

**DRAFT** - If you have indicators to add after the workshop, or updates to listed indicators, please send information to [sjameson@coralseas.com](mailto:sjameson@coralseas.com) before September 15, 2004.

# **A Review of Indicators of Land-based Pollution Stress on Coral Reefs**

## **A Background Paper**

**for the**

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**by**

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# **A review of indicators of land-based pollution stress on coral reefs**

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## **I. Introduction**

The purpose of the paper is to introduce coral reef managers and scientists to the range of available and potential indicators of land-based pollution stress. Indicators are listed by targeted land-based pollution stressor(s) so a particular monitoring and assessment approach is not favored. Indicators to detect cumulative land-based impacts as well as individual land-based stressors are highlighted.

The potential applications of these measures of land-based pollution stress to coral reef ecosystems include permit issuance and review, responsibility allocation, development of indexes of biotic integrity (Jameson et al. 2001) and biological criteria (Jameson et al. 1998) for Clean Water Act regulation, enforcement, conservation decisions, and the garnering of political will for enforcement or regulatory action.

This paper contains information on land-based indicators from the major review efforts by Jameson et al. 1998, 2001 and Hallock et al. in press, as well as many individual papers. Table 1 includes definitions of key terms used in this paper. Table 2 provides a practical example for establishing mean baseline ambient coral reef environmental parameters. Table 3 provides a practical example of how threshold values can be used for monitoring and assessing land-based sources of pollution. Threshold values should be customized, to the highest degree possible, for the location and target species in question.

**Table 1** Key terms used in defining biological condition (adapted from Karr and Chu, 1999).

<b>Term</b>	<b>Definition</b>
Attribute	Measurable part or process of a biological system
Metric	Attribute empirically shown to change in value along a gradient of human influence (i.e., a dose-response context is documented and confirmed)
Endpoint	A measured characteristic that indicates the condition of a biological, chemical or physical system
Multimetric index	An index (expressed as a single numerical value) that integrates several biological metrics to indicate a site's condition (ex., an index of biotic integrity - IBI)
Biological monitoring	Sampling the biota of a place (i.e., coral reef)
Biological assessment	Using samples of living organisms to evaluate the condition of places
Biological integrity	The condition at sites able to support and maintain a balanced, integrated, and adaptive biological system having the full range of elements and processes expected for a region. Biological integrity is the product of ecological and evolutionary processes at a site in the relative absence of human influence (Karr 1996)
Biological criteria	Criteria which define a desired biological condition for a water body and can be used to evaluate the biological integrity of the water body. When adopted by states, they become legally enforceable standards (narrative expressions or numerical values)

**Table 2** Rational, method, and references for establishing mean baseline ambient coral reef environmental parameters. Means should be established by sampling daily over a one week period (minimum). (from Jameson and Hassan 2003)

<b>Parameter</b>	<b>Rational</b>	<b>Method</b>	<b>Reference</b>
<b>Temperature</b>	Affects metabolism of marine organisms and is associated with coral bleaching.	Use simple thermometer, thermistor or other instruments 0.5 m below the surface and at transect depth.	English et al. 1997, Rogers et al. 2001
<b>Salinity</b>	Affects metabolism of marine organisms.	Obtain water samples 30 cm below the surface and at transect depth using small plastic vials for lab analysis using refractometer.	English et al. 1997
<b>pH</b>	Affects corals ability to calcify. Changes may indicate impacts from new pollutants.	pH meter.	Rogers et al. 2001
<b>Turbidity</b>	Affects available light for photosynthesis.	Use nephelometer to check the average of three 100 ml samples.	Rogers et al 2001
<b>Light transmission</b>	Affects available light for photosynthesis.	Use underwater light meter to check values at bottom. K threshold $\leq$ 0.15.	Yentsch et al. 2002
<b>Water transparency</b>	Affects available light for photosynthesis.	Secchi disc measurements to measure vertical and horizontal transparency	Rogers et al. 2001
<b>Current speed &amp; direction</b>	Transports nutrients, pollutants and larval reef organisms.	Drogue drift devices, Rhodamine B or a florescein dye, or current meters.	Rogers et al. 2001
<b>Wind &amp; sea state</b>	Affects the transport pollutants and zonation patterns of marine organisms.	Beaufort Wind Scale and Sea Disturbance Table.	English et al. 1997
<b>Cloud cover</b>	Affects available light for photosynthesis.	Okta estimates.	English et al. 1997

**Table 3** Threshold values for Red Sea coral reef systems (at depths < 100 meters). These values represent the high end of what would be expected for a minimally impaired coral reef. These values do not apply to areas that may experience extreme environmental conditions (i.e., shallow water lagoons behind fringing reefs. <sup>1</sup>Edwards and Head (1987) should be consulted for determining the annual mean temperature and salinity values at various latitudes in the Red Sea corresponding to the monitoring site location. (from Jameson and Hassan 2003)

<b>Parameter</b>	<b>Values not to exceed (unless otherwise stated)</b>
<b>Nutrients</b>	
Soluble reactive phosphorous (SRP) = (PO <sub>4</sub> <sup>-3</sup> )	0.1 µM (Lapointe et al. 1997)
Dissolved inorganic nitrogen (DIN) = (NH <sub>4</sub> <sup>+</sup> +NO <sub>3</sub> <sup>-</sup> +NO <sub>2</sub> <sup>-</sup> )	1.0 µM (Lapointe et al. 1997)
Nitrogen isotope ratio (δ <sup>15</sup> N) of fleshy macroalgae	Clean: 0 ‰, Fertilizer: 0 to +3 ‰, Sewage: +3 to +10 ‰ (Lapointe et al. 2002)
C:N:P ratio of fleshy macroalgae	Shallow and deep samples should have similar tissue % values for C:N:P (Lapointe et al. 2002)
Chlorophyll a	0.15 µg/l (Lapointe et al. 2002)
<b>Suspended Sediments</b>	
Water transparency	K = 0.15 (Yentsch et al. 2002)
Turbidity	2.0 NTU (Telesnicki & Goldberg 1995)
Suspended particulate matter	10.0 mg/l (Rogers 1990)
Sediment deposition rate	10.0 mg/cm <sup>2</sup> /day (Rogers 1990)
<b>Others</b>	
Bunker oil (oil slick)	3.0 ppm (Lane and Harrison 2002)
Dispersed oil	0.5 ppm (Lane and Harrison 2002)
Oil dispersant	8.0 ppm (Lane and Harrison 2002)
Cadmium	0.20 µg/l (Connel and Hawker 1992)
Surfactants	35 µg/l (Connel and Hawker 1992)
PCB (polychlorinated biphenyls)	0.001 µg/l (NYDEC 1985)
Pesticides & herbicides	1.0 ppb (Richmond 1996)
Antifouling agents (TBTO)	0.01 µg/l (Connel and Hawker 1992)
Chlorine (free)	0.20 mg/l (Tomascik 1992)
<b>Environmental Parameters</b>	
Temperature	Not > 1°C above annual sea surface mean <sup>1</sup> and never above 32°C (Edwards 1987)
Salinity	Not > 10‰ above annual mean <sup>1</sup> and never above 41 ppt (Edwards 1987)
pH	Not < 7.8 or > 8.8 (NIOF 1999)
Biological oxygen demand (5 day)	Not > 1.0 mg/l (Bell et al. 1989)
Dissolved oxygen	4.0 - 4.5 ml O <sub>2</sub> /l (Edwards 1987)

*Table 3 note on nutrient threshold values and analysis: Nutrient threshold values are statistical averages. In no case should just single sample values be compared to threshold values. Low level nutrient techniques are required to properly analyze samples. **Nutrient analysis requires high analytical precision and must only be conducted by a qualified laboratory.** In no case should off-the-shelf kits be used to determine nutrient concentrations. See Lapointe and Thacker (2002) for methods.*

## **II. Menu of Available Tools**

The following is a list of available indicators of land-based pollution stress on coral reefs, sorted by the target stressor or stressors.

### **Indicators of salinity stress**

**Pollutant:** Salinity

**Metric:** *In situ* salinity measurements

**Region tested:** Global

**Region applicable:** Global

**References:** English et al. 1997

**Protocol/Endpoint:** Obtain water samples 30 cm below the surface and at transect depth using small plastic vials for lab analysis using refractometer.

**Pollutant:** Salinity

**Metric:** Remote sensing of salinity using aircraft

**Region tested:** Global

**Region applicable:** Global

**References:** Mumby et al. 2004

**Protocol/Endpoint:**

Salinity is measured using aircraft outfitted with the microwave SLFMR (scanning low frequency microwave radiometer) sensor.

### **Indicators of sediment stress**

**Pollutant:** Sedimentation

**Metric:** Sediment deposition and turbidity

**Region tested:** Global

**Region applicable:** Global

**References:** Gardner 1980a&b; Cortes and Risk 1985; Rogers 1983, 1990

**Protocol/Endpoint:** Sediment deposition is monitored (Gardner 1980a&b, Cortes and Risk 1985) by collecting sediment trap samples at and above the substrate to ensure sediment threshold limits of 10 mg/cm<sup>2</sup>/day are not exceeded (Rogers 1983, 1990). Monitor turbidity daily at transect sites using nephelometer to ensure it does not exceed threshold limits of not greater than 1.0-2.0 NTU (Telesnicki & Goldberg 1995). Monitor light transmission using underwater light meter to check values at bottom (K threshold ≤ 0.15 Yentch et al. 2002).

