Research Article

Coral Reef Monitoring: From Cytological Parameters to Community Indices

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Sound-ecosystem-based management of coral reefs is largely based on indicators of reef health state. Currently there are various ecological parameters that serve as reef state indices; however, their practical implications are under debate. In the present study we examine an alternative parameter, the deterioration index (DI), which does not purport to replace the traditional indices but can provide a reliable, stand-alone indication of reef state. Patterns of cytological indices, which are considered as reliable indicators of environmental stressors, have been compared to ten selected reef community indices. The DI showed the highest correlations among community indices to the cytological indices in artificial reefs and high correlation in natural reefs as well. Our results suggest that in cases of lacking adequate monitoring abilities where a full set of community indices cannot be obtained, the DI can serve in many cases as the preferred, stand-alone indicator of coral reef state.

1. Introduction

Coral reefs are in serious decline worldwide [1, 2], and concerns for the future existence of the reefs have driven governments, international organizations, and NGOs to seek ways to prevent or mitigate the degradation of these essential ecosystems. A crucial element of coral reef protection and management is efficient and reliable monitoring; a critical tool for identification of reef deterioration, its causes, and countermeasures. At present, there are various monitoring methods that employ diverse indices of coral reef state (e.g., live cover, species diversity, key species abundances (for more details, see [3, 4])). However, many coral reef ecologists have raised doubts about the usefulness and reliability of the commonly used community parameters (in expressing the actual state of reefs (e.g., [5–9])). These doubts are derived from two major factors: first, the high complexity and natural variability of reef communities and, second, the strong dependency of acquiring reliable reef-state indications on long-term, expensive, and complicated monitoring,

which includes assessment of diverse community variables (e.g., [10]).

It should be stressed that most of the common indices, if examined repeatedly at the same site over several years, indeed provide reliable indications of trends of the coral community state (e.g., [10–13]). However, given the above noted limitations, long-term monitoring programs that include a wide range of community indices are rare. On the other hand, none of the traditional indices can stand alone as a reliable indication of reef community state. Therefore the limitations of the traditional indices require further refinement of their applicability and reliability and/or search for new indices, notably in developing countries where most of the coral reefs are situated but where such countries often lack the resources for long-term, costly monitoring programs.

In a previous study we proposed a new monitoring approach, the deterioration index (DI), as a tool for obtaining a snapshot assessment of coral reef state [14]. The DI is a pure number that compares two of the major processes governing

the reef's coral dynamics: recruitment rate and mortality rate [14]. In the previous study, the DI approach was solely examined on young coral communities that had developed on artificially laid rocks on sandy natural bottoms at similar depths ([14, Figure 1]). The idea of choosing these similar young communities was to avoid complexity effects of different developed reefs. However, the chosen sites differed in their exposure to known anthropogenic disturbances. These differences were used to determine potentially different patterns of monitoring indices (i.e., recruitment rate, mortality rate, species richness, number of coral colonies, and DI) as a response to environmental disturbances. The previous results demonstrated that the DI was best correlated with expectation index, among other indices of reef coral communities and therefore may be considered as best in indicating reef state under a priori known environmental stressors [14].

The DI approach has several advantages (e.g., low cost, simple application, and low proficiency requirements) and disadvantages (i.e., does not cover the entire range of reef community variables and can be applied only if branching corals are present) [14]. However, like the traditional indices, its major problem is that it examines the reef state at the community level, which is more complicated, less understood, and highly debatable [15]. Moreover, community indices are the last to be affected by stressors, as compared with indices of lower biological organization levels (i.e., molecular to cellular). As opposed to community indices, cytological and molecular indices are considered as better understood and more reliable in indicating effects of environmental stressors. Therefore, a practical way to examine community indices may be to compare their quantitative expressions to those of cytological indices, which are better understood and detectable at earlier stages of stress response. In this study ten selected coral reef community indices, including the DI, were compared with three cytological parameters (i.e., micronucleus, single-stranded DNA breaks, and nonspecific esterase activity), assuming that higher correlation with the cytological parameters provides a better indication of the reef actual state. Comparisons were applied through regression analyses between the community-level indices and the acquired data of the three different cytological tests. To the best of our knowledge, the present study is the first to use these tests for assessment of multilevel stress responses in corals. Although not previously used for coral state assessment, cytological parameters as “indicators of authenticity” have been recognized by others (e.g., [16]) as highly reliable in indicating environmental stressors in diverse species [16, 17].

The micronucleus test (MNT) is a major component in modern toxicogenetic screening tests (e.g., [16–18]) and is considered as the only direct indicator of the chromosomal integrity in cells. Any chemical or physical stressors might induce chromosome breaks in mitotic cells and the formation of free acentric chromosome fragments. These fragments show a clastogenic effect or induce spindle apparatus breaks in mitotic cells that produce lagged whole chromosome(s), displaying an eugenic effect. These fragments and/or lagged chromosome(s) form nuclear-like structure(s)

called micronuclei (MN), since they generally contain only 1–10% of diploid DNA. Therefore microscopic counting of MN-containing cells frequency exhibits the level of clastogenic/eugenic action on investigated cells [17]. MN tests (MNTs) have been applied to various types of eukaryotic cells, including plants (e.g., [19]), invertebrates (e.g., [20, 21]), fish (e.g., [22, 23]), and other vertebrates (e.g., [24]). Moreover, due to their high reliability MNTs are widely used as a test in medical and pharmaceutical applications (e.g., [17, 25, 26]).

