those that have survived, without recognizing the biological or physiological phenomena that were acting in such situations. Bradbury et al. (1986) argued that by focusing on quantitative data, ecologists lose vital biological information, sometimes critical to the future of the ecosystem. Following the experience of recent

years, we know that most of the stress-induced initial changes in organisms are qualitative and cryptic, at biochemical-cellular levels, initiated by actual acting factors. The long-term species-count approach, which reveals results of phenomena that acted in the past, is, therefore, inadequate to assess the current state of community health and its potential fate. The actual changes occurring in ecosystems are primarily caused by stresses of anthropogenic nature, and are inherently unpredictable as to their effect (Fishelson, 1995). We postulate that this effect will depend on the nature, strength, duration and frequency of stressors. Interacting with biological taxa such environmental stressors affect the physiology and cytology and, as long recognized by Waddington (1975), ultimately can be ‘canalized’ into the genome and modulate the hereditary attributes of local populations. As a result of this, biota experience changes on two time scales: (1) a long-term evolutionary scale that is modulated by the DNA hereditary map of the genotype and evident in speciation; and (2) small-scale changes evident in phenotype, induced by local adverse stress (see also Stern, 2000). Acting in unison such factors constitute what has been described by German scientists as the ‘*Funktions Gefuge*’ of the organism, a functional entity reflecting the evolutionary processes. In a stable environment the outcome of such developments can be predicted: namely, deviation

in mutation rates will be minimal, and thus population stability will be preserved. This postulation is based on the existence of a stable genotype and a set of physiological defense mechanisms protecting the selected phenotype. In contrast, in stress situations within mutagenic environments, in which increasing quantities of biologically interactive substances are released into the natural environment, the ability of biological defense systems to shield the genotype decreases, together with an increase in mutation rates. This, in turn, results in higher variability and fractionation of phenotypes (Stanton et al., 2000). The more we know of these ‘biological information transfer’ processes (Ryan, 1980), the more we will be able to judge the health of the organisms under study (Fishelson et al., 1994). An example of an anthropogenic induced separation of phenotypes was recently exposed in populations of the patellid gastropods *Cellana rota* and the bivalve *Pteria aegyptia* in the Gulf of Aqaba, Red Sea from polluted (Ardag) and reference (MBL) sites (Fig. 1) According to the levels of studied parameters, the activity of the multi-xenobiotic resistance transport system (MXRtr) and micronucleation (MNT), the distantly related gastropod and bivalve pair of the polluted site are closer to each other than to their conspecific from the reference site. An analogous phenomenon was observed in the Mediterranean species-pair: the gastropod *Patella caerulea*

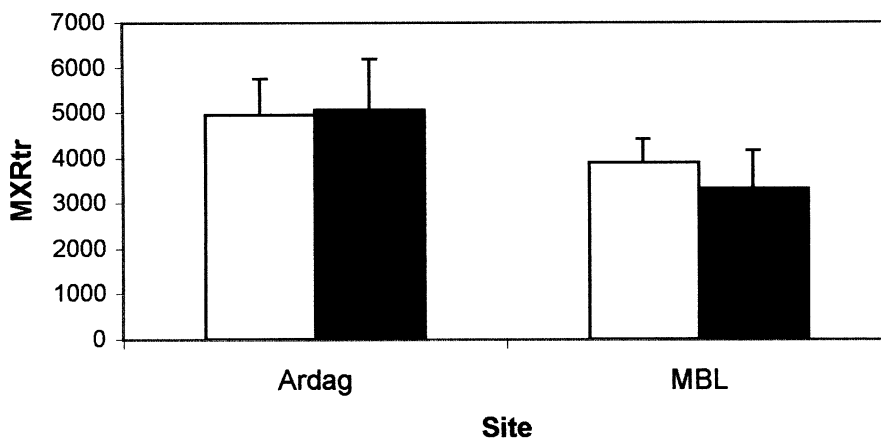


Fig. 1. MXRtr levels of two Red-Sea molluscs (■ *Cellana rota* and □ *Pteria aegyptia*) from a polluted site (Ardag) and reference site (MBL).