**Pollutant: Sedimentation**

**Metric:** suspended sediment, light attenuation, ultraviolet and photosynthetically active radiation

**Region tested:** Global

**Region applicable:** Global

**References:** Andrefouet et al. 2002, Acker et al. 2002, Mumby et al. 2004

**Protocol/Endpoint:** Remote sensing using aircraft and satellites.

Suspended sediment concentration is measured using aircraft outfitted with the hyperspectral sensors AVIRIS (Airborne visible/infrared imaging spectrometer), CASI (Compact airborne spectrographic imager), and ATM (Airborne thematic mapper) and using satellites outfitted with the hyperspectral sensor Hyperion, and the multispectral high resolution sensors IKONOS, and Quickbird; the multispectral medium resolution sensors Landsat TM (Thematic mapper), SPOT (Systeme Probatoire de l'observations de la terre), and IRS (Indian remote sensing satellite); the multispectral low resolution sensors SeaWiFS (Sea wide field-of-view sensor) and MODIS (moderate resolution imaging spectroradiometer); and the radiometer AVHRR (Advanced very high resolution radiometer), ATSR (Along-track scanning radiometere and advanced along-track scanning radiometer).

Light attenuation coefficients is measured using aircraft outfitted with the hyperspectral sensors AVIRIS, CASI, and ATM and using satellites outfitted with the hyperspectral sensor Hyperion, and the multispectral high resolution sensors IKONOS, and Quickbird; the multispectral medium resolution sensors Landsat TM, SPOT, and IRS; the multispectral low resolution sensors SeaWiFS and MODIS; and the radiometer AVHRR, ATSR.

Ultraviolet radiation is measured using meterological satellites outfitted with GOES, GMS, and METEORSAT and the radiometer AVHRR, ATSR.

Photosynthetically active radiation is measured with the hyperspectral sensor Hyperion, multispectral high resolution sensors IKONOS, and Quickbird; the meterological satellites outfitted with GOES, GMS, and METEORSAT and the multispectral low resolution sensors SeaWiFS and MODIS; and the radiometer AVHRR, ATSR.

**Pollutant: Sedimentation**

**Metric:** Tank system for studying sedimentation

**Region tested:** Lab

**Region applicable:** Global

**References:** Anthony KRN

**Protocol/Endpoint:** A tank system is designed to test the long-term exposure of sessile organisms to well-defined ranges of particle loads on a background of natural flowing seawater. Using low technology and a simple mathematical model, the concentration of suspended particulate matter (SPM) and the rate of sedimentation could be predicted and sustained with high precision. The effect of shading was also tested. The high level of environmental control and the constancy of SPM treatment levels were reflected in the absence of tank effects on growth rates and provided sufficient statistical power to detect small differences in growth rates between corals from different treatments.

**Pollutant: Sedimentation**

**Metric:** Sedimentation models

**Region tested:** Global

**Region applicable:** Global

**References:** Kuo et al. 1985, Kuo & Hayes 1991, Booty 2001, DHI 2004

**Protocol/Endpoint:** Use sedimentation and turbidity modeling methods (such as; Kuo et al. 1985, Kuo & Hayes 1991, Booty 2001, DHI 2004) to estimate the sedimentation footprint and turbidity impact on the existing biotic communities. Confirm that sediment and turbidity model input data for grain size are appropriate for local conditions and that sediment blanket thickness threshold (not greater than 1 mm/day, Rogers 1983) and turbidity threshold (not greater than 1.0-2.0 NTU, Telesnicki & Goldberg 1995) are used as standards for the modeling.

DHI (2004) software consists of the following models. MIKE 21 is a 2D engineering modeling tool for rivers, estuaries and coastal waters. MIKE 21 consists of more than twenty modules covering the following areas: Coastal hydrodynamics, Environmental hydraulics, Sediment Processes. MIKE 3 is applicable for simulations of hydrodynamics, water quality and sediment transport in all water bodies where 3D effects are important. MIKE 3 is compatible with MIKE 21 and other DHI Software products, allowing for easy model setup, transfer of boundary data, etc.

LITPACK is a comprehensive software package for the modelling of noncohesive sediment transport in waves and currents. LITPACK includes modules for the simulation of:

- \* Vertical profiles of sediment concentrations in single points
- \* Littoral sediment drift along the coastline
- \* Cross-shore bathymetry profile development
- \* Channel backfilling
- \* Coastline development due to changes in transport capacity

## Indicators of nutrient stress

**Pollutant: Fecal waste and nutrients**

**Metric:** Nitrogen isotope ratios and coprostanol levels in reef organism tissues

**Region tested:** Pacific, Caribbean

**Region applicable:** Global

**References:** Risk et al.1994; Dunn, 1995; Heikoop, 1997; Risk & Erdmann, 2000; Lapointe 1999; Sammarco et al. 1999; Heikoop et al. 1998, 2000,2001; Mendes et al. 1997; Risk et al. 2001; Lapointe and Thacker 2002; Yamamuro 2003; Barile and Lapointe 2004, Lapointe 2004

**Protocol/Endpoint:** Risk et al. (1994) and Dunn (1995) have suggested the determination of stable isotope ratios of  $^{15}\text{N}/^{14}\text{N}$  (denoted  $^{15}\text{N}$ ) in reef organism tissues as an excellent means of specifically evaluating the input of human fecal wastes into reef ecosystems. In studies in Zanzibar and the Maldives, tissues of reef corals from sites with heavy human sewage inputs showed significantly higher  $^{15}\text{N}$  values than coral tissues from relatively “clean” sites (Risk et al. 1994). This technique is based upon the stepwise enrichment of  $^{15}\text{N}/^{14}\text{N}$  ratios along increasing trophic levels, which is caused by the preferential elimination of the lighter isotope  $^{14}\text{N}$  in urine and excretion products and the resulting  $^{15}\text{N}$  increase in organism tissues and faeces (reviewed in Peterson and Fry 1987). The technique is further predicated on the hypothesis that coral reef trophic structures with differing levels of sewage inputs will reflect these differences in the  $^{15}\text{N}$  signal at each trophic level. Those reefs with minimal sewage input should exhibit relatively low  $^{15}\text{N}$  values at each trophic level, indicative of oligotrophic conditions where algal fixation of atmospheric N ( $^{15}\text{N}=0$  by definition) is the major source of nitrogen. Conversely, those reefs which are strongly impacted by inputs of human fecal matter should show enriched  $^{15}\text{N}$  values, as a result of utilization of the relatively high  $^{15}\text{N}$  fecal matter as a primary nitrogen source at the base of the trophic structure. Additional studies currently being conducted on corals in Java and Sulawesi, Indonesia, have substantiated the above results from Zanzibar and the Maldives (Risk personal communication). Extension of the technique to other reef organisms has proved successful as well. Risk and Erdmann (2000) report that stomatopod tissues from the Spermonde Archipelago in Sulawesi show a dramatic, logarithmic increase in  $^{15}\text{N}$  with increasing proximity to Ujung Pandang, a coastal city of over one million residents with no primary sewage treatment.

Continuing research on this bioassay, including comparative work in a number of different regions with varying human population levels (Lapointe and Thacker 2002; Yamamuro 2003; Barile and Lapointe 2004, Lapointe 2004), should result in the eventual calibration of a  $^{15}\text{N}$  index of sewage impacts on coral reefs. The technique has the disadvantage of requiring expensive analytical equipment (mass spectrophotometer), but the extreme sensitivity and replicability of results suggest that this assay could have widespread applicability with a number of reef taxa. Caution must also be used in analyzing data as results could be confounded by fecal material from animals other than humans.

In the Zanzibar/Maldives study, Risk et al. (1994) also suggested analyzing coral tissues for high concentrations of the sterol coprostanol, a breakdown product of cholesterol and hence a potential chemical indicator of human fecal waste. Results from coral sampling in the Maldives were inconclusive, but further research on this method is ongoing (Risk et al. 1994).

*Special note on nutrient threshold values and monitoring: Nutrient threshold values are statistical averages. In no case should just single sample values be compared to threshold values. Low-level nutrient techniques are required to properly analyze samples. Sample analysis should only be conducted by a qualified laboratory capable of high analytical precision. In no case should off-the-shelf kits be used to determine nutrient concentrations. See Lapointe and Thacker (2002) for methods.*

**Pollutant: Nutrients**

**Metric:** Carbon isotope ratios

**Region tested:** Pacific

**Region applicable:** Global

**References:** Risk et al. 1994, Heikoop et al. 2000

**Protocol/Endpoint:** Monitor stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) in nearshore coral tissue to determine the degree to which corals utilize terrigenous carbon as an ultimate food source. Terrigenous influence on diet was measurable out to the edge of the continental shelf.

**Pollutant: Nutrients**

**Metric:** Nutrient water samples

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Lapointe et al. 1997, Lapointe 1999, Lapointe and Thacker 2002, Barile and Lapointe 2004

**Protocol/Endpoint:** Water samples are analyzed for salinity, filtered (0.45  $\mu\text{m}$  GF/F filters) then frozen and subsequently analyzed for DIN and SRP using an autoanalyzer. Also analyze the GF/F filters (above) for phytoplankton biomass (chlorophyll a).

**Pollutant: Nutrients**

**Metric:** C:N:P ratio

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Lapointe et al. 1997, Lapointe 1999, Lapointe and Thacker 2002, Barile and Lapointe 2004

**Protocol/Endpoint:** Using an elemental analyzer, determine Carbon (C), nitrogen (N) and phosphorous (P) ratios (C:N:P) in fleshy macroalgae.