DNA breakage is the outcome of exposure of cellular DNA to diverse endogenous and exogenous agents (e.g., radiation and oxidative stressors) and is reflected in single-stranded DNA breaks (SSB). SSBs frequencies are commonly used to assess environmental genotoxicity, mainly by applying DNA-unwinding assays (e.g., [27–31]). There is a wide range of methods designed to assess SSB, of which we selected two sensitive reliable methods that do not require high amounts of sampled tissues. The first is AO that examines individual cell nuclei under microscopic control, and the second is the fast micromethod with PicoGreen that examines whole-tissue homogenates or lysates [32–36].

The third cytological method is the nonspecific esterase activity test. The activity of enzymes like cholinesterase, nonspecific esterase, and detoxifying enzymes is frequently used as an indication of stress in ecological monitoring [21, 29, 37]. Cholinesterase and nonspecific esterase remain active after formaldehyde fixation [38], which enables the application of the method on preserved materials. The activities of these enzymes have been studied *in vitro* using homogenates or isolated fractions (biochemical approach) and in living cells using fluorogenic substrates (cytochemical approach, e.g., [18, 39, 40]).

The present study is comprised of two stages, separated in time. In the first stage, comparisons between community-level and cellular-level parameters were conducted on ten different ecological indices at the community level (including the DI) and three cellular (cytological) parameters, all of which were calculated on data obtained from young coral reef communities (young artificial reefs (YARs)) developed on deployed boulders that serve as wave-breakers. In the later stage, we focused on evaluating the DI by further comparisons with cytological parameters obtained from six natural, long-standing reefs (developed natural reefs (DNRs)) assuming that the higher the correlation with cytological parameters, the stronger the parameter as an indicator of reef exposure to stressors and thus as a reef state index.

2. Materials and Methods

2.1. Study Sites and Local Anthropogenic Disturbances. Data of community-level and cellular-level parameters were obtained in 2001–2002 from 14 YAR stations at 7 shallow (3–6 m) sites along the 12 km coast of Eilat, from the “North Beach” to the southernmost reef knolls across Taba, and in 2002 from 6 DNR sites (at 3–10 m) along the Aqaba and Eilat coasts (Figure 1). The YAR stations included three stations located at the Peace Lagoon (P1, P2, and P3; see Figure 1), an artificial lagoon located at Eilat's north beach 400 m from

FIGURE 1: The study sites along the coasts of the northern tip of the Gulf of Aqaba (Red Sea). The young coral community sites are marked with an empty circle: Peace lagan: P, three stations; (Y) Yacht Marina, two stations; (N) Naval base, one station; (D) Dekel beach, three stations; (E) Eilat port, one station at the Dolphin Reef 100 m south of the port main quay; (T) (Tur-Yam) old marina, one station; (L) lighthouse beach, three stations. The natural reefs are marked with a filled circle: (MSS) Aqaba's Marine Science Station; (NB) the north beach of Aqaba; (NR) northern part of Eilat's marine nature reserve; (JG) the Japanese Gardens at the southern part of Eilat's nature reserve (north of the underwater observatory); (UO) knolls south of the underwater observatory; (IUI) The Interuniversity Institute for Marine Sciences in Eilat.

fish cages, which were a major source of nutrients (these cages were removed in 2007). The turbidity in this site was high due to high eutrophication from the fish cages and advection of sand from the shore by the north winds. Two stations were located on the eastern breakwater of Eilat's yachting marina (Y1 and Y2; see Figure 1). The sixth station was located near the naval base (N1; see Figure 1), where detergents and oils are regularly washed to the water. North of Eilat's port there were three stations (D1, D2, and D3; see Figure 1) exposed to various potential stressors (notably the construction was carried out six to seven years prior to sampling). South of Eilat's port, there was one station (E2; see Figure 1). From station E2 southwards there are no known major pollution sources. The next station was at the "glass-bottom boat" marina (T1; see Figure 1). The southernmost final three stations were at the Lighthouse beach (L1, L3, and L4; see Figure 1) and were considered as control sites due to their remote location from any known disturbance hotspot.

The six DNR stations along the Aqaba and Eilat coastline were monitored using the DI method. The stations included (from Aqaba to Eilat; see Figure 1) the marine science station (MSS) reef—a protected area encompassing the edge of the marine reserve along the Jordanian coast. This site represents the typical fringing reef of the Gulf of Aqaba—a well-developed reef composed of almost all the recorded species along the Jordanian coast. The reef flat is quite wide with a reef front and a reef slope. The northernmost station

(north beach (NB)) is characterized mainly by a sandy bottom, with scattered patches of grass beds and some dispersed reef patches. Eilat's nature reserve is the only fringing reef at Eilat. It features a 10–50 m wide shallow lagoon relatively isolated from the open sea, a few meters wide reef flat ending in a 3–5 m deep vertical reef crest, and an outer slope with partial coral cover. Two stations were within the nature reserve located at its northern part (NR) and in the south (the Japanese Gardens (JG)). The final two stations comprised the well-developed rich knolls at 3–8 m depth near the underwater observatory (UO) and a reef on the slope opposite the Interuniversity Institute for Marine Sciences marine laboratory (IUI). We selected reefs in relatively shallow water (though deeper than the YAR), which were big enough to allow a DI transect. We avoided too small knolls that exist in several highly polluted sites at both Aqaba's and Eilat's coasts and thus were left with only one site, NR, representing such reefs. The results (see below), however, show major differences between the selected reefs and accordingly provide useful data for further comparisons.