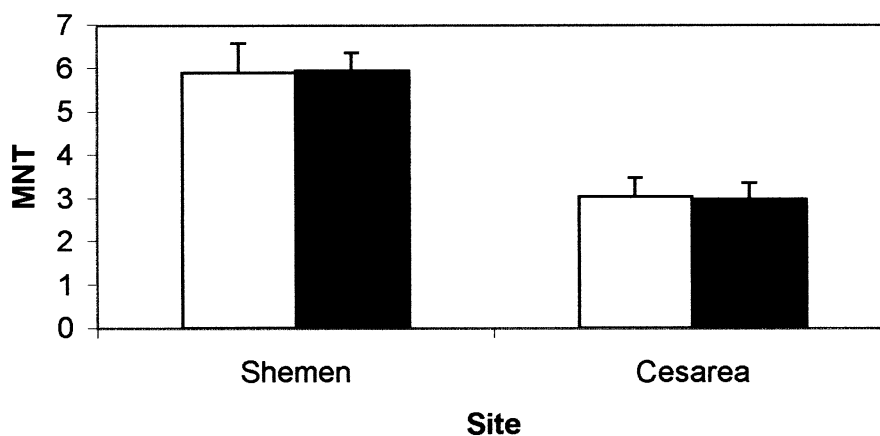


Fig. 2. MNT of two Mediterranean Sea mollusks (■ *Patella caerulea* and □ *Brachidontes pharaonis*) from a polluted (Shemen) and reference site (Cesarea).

and bivalve *Brachidontes pharaonis* from polluted (Shemen) and reference (Cesarea) sites (Fig. 2). A comparison of the relation between levels of MNT and MXRtr for all four species of mollusks

from polluted and reference sites in the Mediterranean and Red Sea reveals a strong separation between the phenotypes of both localities (Fig. 3). According to the studied parameters, the groups

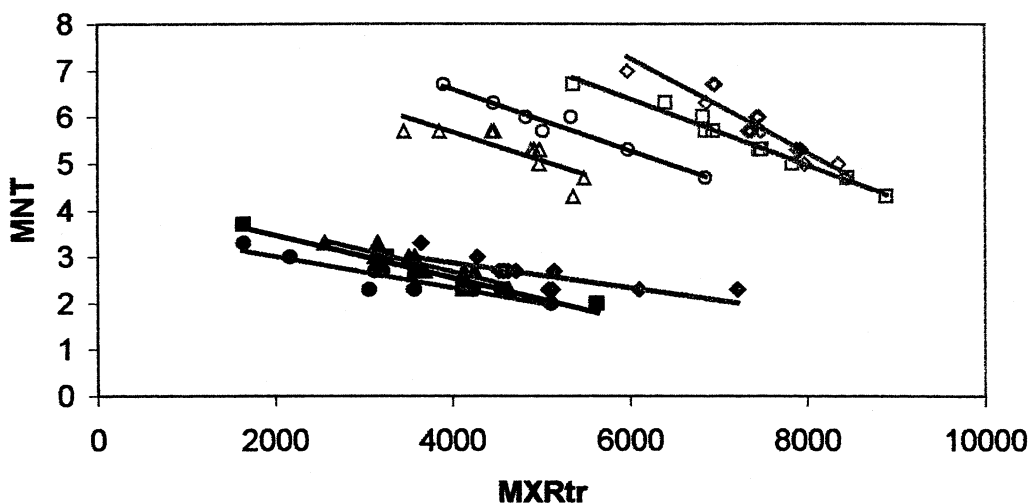


Fig. 3. Multi-xenobiotic resistance transporter (MXRtr) vs. levels of micronucleation (MNT) in four species of Mediterranean and Red Sea mollusks from a polluted (full symbols) and reference sites (empty symbols).

Red-Sea species		Mediterranean-Sea species	
○ <i>Ptereria</i> /MBL	● <i>Ptereria</i> /ARDAG	□ <i>Brachidontes</i> /Cesarea	■ <i>Brachidontes</i> /Shemen
△ <i>Cellana</i> /MBL	▲ <i>Cellana</i> /ARDAG	◇ <i>Patella</i> /Cesarea	◆ <i>Patella</i> /Shemen

Table 5

The number of markers featuring each of the observed expression patterns is displayed according to the size (either larger or smaller than 500 base pairs), and demonstrated by the various differential bands: – indicates lack of expression; +, ++, and +++ indicate low, moderate and high levels of expression, respectively

Expression pattern		Number of markers	
Polluted site	Reference site	>500 bp	<500 bp
Qualitative markers:			
+	–	3	1
–	+	10	0
Quantitative markers:			
++	+	2	3
+	++	2	2
+	+++	2	1

sampled from polluted sites form a cluster separated from the groups taken from the cleaner sites.