**Pollutant: Nutrients**

**Metric:** Community composition

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Lapointe et al. 1997, Lapointe 1999, Lapointe and Thacker 2002, Lapointe et al. 2002, Barile and Lapointe 2004

**Protocol/Endpoint:** Correlates increased nitrogen and phosphorous loads with changes in community composition. Techniques include: using video transect imagery using the point-count method and quantitative quadrat sampling to estimate biomass and species composition of reef macroalgae.

**Pollutant: Nutrients**

**Metric:** Productivity rates

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Lapointe 1995, 1999

**Protocol/Endpoint:** Nutrient enrichment assays involved analysis of macroalgal primary productivity measured by changes in dissolved oxygen using experimental procedures described by Lapointe 1995. These experiments determined the effects of DIN (dissolved inorganic nitrogen) and SRP (soluble reactive phosphorous) enrichment on P<sub>max</sub> (photosynthetic capacity at light saturation) for algae which dominated shallow hard substrata on the back reef. Factorial enrichment treatments included overnight pulsing with NO<sup>3</sup>, SRP, NO<sup>3</sup> and SRP, and a control without added nutrients.

**Pollutant: Nutrients**

**Metric:** Alkaline phosphatase activity

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Lapointe 1999

**Protocol/Endpoint:** Alkaline phosphatase is an exoenzyme produced by P-deficient macroalgae that allows them to utilize ambient dissolved organic phosphorus (DOP) pools as a source of SRP for growth; accordingly, it is useful as a means to gauge the degree of P-limited productivity of coral reef macroalgae. Alkaline phosphatase activity (APA) was measured on a variety of dominant macroalgae by the spectrophotometric method described in Lapointe and O'Connell (1989). For comparison, APA was measured in an oligotrophic system in Belize (Lapointe et al. 1993). APA was also measured in following overnight nutrient pulses to assess metabolic responses to DIN and SRP enrichment, which could potentially have different effects on the status of P-limited productivity.

**Pollutant: Nutrients**

**Metric:** Chlorophyll-*a* concentration and algal blooms

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Lapointe and Thacker 2002, Barile and Lapointe 2004

**Protocol/Endpoint:** GF/F filters used for filtering water samples were frozen and subsequently analyzed for phytoplankton biomass (chlorophyll-*a*). Typical values in Caribbean “blue water” are <0.1µg/l.

**Pollutant: Nutrients**

**Metric:** Chlorophyll-*a* concentration and algal blooms

**Region tested:** Global

**Region applicable:** Global

**References:** Mumby et al. 2004

**Protocol/Endpoint:** Remote sensing using aircraft and satellites.

Chlorophyll-*a* concentration is measured using aircraft outfitted with the hyperspectral sensors AVIRIS(Airborne visible/infrared imaging spectrometer) , CASI (Compact airborne spectrographic imager), and ATM (Airborne thematic mapper) and using satellites outfitted with the hyperspectral sensor Hyperion, and the multispectral high resolution sensors IKONOS, and Quickbird; the multispectral medium resolution sensors Landsat TM (Thematic mapper), SPOT (Systeme Probatoire de l’observations de la terre), and IRS (Indian remote sensing satellite) ; and the multispectral low resolution sensors SeaWiFS (Sea wide field-of-view sensor) and MODIS (moderate resolution imaging spectroradiometer).

Algal blooms are measured using aircraft outfitted with the hyperspectral sensors AVIRIS(Airborne visible/infrared imaging spectrometer) , CASI (Compact airborne spectrographic imager), and ATM (Airborne thematic mapper) and using satellites outfitted with the hyperspectral sensor Hyperion, the multispectral medium resolution sensors Landsat TM (Thematic mapper), SPOT (Systeme Probatoire de l’observations de la terre), and IRS (Indian remote sensing satellite) ; and the multispectral low resolution sensors SeaWiFS (Sea wide field-of-view sensor) and MODIS (moderate resolution imaging spectroradiometer).

**Pollutant: Nutrients**

**Metric:** Human population concentration in the watershed

**Region tested:** Global

**Region applicable:** Global

**References:** Walsh 1984

**Protocol/Endpoint:** The nutrient load of a river is directly proportional to the human populations in the watershed.

**Pollutant: Nutrients**

**Metric:** Nutrient transport modeling

**Region tested: Global**

**Region applicable:** Global

**References:** Knisel et al. 1993, Brunell et al. 1996, Booty 2001

**Protocol/Endpoint:** The modified GLEAMS model (Knisel et al. 1993, Brunell et al. 1996), or other fate and transport type models such as RAISON (Booty 2001), can be used as a crude tool to predict the movement of nutrients and pesticides on the surface of the golf course. The GLEAMS model was developed to predict the fate of agricultural chemicals and determine the best management practices for agricultural land use. With some modifications to the program and the input parameters, GLEAMS can predict the movement of nutrients and pesticides into the water system of a golf course and which chemicals may pose a direct threat to the quality of surface waters. When the construction of a course is proposed, the input requirements can be obtained through soil testing and site plans (Balogh and Walker 1992, Balogh and Watson 1992, Balogh and Anderson 1992). The GLEAMS model could be used to determine the best management practices for the course, the best locations of ponds, and the most effective grading of the land.

Note: GLEAMS type models can also be used to model an existing course to assist golf course managers in choosing the best management practices in order to preserve surface water quality.

## **Indicators of heavy metal stress**

**Pollutant: Heavy metals**

**Metric:** Tolerance limits for heavy metals

**Region tested:** Global

**Region applicable:** Global

**References:** Peters et al. 1997

**Protocol/Endpoint:** Heavy metal pollution cause physiological stress, reduced reproductive success and mortality in fish and invertebrates.

**Research recommendation:** Since tolerance limits are species specific, more research is needed on different species from various coral reefs around the world.

## **Indicators of petroleum stress**

**Pollutant:** Petroleum stress

**Metric:** Tolerance limits for petroleum stress

**Region tested:** Global

**Region applicable:** Global

**References:** Lane and Harrison 2002

**Protocol/Endpoint:** Petroleum pollution cause physiological stress, reduced reproductive success and mortality in fish and invertebrates. Lane and Harrison 2002 provide tolerance limits for coral exposed to bunker oil (oil slick), dispersed oil, and oil dispersant.

**Research recommendation:** Since tolerance limits are species specific, more research is needed on different species from various coral reefs around the world.

## **Indicators of herbicide and pesticide stress**

**Pollutant:** Herbicides and pesticides

**Metric:** Water chemistry

**Region tested:** Global

**Region applicable:** Global

**References:** Stewart and Anderson 1998

**Protocol/Endpoint:** Monitor water for the presence of herbicides and pesticides using mass spectrometer techniques on surface and bottom seawater samples.

**Pollutant:** Herbicide (Irgarol 1051)

**Metric:** Gas chromatograph with mass selective detector

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Owen et al. 2002

**Protocol/Endpoint:** Irgarol 1051, a potent inhibitor of coral photosynthesis, was detected in subsurface waters around marinas, harbors and coastal waters of the Florida Keys, Bermuda and St. Croix using a HP 6890 gas chromatograph with 5973 mass selective detector equipped with an autosampler and HP-5MS capillary column (30 m length, 250 µm diameter in selected ion monitoring mode).

**Pollutant:** Herbicides and pesticides

**Metric:** Ecotoxicological risk assessment

**Region tested:** Atlantic

**Region applicable:** Global

**References:** Scott et al. 2002

**Protocol/Endpoint:** Ecotoxicological studies found evidence of pesticide runoff, particularly endosulfan, in south Florida estuaries.

**Pollutant:** Any metallic biocide or fungicide (i.e., Tributyltin)

**Metric:** Gastropod imposex

**Region tested:** Caribbean, Pacific, Indian Oceans

**Region applicable:** Global

**References:** Ellis and Pattisina 1990, Davies and Minchin 2002, Bech 2002 a & b, Gibson and Wilson 2003, Bech et al. 2002

**Protocol/Endpoint:** A well-substantiated bioassay with extreme sensitivity and response specificity is the evaluation of gastropod imposex as an indicator of metallic biocide or fungicide pollution in marine ecosystems around the world (Ellis and Pattisina 1990, Foale 1993, Gibbs and Bryan 1994). Imposex is the imposition of male sexual characteristics on female marine snails; its occurrence in snail populations generally signals exposure to a metallic biocide or fungicide such as tributyltin, an extremely toxic biocide, which is still used in antifouling paints in a number of developing countries lacking strong environmental protection laws. Imposex as a result of metallic biocide or fungicide exposure (often at concentrations below the limits of chemical analytical detection) has been reported from over 45 species of neogastropod, including reef-associated species of the genera *Thais* and *Vasum* (Ellis and Pattisina 1990, Evans et al. 1995). The occurrence and severity of imposex in a particular population is usually quantified using both frequency of imposex in females and the relative penis size index (RPS Index), calculated by dividing the mean ratio of penis weight to body weight for all females sampled by the mean ratio for the males (Foale 1993; note that other authors - e.g., Ellis and Pattisina 1990 - use penis length in calculating the RPS index instead). In populations which have not been exposed to metallic biocide or fungicide, both the frequency of female imposex and the RPS index is expected to be zero (or nearly so), as unaffected females do not normally develop a penis. In populations with metallic biocide or fungicide exposure, the frequency of imposex often reaches 100%, at which point the RPS index is necessary to differentiate the severity of exposure between populations (Ellis and Pattisina 1990).