2.2. Cytological Tests. We sampled *Stylophora pistillata* and *Pocillopora damicornis* for the cytological tests. These two species were chosen for two major reasons. First, they were common in all the studied sites. Second, they can be sampled without causing major damage to the colony. Two pieces per colony from three colonies of each species were sampled from each of the studied coral reef sites (14 young reef sites and six natural reef sites, as described in the previous section; see Figure 1). Three samples per site (one per sampled colony) were fixed in methanol for the SSB test and the other three in 4% formaldehyde (diluted with filtered sea water, pH 7.6) for the MNT and esterase activity tests.

Soft tissues of the fixed corals were separated from the skeleton in water under a dissecting microscope by shaking and using an aquarelle brush. The resulting suspension of cells and small pieces of tissue were used for the different cytological tests. Cells for microfluorometric measurements were detected in the suspension by moving the object glass. Corresponding cells were superimposed by relevant measurements of the diaphragm (which allows the measuring of single cells within a small complex). Prior to the measurements, the microfluorometer was calibrated using three etalons: uranium glass, microcuvettes with standard fluorescein solutions [29, 37], and a multispectral fluorescence microscopy standard kit (MultiSpeck by Molecular Probes).

For the micronuclei test (MNT), nuclei were stained with DNA-specific fluorochrome ethidium bromide. From each colony, 3,000 cells were examined under a Nikon epifluorescent microscope (excitation filter 510–560 nm, dichroic mirror 580 nm, barrier filter 580 nm, and objective Fluor 40 × 0.85) to calculate frequency of micronuclei-containing cells. In this test the cells are the replica and the results express the proportions of micronuclei-containing cells.

Examination of single-stranded DNA breaks (SSBs) *in situ* using Acridine Orange (AO) and two-wavelength microfluorometry was carried out according to Bresler et al. [29, 30], and Darzynkiewicz [33]. Cells were treated with

TABLE 1: Results of the regression between the cytological tests of samples from the young coral communities. Samples were collected from *Stylophora pistillata* (Sty) and *Pocillopora damicornis* (Po). In each square, the top figure is r^2 and the bottom is the P value. MNT: micronucleus test, SSBs: $-\log$ single strand and Es a: Esterase activity.

Es a Sty	r^2	0.944			
	P	<0.001			
SSBs Po	r^2	0.890	0.943		
	P	<0.001	<0.001		
SSBs Sty	r^2	0.895	0.948	0.998	
	P	<0.001	<0.001	<0.001	
MNT Po	r^2	0.603	0.716	0.851	0.846
	P	0.001	<0.001	<0.001	<0.001
MNT Sty	r^2	0.529	0.643	0.783	0.769
	P	0.003	0.001	<0.001	<0.001
		Es a Po	Es a Sty	SSBs Po	SSBs Sty
					MNT Po

RNAse A (100 units) at 37°C for 1 h in buffered salt solution, incubated in phosphate-citric acid buffer at pH 2.6 for 80 sec, and stained in 20 μ M Acridine orange (AO) solution in the same buffer for 5 min. A drop of this cell suspension on object glass was used for two-wavelength microfluorometry. The object under the fluorescent microscope/microfluorometer was moved to superpose ectodermal cells with measuring diaphragm and measures of both green and red fluorescence of each nucleus were taken. Excitation of AO was achieved with an interference filter 450–490 nm, and fluorescence of AO-ds DNA complex with a barrier filter 520–540 nm, fluorescence of AO-ss DNA complex with a barrier filter 590–620 nm and objective Fluor 40 $\times$ 0.85. In total, 120 cells (50 per colony times 3 colonies) were measured for each sampling site. The fraction of dsDNA (Fds) was calculated as the ratio of green fluorescence intensity of dsDNA to fluorescence of total DNA (green + red fluorescence). The negative log of Fds ($-\log$ Fds) is proportional to the relative number of ssDNA breaks. The relative number of breaks (n) may be calculated as $n = -\log$ Fds $I / -\log$ Fds J , where the “ I ” and “ J ” indicated the different compared sites [29].