As for RNA-level gene expression, out of several hundred bands observed in 24 gels (a total of 14 different primers), 26 differential bands (i.e. markers) were observed in nine of these gels, representing qualitative as well as quantitative differences in gene expression (Table 5) between the populations from polluted and reference sites. Such cryptic changes in the biological taxa can also induce pathological changes (Manelis et al., 1993), such as genotoxicity marked by micronucleation and chromosomal damage (Bresler and Fishelson, 1994; Bresler and Yanko, 1995). Some of the polluting substances interfere with oxidation, desulfurization, sulfoxidation and methylation, and if not subjected to detoxification, additional malfunctions occur. The established numerical ecology

does not recognize these crucial factors and Bradbury et al. (1986) have already noted the need for a technique to enable the collection of biological qualitative data for systematic analyses of the ecosystems.

Our ability to determine these subtle and cryptic changes before the complete or partial extinction of taxa is a function of analytical scaling and methodology. The monitoring of biochemical and physiological deviations from the normal, or what Mayr (1982) calls ‘soft data’, may provide an immediate means for evaluating community health and identifying the possible nature of the stressors long before the disappearance of sensitive taxa. The various methods of chemical ecology that can be employed include techniques that enable us to determine impact areas and specificity of stressors. For example, along the shore of Haifa Bay at a chemical PVC factory site, levels of mercury found in the sand-dwelling mussel, *Donax trunculus* were 25 times higher than in conspecifics from clean sites and their cytological and physiological attributes differed from those in the control area (Table 6). By analyzing such deviations from the norm, we can begin to describe quantitatively, and in biological terms, the responses of organisms to human disturbance. This is particularly important in the tropics and the subtropics, where high temperatures and an intensive solar input, acting synergistically, induce a very high metabolic turnover. In such situations, various alien xenobiotics are more quickly incorporated into the metabolic cycles of the organisms, inactivating their defense systems, disrupting their endocrine activities or destroying their progeny, and increasing the ‘nat-

Table 6

Permeability of epithelia of *Donax trunculus* to acid fluorescein (compared to 100% of a clean site)

	Site		
	Clean	Organic pollution*	Chemical pollution**
Mantle	100	170.3 (±55)	2159.5 (±417)
Hepatopancreas	100	320 (±132)	462 (±150)
Gills	100	133 (±23)	198 (±67)
Lysosomal activity	100	68 (±12)	53 (±15)
Micronucleation	100	211 (±8.6)	268 (±8.5)

\* Domestic effluents.

\*\* Polychlorinated compounds.

ural' selection. Given sufficient time, this process can lead to a type of 'punctuated branching' (Levin, 1984) or 'punctuated evolution' (see Moore, 1984). Consequently, according to the neo-Darwinian approach, novel succession will ultimately be manifested in the biology and physiology of the new phenotype. Nevo and Laevi (1989) described such phenomena in several enzymes of gastropods from Israeli shores. Nevo (1995) discussed in detail what he termed 'trends of increasing diversity' in extreme and unpredictable habitats, especially the higher heterozygosity. Such divergences are possibly the first detectable stages of selection. Mayr (1967) was aware of this, recognizing the ability to vary as the principal qualities of the genome and epigenetic system of the organism. Weingarten (1985) named this the '*Wirkfeld*' (sphere of influence) or '*Auseinandersetzung mit der Umwelt*' (interaction with nature).

It can be postulated that if additional isolating mechanisms participate in this process, such as non-mixing of progeny from various sites, the long-term action of such selection can, in fact, produce a behavioral or physiological isolation that can finally separate between the original and produced genotype. This type of selection is analogous to the directional evolution of bacteria (Mortlok, 1982) or enhancing evolution of plants (Stanton et al., 2000). Such situations produce what Balon (1990) names 'least stabilized thresholds in which changes (epigenetic) are possible to occur'. In bacteria and other parasites, this will increase their resistance to anti-parasitic toxins, and in the mollusks, this leads to resistance to xenobiotics, establishing new morphs in the population. According to Moore (1984), exposing such evolutionary changes in nature is a matter of luck. Looking on the 'bright' side of the negative stress, therefore, this, in fact, reorganizes the genetic composition of a population, disposing of some of it, and supporting genotypes able to withstand the hostile stressors. We cannot predict how these biochemical or physiological separations in such allopatric situations will influence the evolution of the populations, because, as stated by O'Hara (1993) such judgments can only be made retrospectively. However, as stated by Lewontin (1965): 'changes take place to increase the fitness

of populations in today's environment, but the results of that change is a population in the next generation living in tomorrow's environment' (p. 304). By implementing the types of techniques used in our studies, which resemble the prophylactic methodologies of health investigation in humans, we can reveal and compare environmental health phenomena in various regions and generations. In this way, modern ecology will become able to contribute to sustainable development and preservation of natural biological diversity, and limit the destructive, anthropogenic impact on marine as well as terrestrial ecosystems.

The wiser man must also become more human, moving from egocentrism to ecocentrism, and using but not abusing nature.

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