In monitoring situations where metallic biocide or fungicide exposure is a concern, measurement of gastropod imposex is a fully-developed bioassay with proven applicability to coral reef systems. The protocol is fast, inexpensive, and the results are easily interpreted. The only potential problem with its use can be the collection of sufficient sample sizes of the snails, which typically prefer “rocky shore” habitats (Evans et al. 1995).

**Pollutant: Herbicides and pesticides**

**Metric:** Pesticide transport modeling

**Region tested:** Global

**Region applicable:** Global

**References:** Knisel et al. 1993, Brunell et al. 1996, Booty 2001

**Protocol/Endpoint:** The modified GLEAMS model (Knisel et al. 1993, Brunell et al. 1996), or other fate and transport type models such as RAISON (Booty 2001), can be used as a crude tool to predict the movement of nutrients and pesticides on the surface of the golf course. The GLEAMS model was developed to predict the fate of agricultural chemicals and determine the best management practices for agricultural land use. With some modifications to the program and the input parameters, GLEAMS can predict the movement of nutrients and pesticides into the water system of a golf course and which chemicals may pose a direct threat to the quality of surface waters. When the construction of a course is proposed, the input requirements can be obtained through soil testing and site plans (Balogh and Walker 1992). The GLEAMS model could be used to determine the best management practices for the course, the best locations of ponds, and the most effective grading of the land.

Note: GLEAMS type models can also be used to model an existing course to assist golf course managers in choosing the best management practices in order to preserve surface water quality.

## **Indicators of bacteria stress**

**Pollutant: Bacteria and nutrients**

**Metric:** Bacteriophage tracers

**Region tested:** Florida Keys

**Region applicable:** Global

**References:** Paul et al. 1995, 1997, 2000; Griffin et al. 1999

**Protocol/Endpoint:** Use bacteriophage tracers to demonstrate that injection of partially treated wastewater into highly porous limestone results in contamination of Florida Keys coastal waters. Viral tracers were recovered from marine surface waters in as little as ten hours after seeding of waste disposal systems. These studies provided direct evidence for both pathogen and nutrient contamination of coastal environments. Szmant and Forrester (1996) argue that nutrient pollution from the coastal zone of the Florida Keys is quickly taken up by nearshore biotic communities and does not reach the reef tract.

## Indicators of mixture stress

**Pollutant:** Mixture

**Attribute:** Percent cover of lifeforms

**Region tested:** Global

**Region applicable:** Global

**References:** English et al .1997; Rogers et al 2001

**Protocol/Endpoint:** Use quantitative lifeform baseline data (English et al. 1997, Rogers et al. 2001) to monitor change in percent cover of lifeforms.

**Pollutant:** Mixture

**Attribute:** Deterioration Index (for branching coral)

**Region tested:** Red Sea

**Region applicable:** Global

**References:** Ben-Tzvi et al. 2004

**Protocol/Endpoint:** The Deterioration Index (DI) is based on the ratio between mortality and recruitment rates of branching corals. It provides a comparable quantitative assessment of the deterioration process in a coral community and its intensity. To include massive and encrusting corals in the DI determination of erosion and accretion rates is required so dead corals can be recognized and their mortality time estimated.

## III. Menu of Potential Tools

### Indicators of sediment stress

**Pollutant:** Sedimentation

**Attribute:** Gene expression of fructose-1,6-bisphosphatase and succinae-dehydrogenase in the azooxanthellate octocoral *Dendronephthya klunzingeri*

**Region tested:** Red Sea

**Region applicable:** Global

**References:** Ammar et al. (2000)

**Protocol/Endpoint:** Ammar et al. (2000) assayed for gene expression of fructose-1,6-bisphosphatase and succinae-dehydrogenase in octocoral by isolating the cDNA for both compounds. The levels of expression of these enzymes were correlated with stress from sedimentation.

**Research recommendation:** The correlation, gene expression and physical stress will be studied in more detail to clarify if the molecular biological parameter can be used as a biomarker for the assessment of pollution in general.

**Pollutant: Sedimentation**

**Attribute:** Histopathological Indices

**Region tested:** Lab

**Region applicable:** Global

**References:** Vargas-Angel et al. 2004

**Protocol/Endpoint:** Lab sediment exposed corals were histologically assessed for the relative condition/integrity of tissues and selected cellular elements, including: epidermis, gastrodermis, mucos secretory cells, amount and color of mucoid secretions, zooxanthellae, and nuclei. Slight adverse effects were noted as early as week one for all species studied.

**Research recommendation:** These indices offer potential for coral health assessment and warrant further study.

**Pollutant: Sedimentation**

**Attribute:** Coral growth rate

**Region tested:** Pacific, Caribbean

**Region applicable:** Global

**References:** Brown and Howard 1985, Hudson 1981, Cortes and Risk 1985, Rogers 1990, Brown et al. 1990, Tomascik and Sander 1985, Risk et al. 1995, Edinger 1991

**Protocol/Endpoint:** A number of studies have suggested that coral growth rate is one of the most relevant individual-based parameters for measuring declining environmental quality on reefs (reviewed in Brown and Howard 1985). Despite this assertion, the literature provides conflicting evidence of the effects of stress on coral skeletal extension rates. For example, though a number of workers (e.g., Hudson 1981, Cortes and Risk 1985, Rogers 1990) have suggested that massive corals demonstrate a decrease in growth rate under environmental stress such as increased siltation, Brown et al. (1990) found no apparent effect of increased sedimentation on growth rates of *Porites* on a reef in Thailand which had experienced significant coral mortality due to dredging. Still others (Tomascik and Sander 1985, Risk et al. 1995) report that corals from eutrophied and sedimented sites often demonstrate an initial increase in growth rate due to increased nutrient availability and the use of particulate matter as a food source (though corals on the most eutrophied sites in these studies did show a reduction in growth rates). Edinger (1991) has termed this the “Janus effect”, whereby nutrient enhancement can increase coral growth rates up to a certain critical level, after which eutrophication becomes deleterious and growth rates decline.

**Research recommendation:** Obviously, this phenomena is in need of further research before coral growth rates can be interpreted reliably and their measurement properly calibrated for use in water quality assessment.

**Pollutant: Drilling discharges (sedimentation)**

**Attribute:** Coelobite Index

**Region tested:** Pacific (Philippines)

**Region applicable:** Global

**References:** Choi 1982

**Protocol/Endpoint:** Choi (1982) proposed that coelobite communities (reef cavity-dwellers such as foraminifers, bryozoans, tunicates, molluscs, sponges and serpulid worms) also respond in a sensitive manner to environmental stress, though in an opposite manner from that of internal bioeroders. His study on the effects of offshore drilling on coral rubble-dwelling coelobite communities showed a dramatic decrease in abundance of coelobites with proximity to the well-head, which he suggests is an effect of the greater concentration of drilling discharges close to the well-head. Drilling discharges were postulated to affect coelobites by direct smothering and/or iron toxicity. In order to characterize the effects on community structure, Choi developed a numerical index whereby he assigned points to each community (rubble piece) sampled based upon the presence/absence and abundance of various coelobite groups. Using the results of his study, he calibrated the index and assigned interpretive meanings to various scores (e.g., scores of 10 or higher indicate a “healthy” or “recovering” coelobite community). While the widespread applicability of this bioassay has yet to be demonstrated (offshore drilling is a relatively uncommon stress to reefs), it may have potential for monitoring sedimentation stress on reefs. The method is particularly noteworthy in that it is one of the only examples of a calibrated numerical index of reef community health.

**Research recommendation:** Further research should focus on determining the sensitivity of this response relative to the hard coral community response to sedimentation (i.e., does it provide an early warning of increasing sedimentation, or is this parameter more easily measured by simple sediment traps?).

## Nutrients

**Pollutant: Nutrients**

**Attribute:** Internal bioeroders

**Region tested:** Caribbean (Barbados, Grand Cayman), Pacific (Great Barrier Reef)

**Region applicable:** Global

**References:** Rose and Risk 1985, Sammarco and Risk 1990, Risk et al. 1995, Holmes 1997

**Protocol/Endpoint:** Internal bioeroders, have been thoroughly investigated and have demonstrated a consistent, graded response of increasing abundance with increasing eutrophication on reefs (Rose and Risk 1985, Sammarco and Risk 1990, Risk et al. 1995, Holmes 1997). Holmes (1997) found that the proportion of dead coral rubble invaded by clionid sponges, as well as the number of invasions per rubble sample, increased dramatically with increasing eutrophication on reefs of Barbados. Rose and Risk (1985) found similar results with *Cliona* infestations of live *Montastrea cavernosa* heads in the Grand Caymans, while Sammarco and Risk (1990) and Risk et al. (1995) suggested that

distinctive cross-continental shelf patterns of bioerosion (by sponges and bivalves) in *Porites* and *Acropora* on the Great Barrier Reef were explained primarily by increasing organic input with proximity to the mainland.

**Research recommendation:** Though this group has not yet been formally proposed for inclusion in biomonitoring programs, results of the above research suggest that internal bioeroders provide a sensitive assessment of increasing eutrophication on reefs and that development of a rigorous bioassay could be accomplished with minimal additional research.

**Pollutant: Nutrients**

**Attribute:** Coral fecundity, fertilization rate

**Region tested:** Caribbean, Pacific, Indian

**Region applicable:** Global

**References:** Tomascik and Sander 1987b, Ward 1997, Ward and Harrison 1999,

**Protocol/Endpoint:** Recent results from the large-scale ENCORE experiment show conclusively that increased ammonium and phosphate levels in reef environments have strongly negative effects on coral fecundity and fertilization rate. In experiments with several acroporid species, corals subject to increased nutrient levels had significantly smaller and fewer eggs and less testes, and fertilization rates were reduced.