Examination of SSBs breaks using micromethod with PicoGreen (Molecular Probes) was based on a method described by Batel et al. [35] and Jakšić and Batel [36]. In brief, 25 μ L of cell suspension (3000 ± 120 cells) were mixed in a microcuvette with 25 μ L of lysing solution (9 M urea, 0.1% SDS, and 0.2 M EDTA pH 10.0) containing PicoGreen (20 μ L of original dye stock solution per one mL of lysing solution), and the microcuvette was then incubated on ice in the dark for 40 min. DNA unwinding was started by adding 250 μ L 0.025 M NaOH solution (pH changed to 12.4), and green fluorescence was measured at room temperature every 60 s for 20 min in a microfluorometer at 450–490 nm excitation filter, a 505 nm dichroic mirror, and a 520–550 nm barrier filter using the same fluorescent microscope/microfluorometer used for AO. Three probes (homogenates from three colonies collected from each

station) from each station were measured. The fraction of dsDNA (Fds) in a given preparation was calculated as the ratio of PicoGreen-dsDNA fluorescence after unwinding to PicoGreen-dsDNA fluorescence before unwinding (at time 0). The negative log of Fds ($-\log$ Fds) is proportional to the relative number of DNA unwinding points. The ratio $-\log$ Fds in a given site to $-\log$ Fds in the reference site shows the relative number of strand breaks per unwinding units [29]. It should be noted that the coral samples for DNA examinations (for both OA and PicoGreen) were fixed by alcohol which extracted chlorophyll and highly decreased autofluorescence. Moreover, tissue for the PicoGreen method was lysed and diluted, thus, the influence of autofluorescence in these measurements is negligible.

Nn-specific esterase activity was determined with fluorogenic substrate fluorescein diacetate (FDA) [29, 30]. We normalized esterase activity (i.e., fluorescence intensity of liberated fluorescein) by standardization of all conditions (substrate concentration, time of incubation, temperature, etc.) prior to any measurements. Cells were incubated with a final concentration of 10 μ M of FDA for 10 min. For each species in each site, 120 cells (40 cells from each of 3 colonies) were obtained. However, as it is possible to distinguish between ectodermal cells, gastrodermal cells, and zooxanthellae under the microscope, no measurements of zooxanthellae or gastrodermal cells were taken. Liberated fluorescein was determined by microfluorometry using fluorescent microscope/microfluorometer (excitation filter 450–490 nm, dichroic mirror 510 nm, barrier filter > 520 nm, and objective Fluor 40 $\times$ 0.85). Esterase activity can be displayed as micromoles of product (liberated fluorescein) per cell per time (10 min in this study).

2.3. Coral Community Surveys. The censuses of the YAR were conducted by three fixed belt transects of 6 m² (10 m $\times$ 0.6 m) each. All stony corals within the transect were counted and identified to species level [41]. The longest diameter of all living branching corals was measured. All dead branching corals that could be identified as such were also counted. Live coral cover was quantified using a line intercept [42] along the line of the same belt transects. The transect data of the “young reefs” served for calculating the community-level indices: (1) Simpson’s index (d) [43]), (2) Shannon’s index (H) [44]), (3) d/d max [45], (4) H/H max [45], (5) coral colony abundance (number of colonies per m²), (6) percentage of live coral cover, (7) average size (the longest diameter) of branching corals (an indicator of the corals age), (8) percentage of small (< 3 cm) branching corals from all living branching corals (an indicator of recruitment to the reef and reef renovation), (9) the percentage of dead branching colonies from all (living and standing dead) branching colonies, and (10) the deterioration index (DI) for which the ratios between the proportions of dead branching (i.e., coral skeletons) and small branching corals are calculated using the following formula:

$$DI = \frac{DC (DC + LC)}{SC/LC}, \quad (1)$$

FIGURE 2: The results of the cytological test obtained from the two coral species at the fourteen young coral communities study stations. Micronucleus test (frequencies of micronuclei-containing cells, %), $n = 9000$ cells per sampling site. Frequencies of single-stranded DNA breaks ($-\log Fds$), $n = 120$ cells per sampling site (samples stained with Acridine orange). Esterase activities (arbitrary units), $n = 120$ cells per sampling site. Bars are 95% confidence interval. Stations are Peace Lagoon, southern breakwater (P1); Peace Lagoon, end of southern breakwater (P2); Peace Lagoon, eastern breakwater (P3); Yachts marina, south side of breakwater (Y1); Yachts marina, east side of breakwater (Y2); Naval port (N1); Dekel beach, plain (D1); Dekel beach, slope (D2); Dekel beach, large rocks (D3); Dolphin Reef (E2); Tur Yam (T1); Lighthouse beach, small rocks (L1); Lighthouse beach, large rocks (L3); Lighthouse beach, southern slope (L4).

where the mortality rate is the proportion of dead colonies (DC) out of the total number of living and dead corals (DC + LC), and the recruitment rate is given by the ratio between the number of the smallest detectable living corals (up to 3 cm (SC) and total number of all living corals (LCs) [14].

Regression was used to examine the relations between (1) the different cytological parameters (“within cytological”), where the average result per each test per site was examined against the results of the other tests at the same site, (2) the different community-level indices (“within community”), where each parameter’s result obtained from each transect was compared with all other parameters’ results of the same transect, and (3) the cytological parameters with the community indices (“between cytological-community”), where averages of results obtained from the three transects of each site were examined against the results of the cytological tests obtained from the samples collected at the same site. These comparisons were conducted, respectively, to (1) verify the usefulness of the cytological parameters as “stress indicators” (since it is the first time that these tests were conducted on corals), (2) examine to what extent there are similarities among the community level indices, and (3) determine which of the community indices best indicates early stages of stress and also best correlates with cellular-level changes.

At the DNR we applied the DI using the same three 6 m² belt transects at each station. Regression was used to compare between the averages of the different cytological parameters and between them and the average DI obtained from the same site.

3. Results

The cytological test results of the two coral species, *S. pistillata* and *P. damicornis*, showed high variability among the YARs and the DNRs (Figures 2 and 3). The high variability and pattern among sites was in accordance with the known disturbance hotspots at the different sites. The three cytological parameters (four different tests looked at in the DNR) show significant correlations among themselves for both species ($P < 0.05$; see Tables 1 and 2), with parameters highly correlated among samples from the DNR (r^2 up to 0.99; see Tables 1 and 2).

The results of the different community-level indices calculated for the “young reefs” have a clear inconsistency (Table 3). This inconsistency is expressed in significantly different values of the different indices at a given station for each of the studied sites; that is, the different indices provided contradictory indications of the reef state for several stations (Table 3). It should be noted that the community indices

TABLE 2: Results of the regression between the cytological tests of samples from the natural reefs. Samples were collected from *Stylophora pistillata* (Sty) and *Pocillopora damicornis* (Po). In each square, the top figure is r^2 and the bottom is the P value. MNT: micronucleus test, SSBs: –log single strand breakage (A) with a acridine orange and (P) with PicoGreen. Es a: esterase activity.

Es a Sty	r^2	0.846						
	P	0.009						
SSBs Po (A)	r^2	0.914	0.914					
	P	0.003	0.003					
SSBs Sty (A)	r^2	0.816	0.976	0.952				
	P	0.014	<0.001	0.001				
SSBs Po (p)	r^2	0.900	0.956	0.958	0.957			
	P	0.004	0.001	0.001	0.001			
SSBs Sty (P)	r^2	0/872	0.988	0.914	0.960	0.976		
	P	0.006	<0.001	0.003	0.001	<0.001		
MNT Po	r^2	0.747	0.775	0.929	0.868	0.822	0.743	
	P	0.026	0.021	0.002	0.007	0.013	0.027	
MNT Sty	r^2	0.786	0.872	0.944	0.929	0.929	0.859	0.969
	P	0.019	0.006	0.001	0.002	0.002	0.008	0.001
		Es a Po	Es a Sty	SSBs Po (A)	SSBs Sty (A)	SSBs Po (p)	SSBs Sty (P)	MNT Po

TABLE 3: Average values ($\pm$ standard deviation) of ten indices obtained at the study stations of young coral communities. Stations are (from top to the bottom) Peace Lagoon, southern breakwater (P1); Peace Lagoon, end of southern breakwater (P2); Peace Lagoon, eastern breakwater (P3); Yacht marina, south side of breakwater (Y1); Yacht marina, east side of breakwater (Y2); Naval port (N1); Dekel beach, plain (D1). Dekel beach, slope (D2); Dekel beach, large rocks (D3); Dolphin Reef (E2); Tur-Yam (T1); Lighthouse beach, small size rocks (L1); Lighthouse beach, large size rock (L3); Lighthouse beach, southern slope (L4).