**Research recommendation:** Though the authors did not suggest that these coral parameters be used as a bioassay of eutrophication, these results corroborate earlier suggestions that coral fecundity and fertilization rate may be used as sensitive indicators.

**Pollutant: Nutrients**

**Attribute:** Coral fecundity and recruitment

**Region tested:** Caribbean, Pacific

**Region applicable:** Global

**References:** Brown 1988, Tomascik and Sander 1987b

**Protocol/Endpoint:** Two further individual-based coral parameters which Brown (1988) proposes as potentially useful indicators of sublethal stress on coral reefs are coral fecundity and recruitment. Tomascik and Sander (1987b) suggest that coral fecundity is decreased on reefs subject to increased eutrophication.

**Research recommendation:** Again, this area of research seems particularly promising, and is just starting to be applied in a systematic, calibrated fashion to water quality biomonitoring efforts on reefs. Research is needed to determine the effects of pollutants on corals and coral reefs through the use of fertilization and recruitment bioassays. The use of adult corals (measuring growth rates, fecundity, and symbiotic associations) as well as their gametes and larvae as ecological indicators addresses the concern that mortality is a crude measure of environmental stress. Determining and measuring sublethal effects allow for a more proactive approach to monitoring and management.

**Pollutant: Nutrients**

**Attribute:** Coral settlement rate

**Region tested:** Caribbean, Pacific, Indian

**Region applicable:** Global

**References:** Ward and Harrison 1997, Ward and Harrison, 1999

**Protocol/Endpoint:** Further results from the ENCORE experiment show that settlement tiles placed in reef environments subject to increased levels of ammonium and of ammonium and phosphate have significantly reduced settlement of coral spat.

**Research recommendation:** Though not yet developed into a biomonitoring protocol, use of settlement tiles for water quality monitoring of nutrient inputs to reef environments is a promising technique worthy of further research.

**Pollutant: Nutrients**

**Attribute:** Coral settlement

**Region tested:** Pacific

**Region applicable:** Global

**References:** Richmond 1993, McKenna et al 2001

**Protocol/Endpoint:** Selected organophosphates can inhibit settlement of coral larvae. McKenna et al. (2001) developed procedures for using this information in assessment.

**Research recommendation:** Since corals spawn annually, availability of larvae restrict the widespread applicability of such methods.

**Pollutant: Nutrients**

**Attribute:** Giant clam zooxanthellae

**Region tested:** Pacific, Indian

**Region applicable:**

**References:** Koop et al. 2001, Ambariyanto and Hoegh-Guldberg 1996, 1997, Ambariyanto 1996, Belda et al. 1993b, Belda-Baillie et al. 1998

**Protocol/Endpoint:** Giant clam zooxanthellae populations are generally considered to be N-limited. Results from the ENCORE experiment demonstrate conclusively that zooxanthellae in *Tridacna maxima* show a number of interrelated responses to increased ammonium, including an increase in the density and chlorophyll content of zooxanthellae, a decrease in the average size of zooxanthellae, and a decrease in the starch sheath surrounding the pyrenoid of the zooxanthellae chloroplasts.

**Research recommendation:** This sensitive response of giant clam zooxanthellae populations make them an excellent candidate for development as bioindicators of nutrient enrichment. Monitoring the size of clam zooxanthellae seems particularly promising, as it is quick, easy and does not harm the clam.

**Pollutant: Nutrients**

**Attribute:** Giant clam shell growth rate

**Region tested:** Pacific, Indian

**Region applicable:** Pacific, Indian

**References:** Ambariyanto 1996, Belda et al. 1993a, Koop et al. 2001

**Protocol/Endpoint:** Results from the ENCORE experiment show that giant clams (*T. maxima*) exposed to increased levels of ammonium have significantly increased shell growth rates. This parameter is easy and inexpensive to monitor. .

**Research recommendation:** With proper calibration this attribute could be an excellent metric for monitoring programs concerned with nutrient enrichment.

**Pollutant: Nutrients**

**Attribute:** Coral growth rate

**Region tested:** Pacific, Caribbean

**Region applicable:** Global

**References:** Brown and Howard 1985, Hudson 1981, Cortes and Risk 1985, Rogers 1990, Brown et al. 1990, Tomascik and Sander 1985, Risk et al. 1995, Edinger 1991

**Protocol/Endpoint:** A number of studies have suggested that coral growth rate is one of the most relevant individual-based parameters for measuring declining environmental quality on reefs (reviewed in Brown and Howard 1985). Despite this assertion, the literature provides conflicting evidence of the effects of stress on coral skeletal extension rates. For example, though a number of workers (e.g., Hudson 1981, Cortes and Risk 1985, Rogers 1990) have suggested that massive corals demonstrate a decrease in growth rate under environmental stress such as increased siltation, Brown et al. (1990) found no apparent effect of increased sedimentation on growth rates of *Porites* on a reef in Thailand which had experienced significant coral mortality due to dredging. Still others (Tomascik and Sander 1985, Risk et al. 1995) report that corals from eutrophied and sedimented sites often demonstrate an initial increase in growth rate due to increased nutrient availability and the use of particulate matter as a food source (though corals on the most eutrophied sites in these studies did show a reduction in growth rates). Edinger (1991) has termed this the “Janus effect”, whereby nutrient enhancement can increase coral growth rates up to a certain critical level, after which eutrophication becomes deleterious and growth rates decline.

**Research recommendation:** Obviously, this phenomena is in need of further research before coral growth rates can be interpreted reliably and their measurement properly calibrated for use in water quality assessment.

**Pollutant: Nutrients**

**Attribute:** Composition of reef sediments

**Region tested:** Atlantic

**Region applicable:** Global

**References:** McKee et al. 1956, Lidz and Hallock 2000

**Protocol/Endpoint:** The composition of reef sediments as a whole reflect benthic community structure and function. Mixotrophic organisms, particularly those that calcify, are most competitive in low nutrient environments. Therefore, reef sediments are dominated by readily identifiable skeletal components, particularly larger foraminifers (McKee et al. 1956). As nutrient supplies increase, skeletal fragments of calcareous algae and herbivorous gastropods increase in abundance, as do bioeroded coral fragments. When nutrient supplies increase even further, such that fleshy algae, boring

sponges and bivalves become increasingly prevalent, recognizable coral and larger foraminiferal constituents become increasingly rare, while unrecognizable fragments, along with shells and fragments of heterotrophic foraminifers and bivalves, become dominant. Because carbonate sediment composition reflects community structure and function, the technique can be applied as an assessment tool worldwide.

**Research recommendation:** Further evaluation is needed to produce a standardized protocol.

## Indicators of heavy metal stress

**Pollutant:** Heavy metal stress

**Attribute:** Activity levels of carbonic anhydrase (CA)

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Gilbert and Guzman 2001

**Protocol/Endpoint:** Activity levels of CA were assessed in anemones *Condylactis gigantea* and *Stichodactyla helianthus* with lab exposure to copper, nickel, lead, and vanadium, and also in animals collected from polluted vs. minimally impaired field sites. CA activity was found to be decreased with increase in metal concentration and also in animals collected from the polluted field site.

**Research recommendation:** Preliminary assessments to adapt the CA assay for use in *Montastraea cavernosa* showed decreased CA activity in specimens from the polluted field site and provide an avenue for future research aimed at more thoroughly describing coral CA activity for potential application in bioindication.

## Indicators of cellular and genetic stress

**Pollutant:** Cellular stress

**Attribute:** Molecular Biomarker System (MBS)

**Region tested:** Atlantic (Florida)

**Region applicable:** Global

**References:** Downs et al. 2000, 2001a, 2001b, 2002a, 2002b, in press;

Fauth et al. 2004b, Anderson et al. 2001

**Protocol/Endpoint:** Assays specific cellular and molecular parameters to assess the physiological status of coral reef organisms challenged by heat, oxidative, or xenobiotic stress and indicates if defenses have been mounted against a particular stressor or stressors (i.e., pesticide, heavy metal, PAH). This technology aids in the accurate diagnosis of coral condition because each parameter is physiologically well understood.

The MBS technology is relatively inexpensive, easy to implement, precise, and can be quickly adapted to a high throughput robotic system for mass sample analysis. The MBS:

- distinguished the separate and combined effects of heat and light on the two coral symbionts, a scleractinian coral and a dinoflagellate algae (zooxanthellae) (Downs et al. 2000);
  - assessed the physiological status of grass shrimp challenged with exposure to heat stress, cadmium, atrazine, and water-accommodating fraction of either diesel fuel or bunker fuel No. 2 (Downs et al. 2001a);
  - assessed the physiological status of mud snails challenged by exposure to high temperature, cadmium, atrazine, endosulfan and the water-accommodating fraction of bunker fuel No. 2 (Downs et al. 2001b);
  - determined the cellular physiological stress of bivalves in an area experiencing a 10 year chronic exposure of spilled *Exxon Valdez* crude oil in Prince William Sound (Downs et al. 2002a; and
  - showed that coral bleaching is tightly coupled to the antioxidant and cellular stress capacity of the symbiotic coral, supporting the mechanistic model that coral bleaching may be the final strategy to defend corals from oxidative stress (Downs et al. 2002b); and
  - assess the health of the coral reef ecosystem in the Florida Keys (Wodley et al. 2004b).
- Research recommendation:** Advancing the wide-scale application of cellular-based metrics requires progress in three areas: knowledge of fundamental processes, technology development and application, and validation of concept and technology (i.e., an applied method of investigation) to real world conditions.