Simpson d	Shannon H	$d/d_{\max}$	$H/H_{\max}$	Coral abundance	Coral live cover %	Branching coral size cm	% small branching	% dead branching	DI
10.3 $\pm$ 0.9	2.7 $\pm$ 0.1	0.53 $\pm$ 0.03	0.87 $\pm$ 0.00	17.1 $\pm$ 1.2	17.4 $\pm$ 3	13.7 $\pm$ 2.3	0.6 $\pm$ 1.2	62 $\pm$ 8.5	39.9 $\pm$ 22
10.8 $\pm$ 3.6	2.6 $\pm$ 0.4	0.57 $\pm$ 0.11	0.88 $\pm$ 0.03	15.2 $\pm$ 10.2	18.2 $\pm$ 12.4	18.1 $\pm$ 2.2	1.3 $\pm$ 1.5	31.5 $\pm$ 1.6	29.5 $\pm$ 15.6
4.8 $\pm$ 2	1.6 $\pm$ 0.4	0.79 $\pm$ 0.1	0.93 $\pm$ 0.02	1.9 $\pm$ 1.5	1.3 $\pm$ 1.7	7.6 $\pm$ 3.6	0	90.3 $\pm$ 12.4	Infinite
16.4 $\pm$ 2.3	3.1 $\pm$ 0.1	0.4 $\pm$ 0.05	0.87 $\pm$ 0.02	29.3 $\pm$ 0.7	15.8 $\pm$ 5.8	9.1 $\pm$ 0.3	12.3 $\pm$ 9	22.2 $\pm$ 6	1.2 $\pm$ 0.6
3.2 $\pm$ 0.7	2.1 $\pm$ 0.3	0.1 $\pm$ 0.02	0.61 $\pm$ 0.006	32.8 $\pm$ 3.5	24.5 $\pm$ 7.4	6.4 $\pm$ 2	87.3 $\pm$ 30	12 $\pm$ 2	0.2 $\pm$ 0.04
7.9 $\pm$ 1.3	2.5 $\pm$ 0.1	0.3 $\pm$ 0.06	0.76 $\pm$ 0.05	44.4 $\pm$ 2.7	11.9 $\pm$ 3	11.6 $\pm$ 1.2	2.3 $\pm$ 2	13.5 $\pm$ 7.6	7.8 $\pm$ 12.4
8.7 $\pm$ 1.7	2.6 $\pm$ 0.1	0.4 $\pm$ 0.04	0.82 $\pm$ 0.03	17.4 $\pm$ 5.1	2 $\pm$ 0.3	4.8 $\pm$ 0.2	21 $\pm$ 3.6	46.7 $\pm$ 10	1 $\pm$ 0.2
9.7 $\pm$ 0.3	2.7 $\pm$ 0.1	0.4 $\pm$ 0.03	0.82 $\pm$ 0.01	20.4 $\pm$ 1.8	19.1 $\pm$ 4.8	11.3 $\pm$ 1.9	17 $\pm$ 9.6	42.3 $\pm$ 4.6	2.8 $\pm$ 1.5
3.7 $\pm$ 0.4	2 $\pm$ 0.1	0.2 $\pm$ 0.02	0.67 $\pm$ 0.01	19.2 $\pm$ 9.8	8.7 $\pm$ 4.3	5.5 $\pm$ 2	51.3 $\pm$ 19.9	32 $\pm$ 13	0.5 $\pm$ 0.26
6.6 $\pm$ 0.3	2.2 $\pm$ 0.1	0.4 $\pm$ 0.05	0.79 $\pm$ 0.01	20.8 $\pm$ 2	15.5 $\pm$ 3	8.6 $\pm$ 1.2	33 $\pm$ 11.5	26.7 $\pm$ 15.4	1 $\pm$ 0.61
5.2 $\pm$ 1.9	2 $\pm$ 0.3	0.4 $\pm$ 0.13	0.77 $\pm$ 0.1	8.1 $\pm$ 1.7	6.2 $\pm$ 0.6	8.2 $\pm$ 1.6	6 $\pm$ 2	19.5 $\pm$ 6.2	1 $\pm$ 0.39
8 $\pm$ 0.7	2.5 $\pm$ 0.1	0.4 $\pm$ 0.04	0.83 $\pm$ 0.01	14.8 $\pm$ 5.2	5.1 $\pm$ 1.4	8.2 $\pm$ 0.9	14.3 $\pm$ 2.5	49.4 $\pm$ 8	1.9 $\pm$ 0.66
6.5 $\pm$ 1.8	2.3 $\pm$ 0.2	0.3 $\pm$ 0.06	0.76 $\pm$ 0.06	15.1 $\pm$ 0.8	11 $\pm$ 5.4	7.7 $\pm$ 0.4	18.7 $\pm$ 6.7	41.9 $\pm$ 13	1.1 $\pm$ 0.48
5.5 $\pm$ 1.2	2.3 $\pm$ 0.2	0.3 $\pm$ 0.06	0.74 $\pm$ 0.05	18.6 $\pm$ 3.4	6.6 $\pm$ 2.8	6.4 $\pm$ 0.1	41 $\pm$ 13	39.2 $\pm$ 6.5	0.7 $\pm$ 0.18

revealed no correlations among themselves ($r^2 < 0.1$; see Table 4). Moreover, correlations with the cytological tests in the “young reefs” were only found in the DI, which was in significant correlation ($P < 0.05$) with two of the three cytological tests (i.e., MNT and SSBs; see Table 5). The DI values of DNR showed high variation, including high values at the JB and NR sites, which are protected sites (Table 6). The correlation tests in the “developed reefs” revealed a clearer picture, where very high correlations were found between the DI values and the cytological tests obtained from these reefs (Table 7).

4. Discussion

The results of the present study show an inconsistency among the different ecological indices in revealing the reef state as well as in discriminating between relatively healthy and disturbed sites. This inconsistency is expressed by the obtained correlations, which show weak or no dependence between some of the different community-level indices (Table 4). Moreover, although some indices show significant correlations with the majority of other indices (e.g., DI and coral abundance), the r^2 values of

TABLE 4: Results of regression between the ecological indices calculated for each transect of the fourteen stations of young coral communities. In each square the top figure is r^2 and the bottom is the P value. Significant correlation ($P < 0.05$) is marked in bold.

Shannon's index	r^2	0.804								
	P	<0.001								
$d/d_{\max}$	r^2	0.079	0.004							
	P	0.053	0.668							
$H/H_{\max}$	r^2	0.364	0.131	0.783						
	P	<0.001	0.011	<0.001						
Coral abundance	r^2	0.087	0.249	0.302	0.126					
	P	0.042	<0.001	<0.001	0.013					
Coral live cover	r^2	0.007	0.029	0.092	0.084	0.133				
	P	0.571	0.250	0.036	0.046	0.011				
Branch avr. size	r^2	0.182	0.087	0.190	0.173	0.005	0.027			
	P	0.003	0.044	0.002	0.004	0.621	0.271			
% small branch	r^2	0.164	0.017	0.542	0.404	0.027	<0.001	0.569		
	P	0.005	0.330	0.000	0.000	0.272	0.335	<0.001		
% dead branch	r^2	0.004	0.007	0.373	0.311	0.183	0.089	0.012	0.049	
	P	0.636	0.578	<0.001	<0.001	0.002	0.040	0.456	0.134	
DI	r^2	0.002	0.099	0.516	0.249	0.137	0.076	0.097	0.276	0.525
	P	0.767	0.029	<0.001	<0.001	0.010	0.058	0.033	<0.001	<0.001
		Simpson's index	Shannon's index	$d/d_{\max}$	$H/H_{\max}$	Coral abundance	Coral live cover	Branch avr. size	% small branch	% dead branch

TABLE 5: Results of the regression between the ecological indices obtained from the young coral communities and the cytological test results of samples from these communities. Correlation tests were conducted between the average values of each of the indices and the cytological tests at each station. In each square, the top figure is r^2 and the bottom is the P value. Bold figures mark significant correlations ($P < 0.05$). In the cytological tests, MNT: micronucleus test, SSBs: $-\log$ of single strand breakage value, Es a: esterase activity, Sty: *Stylophora pistillata* and Po: *Pocillopora damicornis*.

		MNT Sty	MNT Po	SSBs Sty	SSBs Po	Es a Sty	Es a Po
Simpson's index	r^2	0.003	0.055	0.023	0.022	0.015	0.018
	P	0.843	0.420	0.601	0.612	0.675	0.643
Shannon's index	r^2	0.006	0.001	0.002	0.002	0.004	0.004
	P	0.794	0.902	0.880	0.880	0.823	0.823
$d/d_{\max}$	r^2	0.177	0.233	0.187	0.181	0.159	0.173
	P	0.134	0.080	0.123	0.129	0.157	0.139
$H/H_{\max}$	r^2	0.081	0.133	0.090	0.086	0.073	0.090
	P	0.326	0.200	0.296	0.308	0.349	0.297
Coral abundance	r^2	0.004	0.011	<0.001	<0.001	0.011	0.013
	P	0.821	0.724	0.980	0.991	0.720	0.695
Coral live cover	r^2	0.049	0.076	0.051	0.057	0.043	0.013
	P	0.446	0.340	0.437	0.413	0.475	0.700
Branch avr. size	r^2	0.271	0.287	0.173	0.178	0.086	0.043
	P	0.056	0.065	0.139	0.133	0.309	0.043
% small branch	r^2	0.206	0.239	0.154	0.153	0.080	0.079
	P	0.103	0.076	0.165	0.167	0.328	0.329
% dead branch	r^2	0.103	0.083	0.075	0.078	0.092	0.096
	P	0.264	0.318	0.344	0.335	0.292	0.281
DI	r^2	0.380	0.426	0.311	0.312	0.257	0.228
	P	0.019	0.011	0.038	0.038	0.064	0.085
		MNT Sty	MNT Po	SSBs Sty	SSBs Po	Es a Sty	Es a Po

FIGURE 3: The average results of the four cytological tests on samples of *Stylophora pistillata* and *Pocillopora damicornis* collected at six of the natural reef sites along the northern part of the Gulf of Aqaba. (a) Micronucleus test (frequencies of micronuclei-containing cells, %), $n = 9000$ cells per sampling site. (b) Frequencies of single-stranded DNA breaks ($-\log Fds$), $n = 120$ cells per sampling site (samples stained with Acridine orange). (c) Frequencies of single-stranded DNA breaks ($-\log Fds$) determined by micromethod with PicoGreen, $n = 9$ lysate probes of 3000 ± 120 cells each per sampling site. (d) Esterase activities (arbitrary units), $n = 120$ cells per sampling site. Bars show 95% confidence limits. Stations are IUI: Eilat's marine lab, UO: south of the underwater observatory of Eilat, JG: the Japanese gardens at the south part of Eilat's marine nature reserve, NR: the north part of Eilat's marine nature reserve, NB: the north beach of Aqaba, MSS: Aqaba's marine station.

TABLE 6: DI values obtained from DNR.

Site	DI
IUI	0.82
UO	0.59
JG	3.18
NR	3.34
NB	0.19
MSS	1.42

most of these correlations are very low (<0.3). Of the various indices, only the evenness indices show better correlations with most other indices and strong correlations with the diversity indices (as may be expected from their common components). In a previous study we showed that some of these indices do not always enable discrimination

between sites exposed to different levels of disturbances [14]. A comparison between some of the indices (Table 3) indicates relatively different states of health in the same site. For instance, the d and H values found at P stations are relatively high and do not indicate a noticeable decline of coral community in this site (which was also expressed by the DI). On the other hand, at the relatively healthy L sites, these three indices (d , H , and DI) show the same pattern, with lower levels of d and H . The above-noted inconsistencies and the difficulty of discriminating disturbed sites from relatively healthy sites emphasize the problem of using traditional community indices for snapshot assessments of coral reefs, as was discussed earlier in the literature (e.g., [5, 9]).

It is well known that anthropogenic stressors may cause diseases, decrease in health, enhanced lethality, and alterations of community structure in coral reef communities

TABLE 7: Results of the regression between the DI and the results of four cytological tests of coral samples at six natural coral reef sites. MNT: micronucleus test. SSBs: –log single-stranded DNA breaks test where A stands for Acridine orange stained samples and P for PicoGreen stained samples. Es Ac: esterase activity test. Sty: *Stylophora pistillata*, and Po: *Pocillopora damicornis*. –log 10 of the results was tested for the correlations. R^2 and P are given for the correlation of each test result with the DI at six sites (IUI, UO, JG, NR, NB, and MSS).