**Pollutant: Cellular stress**

**Attribute:** Molecular Diagnostic System (MDS)

**Region tested:** western Atlantic

**Region applicable:** Global

**References:** Woodley et al. 2004a, Fauth et al. 2004a

**Protocol/Endpoint:** Like the MBS above, the MDS is an array of biomarkers of known biochemical, cellular and physiological processes and are tailored to individual species and likely sources of environmental stress. The system is designed with redundancy; several parameters from differing biochemical pathways characterize physiological status, thereby allowing concordance among markers for a more reliable diagnosis.

**Research recommendation:** see above

**Pollutant: Cellular stress**

**Attribute:** Rapid Assessment of Marine Pollution (RAMP)

**Region tested:** Global

**Region applicable:** Global

**References:** Dankward et al. 1998, Wells et al. 2001, Galloway et al. 2002

**Protocol/Endpoint:** Chemical markers measured using immunoassays (i.e., enzyme-linked immunosorbent assays -ELISAs) provide an inexpensive, rapid, and highly selective means of measuring specific chemical compounds (Dankward et al. 1998).

The method involves using antibodies that have been raised to specific types of chemical pollutants. Tests are designed so that the intensity of a color reaction diminishes when the antibody and chemical combine (i.e., diminution in color is used to estimate concentrations of a chemical in samples).

PCB and PAH concentrations were detected in mussels in New Bedford Harbor, MA using RaPID immunoassay (Galloway et al. 2002). Selected analyses were verified using GC/MS. While causality could not be attributed, multivariate canonical correlation analysis indicated that PCB and PAH concentrations were strongly associated with the induction of biomarkers of genotoxicity (micronucleus formation), immunotoxicity (spontaneous cytotoxicity), and physiological impairment (heart rate).

**Research recommendation:** The incorporation of chemical immunoassays with biological monitoring tools into routine management procedures is clearly viable and valuable as a means of identifying toxic impacts of pollutants on biota *in situ*.

**Pollutant: Cellular stress**

**Attribute:** Endocrine disruption in fish

**Region tested:** Atlantic

**Region applicable: Global**

**References:** Hutchinson and Pickford 2002

**Protocol/Endpoint:** A tiered approach is recommended for endocrine disruption risk assessment incorporating a fish 14-day screening assay (Tier 1), fish development and reproduction tests (both tier 2), and a fish full life-cycle test (tier 3). For detection of (anti-)oestrogens, the yolk-precursor protein vitellogenin is an ideal biomarker of exposure and functionally equivalent biomarkers are being sought for (anti-)androgens in fish. At the two higher tiers, impacts are assessed in terms of apical endpoints (e.g. development, breeding behavior and fecundity) and also gonadal histopathology.

**Research recommendation:** Validation of these higher tier tests should include comparison of sensitivity of biochemical and apical endpoints to optimise the value of biomarkers for predicting adverse health effects (e.g. impaired reproduction). The specificity of future OECD fish and amphibian test guidelines for endocrine disruptors needs further consideration through inclusion of mechanistic endpoints based on state-of-the-art molecular endocrinology.

**Pollutant: Cellular stress**

**Attribute:** Biomarker responses in zooplankton

**Region tested:** Freshwater lakes

**Region applicable: Global**

**References:** Fossi et al. 1996, 2001

**Protocol/Endpoint:** Acetylcholinesterase activity (AChE) was determined in homogenates of whole organisms of the copepods *Acartia margalefi*, *A. latisetosa* and the mysid *Siriella clausi*. Preliminary lab work was also carried out on *A. latisetosa* exposed to the organophosphorus insecticide (parathion). AChE activities were two orders of magnitude higher in the zooplankton than in hemolymph samples of the decapod *Carcinus aestuarii* (Fossi et al 1996) indicating that these species have a high metabolic rate, which makes them suitable for biomarker studies.

**Research recommendation:** Two main lines of future research will be to determine the basal activities of biomarkers in several zooplankton species exposed to environmental contaminants in the lab, to study different environments at different contamination levels (coastal areas, harbors, lagoons), and to evaluate the biological marker responses of the selected zooplankton bioindicator species.

**Pollutant: Cellular stress**

**Attribute:** Early warning for coral bleaching

**Region tested:** Pacific

**Region applicable:** Global

**References:** Smith et al. 2004

**Protocol/Endpoint:** Heat shock proteins (HSPs) and antioxidant enzymes (such as copper zinc superoxide dismutase) was used for predicting the occurrence of coral bleaching and diagnosing their cause.

**Research recommendation:** Cellular biomarker data must be cautiously interpreted in the context of seasonal physiological status.

**Pollutant: Cellular stress**

**Attribute:** Virtual cells, organs, and animals

**Region tested:** Lab

**Region applicable:** Global

**References:** Moore 2002

**Protocol/Endpoint:** The development and use of process-based computational simulation models (i.e., virtual cells, organs, and animals), using endosomal/lysosomal uptake and cell injury models, will facilitate the development of a predictive capacity for estimating risk associated with the possibility of future environmental events.

**Research recommendation:** Further research is needed on the effects of physico-chemical speciation on uptake and toxicity, the toxicity of complex mixtures, and linking the impact of pollutants through the various hierarchical levels of biological organization to ecosystem and human health.

**Pollutant: Genetic stress**

**Attribute:** cDNA array

**Region tested:** western Atlantic

**Region applicable:** Global

**References:** Brogdon et al. 2004

**Protocol/Endpoint:** Using a variety of techniques, a cDNA array was developed consisting of 32 gene probes. The known function of these genes allows changes in their expression to be associated with thermal stress, oxidative stress, and sediment stress, among others.

**Research recommendation:** New research will involve correlating gene expression patterns with environmental parameters to track the timing of stress events and to diagnose the main causes of stressors impacting natural populations of corals.

**Pollutant: Genetic stress**

**Attribute:** Coral stress using gene expression

**Region tested:** western Atlantic (Florida)

**Region applicable:**

**References:** Morgan et al. (2001)

**Protocol/Endpoint:**

Uses recent advances in molecular biology to visualize changes in scleractinian mRNA abundance. Stressor-specific probes for mRNA are being developed for quantifying the intensity of stress in corals and diagnosing the most likely stressors.

**Research recommendation:** Transplantation experiments will be conducted to examine how stressors in natural populations induce gene expression.

**Pollutant: Cellular stress**

**Attribute:** Biomarkers and community indices as complementary tools for environmental safety

**Region tested:** Global

**Region applicable:** Global

**References:** Vasseur and Cossu-Leguille 2003

**Protocol/Endpoint:** Reviews results of various field studies to show the relevance of an integrated approach to environmental quality assessment.

**Research recommendation:** Needs extensive research associating biomarkers and ecological studies in ecosystems of different quality in order to validate the use of biomarkers for modeling environmental quality.

## **Indicators of herbicide and pesticide stress**

**Pollutant: Herbicides and Pesticides**

**Attribute:** Coral fecundity and recruitment

**Region tested:** Pacific

**Region applicable:** Global

**References:** Richmond 1993, 1994a, 1994b, 1995, 1996; Peters et al. 1997

**Protocol/Endpoint:** Ongoing research on pollutant effects on coral fecundity and recruitment (primarily out of the University of Guam) is focused on developing practical and effective methods for assessing coral reef condition and developing predictive tools to be applied to coral reef monitoring and management (Richmond 1993, 1994a, 1994b, 1995, 1996; Peters et al. 1997). Presently accepted protocols, including the use of LC-50's on adult corals or coral reef proxies (e.g. Tilapia) are often inappropriate as well as inadequate for understanding the effects of pollutants on coral reefs. Work on cyanide exposure found some effects were not apparent until two weeks after exposure. In larval recruitment bioassays, larvae exposed to the golf course pesticide Chlorpyrifos had high survivorship, but significantly reduced abilities for recruitment. In a coral reef setting, lack of recruitment is equivalent to direct mortality (Richmond personal communication).

Different life-history stages of corals exhibit differential sensitivities to pollutants. Five chemically-mediated steps have been identified that affect the success of coral reproduction and recruitment:

- 1) reproductive synchronization among conspecific corals;
- 2) egg-sperm interactions;
- 3) embryological development;
- 4) larval settlement and metamorphic induction; and
- 5) acquisition of zooxanthellae (for most spawning species).

While adult corals may survive elevated levels of certain pollutants (e.g., organophosphate pesticides), the above five links may be affected by pollutants at extremely low levels. Also, different substances will differentially affect different stages in the reproduction/recruitment cycle: hydrophilic substances will have a greater effect on reproductive synchrony, egg-sperm interactions and embryological development, while hydrophobic/lipophilic substances will affect settlement and metamorphic induction (Richmond 1996).

**Research recommendation:** Again, this area of research seems particularly promising, and is just starting to be applied in a systematic, calibrated fashion to water quality biomonitoring efforts on reefs. Research is needed to determine the effects of selected pesticides, PAH's and other potential pollutants on corals and coral reefs through the use of fertilization and recruitment bioassays. The use of adult corals (measuring growth rates, fecundity, and symbiotic associations) as well as their gametes and larvae as ecological indicators addresses the concern that mortality is a crude measure of environmental stress. Determining and measuring sublethal effects allow for a more proactive approach to monitoring and management.

## Indicators of bacterial stress

**Pollutant:** Bacterial stress

**Attribute:** Coral surface microlayer (CSM)

**Region tested:**

**Region applicable:**

**References:** Frias-Lopez et al. 2002, Lyons et al. 1998, Cooney et al. 2002, Webster et al. 2002, Cochran et al. 1998

**Protocol/Endpoint:** Observes distinct differences among bacterial assemblages in seawater versus healthy, diseased and dead coral surfaces. Lyons et al. 1998 proposed that DNA damage in microorganisms associated with the CSM could be used as an indicator of stress from damaging ultraviolet radiation (UV-B).

**Research recommendation:** Other potential methods include:

- molecular screening procedures to characterize microbial assemblages (Cooney et al. 2002, Frias-Lopez et al 2002);
- assessing for specific indicators of stress such as UV-B damage (Lyons et al 1998) and toxic metals (Webster et al. 2002); and
- detecting lysogenic viral activity in response to pollutants (Cochran et al. 1998);
- development of a prophage induction assay for detecting carcinogenic compounds in marine environments.

Advantages include non-destructive sampling.

**Pollutant: Bacterial stress**

**Attribute:** Coral mucus

**Region tested:**

**Region applicable:**

**References:** Patterson et al. 2002, Frias-Lopez et al. 2002, Lipp et al. 2002

**Protocol/Endpoint:** Terrestrial pathogens are detected in coral mucus. Patterson et al. 2002 found white pox fecal bacterium in coral mucus and Frias-Lopez et al. 2002 found bacterial sequences indicating the terrestrial origin of some bacteria in black-band infected coral surfaces.

**Research recommendation:** Lipp et al. 2002 found coral mucus to accumulate enteric microorganisms and recommended assessing human health risks as well as coral health risks.

## Indicators of mixtures of stress

### *Multi-metric indicators*

**Pollutant: Mixture**

**Attribute:** Biological Response Signatures using Diagnostic Monitoring and Assessment

**Region tested:** US (freshwater)

**Region applicable:** Global

**References:** Yoder and DeShon 2002; Jameson et al 1998,2001, subm a, subm b

**Protocol/Endpoint:** Stressor, exposure and response indicators are used to characterize and quantify the extent and severity of impairments and an interpretive process for determining the associated causes and sources of those impairments. This is accomplished by combining the concepts of the biological response signatures developed by Yoder and Rankin (1995) within a hierarchical process where chemical, physical, and biological indicators from sources of potential stress and the ambient environment are linked to form a rationale diagnosis. The indicator hierarchy used in this process was originally developed by USEPA (1995) and has been described further by Yoder and Rankin (1998).

**Research recommendation:** Needs field testing in the coral reef environment.

**Pollutant:** Mixture

**Attribute:** Coral reef index of biotic integrity

**Region tested:** Global (freshwater)

**Region applicable:** Global

**References:** Karr and Chu 1999; Jameson et al 1998, 2001, subm a, subm b

**Protocol/Endpoint:** An index of biotic integrity (IBI) is an index (expressed as a single numerical value) that integrates several biological metrics to indicate a site's condition, which can then be compared over time or with other sites. Such rankings have an intuitive appeal to resource managers and users, and can be an effective means of galvanizing political willpower towards pollution prevention and conservation activities. The coral reef metrics used in an IBI show a quantitative dose response change in attribute value across a gradient of human influence that is reliable and interpretable. Because the multimetric index is grounded in biological context and situation it can be expressed as a single number (IBI) or the metrics within the IBI can be expressed in a narrative that describes exactly how the biota at a site differs from what might be expected at a minimally disturbed site. The potential for diagnostic uses to identify causes of degradation is present as well.

IBIs are generally dominated by metrics of taxa richness, because structural changes in aquatic systems, such as shifts among taxa, generally occur at lower levels of stress than do changes in ecosystem process (Karr and Chu, 1999). However, IBIs also often include measures of ecological structure, frequency of diseased individuals, etc. and are broad in scope. IBIs can detect many influences in both time and space, reflecting changes in resident biological assemblages caused by single point sources, multiple point sources, and nonpoint sources. They can be useful in monitoring one coral reef or several, and they permit comparisons over a wide geographic area. The wide-ranging responsiveness of IBIs makes them an ideal tool for judging the effectiveness of management decisions (Karr and Chu, 1999).

Multimetric indexes avoid flawed or ambiguous indicators, such as diversity indexes or population size, and they are wider in scope (Karr and Chu, 1999). Diversity indexes are avoided because they combine richness and relative abundance; most IBIs, for example, include both richness and dominance metrics. Density or abundance measures are typically not used because of their high natural variation.

Biological assessment must have a standard (reference condition) against which the conditions of one or more sites can be evaluated. In multimetric biological assessment, reference condition equates with biological integrity. IBIs measure the divergence from biological integrity. When divergence is detected, society has a choice: to accept divergence from integrity at that place and time, or to restore the site. There are few, if any, coral reefs remaining in the world that have not been influenced by human actions. Defining and selecting reference sites, and measuring conditions at those sites, requires a careful sampling and analysis plan.

**Research recommendation:** Needs field testing in the coral reef environment.

**Pollutant: Mixture (nutrients, sediments, pesticides)**

**Attribute:** Framework to identify ecological change and its causes

**Region tested:** Pacific

**Region applicable:** Global

**References:** Fabricus and De'ath in press

**Protocol/Endpoint:** Framework is based on improved methods of statistical estimation (rejecting the use of statistical tests to detect change), and the use of epidemiological causal criteria that are both scientifically rigorous and understood by non-scientists.

Three groups of ecological attributes, benthos cover, octocoral richness, and community structure, were used to discriminate between potential causes of change in water quality. Causal criteria applied to each ecological attribute included: dose-response relationship, strength of association, logical time sequence, consistency across populations, specificity, and agreement with biological facts.

**Research recommendation:** Community structure responded more sensitively to water quality than summary parameters such as benthic cover, indicating that substantial information is contained in the responses of individual species that will require follow-up lab exposure-effects experiments.

**Pollutant: Mixture**

**Attribute:** Coral Reef Exam

**Region tested:** Pacific

**Region applicable:** Global

**References:** Richmond (in prep)

**Protocol/Endpoint:** A Coral Reef Exam was developed to detect potential problems with the health of reefs and identify areas that need further study. The exam is a set of questions addressing coral reef ecosystem parameters that can be evaluated with a reduced budget and little specialized equipment. The surveys take approximately 30-90 minutes per site depending on the level of detail and number of individuals involved and they measure: benthic and pelagic community composition; coral growth rate; symbiotic, competitive, and parasitic interactions; reproduction and recruitment; and reef metabolism. These parameters can be evaluated by a few minimally-trained marine biologists, fishers or other interested individuals. The nested approach of the exam allows individuals to gather as much information as they can based on their level of expertise, an important feature since expertise varies among islands, institutions, agencies and individuals.

Analysis of the results can point resource managers towards the potential stressors of their reef. For example, changes in dominant coral forms can indicate an increase in sedimentation and runoff. Likewise, the disappearance of crabs and shrimps within the branches of certain corals can signal a pesticide problem and would suggest the need for additional water quality testing. If a likely stressor is identified by this exam further investigations in that area would need to be conducted to confirm the initial finding. With this tool, marine resource managers can quickly and inexpensively make an initial assessment of the health and potential contributors to degradation of their coral reefs.

**Research recommendation:** Managers can use this initial assessment to aim further investigation towards appropriate avenues.

**Pollutant:** Mixture

**Attribute:** Healthy Reef Initiative

**Region tested:** Caribbean

**Region applicable:** Global

**References:** McField 2004

**Protocol/Endpoint:** Seventeen indicators are identified for monitoring the Meso-American Barrier Reef (MAR).

**Research recommendation:** Needs field testing and calibration for MBR.

### *Scleractinian coral indicators*

**Pollutant:** Mixture

**Attribute:** Coral Habitat Occupancy Index

**Region tested:** Pacific

**Region applicable:** Global

**References:** Capili et al. 2004

**Protocol/Endpoint:** Coral Habitat Occupancy Index takes into account space available for coral recruitment (i.e., hard substrate with little algae cover).

**Research recommendation:** Most useful if applied with other indices.

**Pollutant:** Mixture

**Attribute:** Percentage hard coral cover, coral diversity indices, and coral vitality indices

**Region tested:** Global

**Region applicable:** Global

**References:** Dodge et al. 1982, DeVantier 1986, Gomez and Yap 1988, Aronson et al. 1994, English et al. 1997

**Protocol/Endpoint:** To date, the majority of coral reef monitoring programs have focused on two primary parameters:

1. Percentage of live hard coral cover and;
2. Various indices of the diversity of benthic cover, either at the species or life-form level (Dodge et al. 1982, DeVantier 1986, Gomez and Yap 1988, Aronson et al. 1994, English et al. 1997).

Many workers have discussed the dangers of relying too heavily on these two state variables (e.g., Dustan and Hallas 1987, McLanahan 1997), and some have even questioned their significance. For example, Brown (1988) describes several studies which measured no effect of severe environmental perturbations on coral community diversity indices (though at least one comprehensive study, that of Tomascik and Sander 1987a, did demonstrate a sensitive response of species diversity to eutrophication stress).