	MNT Sty	MNT Po	SSBs A Sty	SSBs A Po	SSBs P Sty	SSBs P Po	Es Ac Sty	Es Ac Po
R^2	0.915	0.772	0.940	0.930	0.940	0.990	0.926	0.825
P	0.003	0.021	0.001	0.002	0.001	<0.001	0.002	0.012

[5, 21, 29, 30, 46]. Diverse biochemical, cytological, and cytophysiological biomarkers were recommended for the detection of early signs of environmental stress [16, 17, 20, 27, 30, 35]. Since we do not have precise data of the exact type of the existing disturbances, and due to the lack of previous experience in conducting cytological tests on corals, we chose tests that are easy to conduct and examine cellular factors that might be affected by a wide range of disturbances. Three of the chosen tests (2 SSBs and MNT) are assigned to reveal DNA damage. Single-stranded DNA breaks (SSBs) represent the most frequent primary DNA damage in eukaryotic cells by physical or chemical environmental stressors [16, 17, 28]. From the large variety of methods designed to unmask SSB, we selected two sensitive methods, which require a small amount of tissues: the AO that examines individual cell nuclei under microscopic control and the fast micromethod with PicoGreen that examines whole-tissue homogenates or lysates. The third test, MNT, allows direct detection of chromosome alterations in eukaryotic cells of different origin. These irreversible alterations indicate heavy damage to the chromosome, such as chromosome breaks [16, 17, 20–22, 29, 30, 47, 48] that can be induced by diverse environmental stressors. The esterase activity test has been applied due to its wide-range response to a variety of species, such as protozoans, insects, mollusks, and fish.

The high correlation between the different cytological tests of the two examined species (Tables 1 and 2) indicates the consistency of these tests and thus provides some support for their validity in indicating coral health. It should be emphasized that the examined species are r-strategists, the health state of which does not necessarily represent other coral states. However, the results of the cytological tests confirm that these species are suitable as biomonitor species and can indicate differences in environmental conditions between sites.

The regression tests of the natural reefs (DNR) data revealed high r^2 values for the relations between DI and all the examined cytological parameters (Table 7). However, as opposed to the significant correlations between the DI and cytological indices in natural reefs (DNR), the correlation in the artificial reefs (YAR) was weak (only two parameters, MNT and SSBs, and $r^2 = 0.43 - 0.31$; see Table 5). Therefore it may be argued that the DI, although likely to be the most efficient in indicating the stress level of coral communities in snapshot censuses, cannot as yet serve as a stand-alone monitoring tool for every coral reef site. The remarkable difference between the r^2 values of the artificial reefs (YAR) and the natural reefs (DNR) may be explained by the relatively low stability of the YARs and their broad available space for

settlement. The majority of the colonies composing these reef communities are fast-growing, relatively vulnerable “r strategy” species. These species can benefit from available substrates by recruiting in large numbers, but are expected to experience high mortality rates under stress events. Since the recruitment and mortality rates are the factors composing the DI, fluctuations in both can significantly change the DI values and may explain the low correlations found at the YAR communities.

The present study shows that the selected cytological methods can serve as stress indicators in reef corals. Moreover, as noted earlier, cytological tests are well known as efficient tools in indicating stress in many organisms at early stages, including marine invertebrates. However, it should be emphasized that our aim was not to prove the reliability of the cytological methods. In fact, we do not recommend the use of cytological methods for coral reef monitoring. These methods are very expensive and complicated and they require well-equipped laboratories and high expertise. Thus these methods are not likely to be suitable for the monitoring of most coral reef sites. Our goal, by applying the cytological tests, was to determine whether the differences between and within the ecological indices can be explained by well-accepted, reliable monitoring tools (i.e., cytological indices). The results, as indicated, show that DI was the index showing by far the highest correlation with the cytological results from the YAR. Moreover, the somewhat surprising DI values of the DNR (especially the high values in JG and NR sites [49]) are well in accordance with the cytological values as expressed by the relatively high correlation.

Despite the described clear advantages of the DI, it should be noted that it has some major disadvantages, which one should be aware of prior to any attempt of applying it. First, it cannot reveal changes in the community structure, which might be the consequence of anthropogenic disturbances. Second, it depends on the local growth rates of corals, which can significantly differ between different locations and thus should be checked and adjusted prior to any use in new locations. Finally, the presence of at least some branching corals is a prerequisite in order to apply this method. Despite these and other possible disadvantages, our current findings do support the use of DI as a relatively reliable index for assessment of various disturbed coral communities. Our results suggest that the major advantage of the DI may be expressed in those cases where there are insufficient resources and expertise available and where large-scale snapshot monitoring is required.

In summary, it should be stressed that we have no pretension to present the DI as an alternative to all other reef

state indices. We propose the DI as a fast and easy index, yet reliable, which can be applicable in diverse reef sites. However, given the above-listed limitations, the DI cannot be applied in every reef type and certainly cannot provide a comprehensive picture of the state and health of all reef sites). Weighing the advantages and disadvantages of the DI, we propose it as a fast and efficient monitoring tool, which in many cases can identify coral reef state of health and early stages of deterioration. No doubt that further work is required to refine the effectiveness of the DI approach in monitoring various coral reef communities and to calibrate its levels to provide accurate, site-specific, quantitative indications of reef states.

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