**Research recommendation:** In general, these two parameters are now considered to be important to measure, but insufficient as the sole data used in reef assessment (McLanahan 1997). The realization that 100% live hard coral cover is not a standard to which most coral reefs can compare, even in a pristine state, has led a number of workers to suggest the use of coral “vitality” or “mortality” indices which take into account ratios of live and dead coral cover in an estimation of reef “health” (Grigg and Dollar 1990, Dustan 1994, Gomez et al. 1994, Ginsburg et al. 1996, Steneck et al. 1997). Similarly, Aronson et al. (1994) suggest the measurement of reef topographic complexity as a more relevant indicator of reef health than simple percentage live cover. Despite the potential improvements of these methods over measurement of percentage cover and diversity only, and their utility in providing an assessment of reef “health”, neither provide an early warning function of deteriorating environmental conditions. Standardized monitoring procedures quantifying coral abundance and diversity are snapshots in time, and are non-predictive. In many cases, impact studies (e.g. sewer outfalls) have been started years after the insult began. In such a case, we must assume that sensitive species have already been eliminated, and that we have reached a stable state. By carefully choosing sensitive scleractinian coral indicator species and transplanting them back to affected areas (over a gradient) we can choose several sub-lethal indicators (possibly growth rate, fecundity, etc) and determine at what distance these are no longer affected. We can then determine if reaching that level of water/substratum quality is cost effective or possible considering the improved conditions for reef recovery.

Brown (1988) reviews a number of additional coral-focused parameters which may provide an indication of sublethal environmental stress and therefore be of particular use in pollution assessment studies. These include:

- measurement of coral growth (skeletal extension) rates;
- calcification and productivity profiles;
- coral fecundity and recruitment;
- monitoring for zooxanthellae loss, coral diseases and cyanobacterial blooms; and
- measurement of the bioaccumulation function of coral skeletons.

Each of these is briefly reviewed below in the context of the bioindicator criteria presented above.

**Pollutant: Mixture**

**Attribute:** Productivity and calcification profiles

**Region tested:** Pacific

**Region applicable:** Global

**References:** Brown 1988, Barnes 1983, Chalker et al. 1985, McLanahan 1997

**Protocol/Endpoint:** Brown (1988) suggests using productivity and calcification profiles as a means of classifying the status of coral reefs. This bioassay is based upon the concept that healthy reefs operate within narrowly-defined metabolic limits, such that a profile of a reef’s performance with respect to these limits should provide an assessment of its current status (Barnes 1983).

Theory and data show it is possible to measure productivity and calcification from changes in the oxygen concentration and pH of sea water flowing across a reef flat. When more is known about the variations in the respiratory and metabolic quotients of coral reef benthic communities, it should be possible to characterize the metabolic performance of large areas of reef flat by means of a few transects in the day and at night (Barnes 1983).

Chalker et al. (1985) developed a respirometer that can be deployed in situ on coral reefs to a depth of 50 meters for the measurement of primary productivity and calcification by corals, calcareous algae and the communities living on dead scleractinian skeletons using the technique developed by Barnes (1983).

McLanahan (1997) further advocates that the calcium carbonate balance (ratio of carbonate accretion to carbonate erosion) is the “universal currency of reef health and value”.

**Research recommendation:** Though the techniques for measuring these parameters have been developed (e.g., Barnes 1983, Chalker et al. 1985), further research focused on applying these techniques to water quality assessment and reef monitoring are clearly needed.

**Pollutant: Mixture**

**Attribute:** Coral fecundity and recruitment

**Region tested:** Caribbean, Pacific

**Region applicable:** Global

**References:** Brown 1988, Tomascik and Sander 1987b, Pearson 1981, Richmond 1993, 1994a, 1994b, 1995, 1996; Peters et al. 1997

**Protocol/Endpoint:** Two further individual-based coral parameters which Brown (1988) proposes as potentially useful indicators of sublethal stress on coral reefs are coral fecundity and recruitment. A number of studies (reviewed in Pearson 1981 and Brown 1988) have detected reduced coral recruitment and even recruitment failure due to a variety of environmental perturbations.

**Research recommendation:** Again, this area of research seems particularly promising, and is just starting to be applied in a systematic, calibrated fashion to water quality biomonitoring efforts on reefs. Research is needed to determine the effects of selected pesticides, PAH's and other potential pollutants on corals and coral reefs through the use of fertilization and recruitment bioassays. The use of adult corals (measuring growth rates, fecundity, and symbiotic associations) as well as their gametes and larvae as ecological indicators addresses the concern that mortality is a crude measure of environmental stress. Determining and measuring sublethal effects allow for a more proactive approach to monitoring and management.

**Pollutant: Mixture**

**Attribute:** Coral population colony size structure

**Region tested:** Caribbean, Pacific, Indian

**Region applicable:** Global

**References:** Bak and Meesters 1998

**Protocol/Endpoint:** Colony size frequencies of coral populations can be modeled by log normal distributions. Under “normal” conditions, colony size structure is skewed to the right, with high frequencies of small coral colonies. Evidence from a comparison of coral colony size frequencies from degraded and “less degraded” reefs suggests that under deteriorating environmental conditions, modal coral colony size increases, indicating changes in mortality and recruitment patterns that result in relatively fewer small and more large coral colonies.

**Research recommendation:** none

**Pollutant: Mixture**

**Attribute:** Coral morphology triangles

**Region tested:** Caribbean, Pacific, Indian

**Region applicable:** Global

**References:** Edinger and Risk 1999

**Protocol/Endpoint:** Adapted from a terrestrial plant ecology methodology, this technique classifies coral reefs according to their conservation value using r-K-S (ruderal/competitor/ stress-tolerator) ternary diagrams based upon the relative abundance of standardized coral morphology categories on each reef. Technique has been calibrated for Indonesian reefs, and assigns a conservation value to each reef based upon its position in an r-K-S ternary diagram. Has the advantage that it does not require coral taxonomic knowledge, but instead utilizes the considerable database of life forms transect data which is commonly collected in monitoring programs of many Indo-Pacific countries.

**Research recommendation:** none

**Pollutant: Mixture**

**Attribute:** *Vibrio shiloi* indicator

**Region tested:** Mediterranean

**Region applicable:** Global

**References:** Rosenberg and Loya 1999

**Protocol/Endpoint:** *Vibrio shiloi* as causative agent of *Oculina patagonica* bleaching. Studies using the coral *Oculina patagonica* have linked coral bleaching with a bacterial disease caused by *Vibrio shiloi*. The disease can be blocked by antibiotics. Elevated seawater temperature is a critical factor for this disease. From 16-20°C the disease does not occur, whereas from 25-30°C even a few *Vibrio shiloi* can cause the disease. Increased temperature without the bacteria is insufficient to cause bleaching because antibiotics prevent the bleaching even at elevated seawater temperatures. Elevated temperature triggers bacterial adhesion to coral surface and allows infection to proceed. Mediterranean coast of Israel.

**Research recommendation:** none

**Pollutant: Mixture**

**Attribute:** Zooxanthellae loss

**Region tested:** Pacific

**Region applicable:** Global

**References:** Gates and Brown 1985, Brown 1988, Jones 1997

**Protocol/Endpoint:** One oft-cited response of zooxanthellate reef organisms to a variety of stresses (both natural and anthropogenic) is the expulsion of symbiotic zooxanthellae, or “bleaching” (e.g., Gates and Brown 1985). As this phenomenon is both widespread and easily measured in a quantitative fashion, Brown (1988) and Jones (1997) have suggested that bleaching can serve as an excellent bioassay for assessing environmental stress on corals. Unfortunately, although zooxanthellae loss is a sensitive, sublethal response of corals to a wide range of environmental stressors (including temperature and salinity fluctuations and marine pollution), it is precisely this lack of a response specificity which limits its usefulness in bioassays.

**Research recommendation:** Bleaching may provide an early indication of stressful conditions upon a reef, but additional bioassays must be employed in order to identify the specific nature of the stressor and thus initiate corrective management actions.

**Pollutant: Mixture**

**Attribute:** Coral diseases and cyanobacterial blooms

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Richardson 1996, Ben-Haim and Rosenberg 2002

**Protocol/Endpoint:** Monitoring the frequency and severity of occurrences of coral diseases has been proposed as an important metric of reef health (Richardson 1996). Particularly in the Florida Keys and Caribbean reef province as a whole, coral diseases such as black, white and red band disease are thought to have played an important role in reef degradation. Unfortunately, the causal factors involved in coral diseases and cyanobacterial blooms are poorly understood, though circumstantial evidence suggests that eutrophication may play a role, especially in cyanobacterial blooms. Nonetheless, incidence of coral diseases and algal/bacterial blooms are certainly an indicator of coral health, and clearly merit consideration.

**Research recommendation:** Current research is focusing on determining the causal agents of coral diseases, as well as the relationship of disease incidence to surrounding water quality (Richardson 1996). Results from this research should determine the ultimate utility of these potential bioindicators. Further work should also focus on developing a standardized protocol for measuring incidence and severity of diseases and blooms, as well as interpretation of results.

**Pollutant: Mixture****Attribute:** Bioaccumulation of metals, phosphorus in coral skeletons**Region tested:** Caribbean, Pacific**Region applicable:** Global**References:** Brown and Holley 1982, Dodge et al. 1984, Brown 1988, Hanna and Muir 1990

**Protocol/Endpoint:** This bioassay relies upon the bioaccumulating function of hard coral skeletons. A number of studies have revealed the tendency of hard corals to incorporate seawater contaminants such as trace metals and phosphorus into their skeletons during normal growth (Dodge et al. 1984, Brown 1988, Hanna and Muir, 1990). These studies have demonstrated that corals incorporate these contaminants in proportion to their ambient concentrations in the surrounding seawater, suggesting corals may be faithful long-term recorders of environmental water quality. Note, however, that at least one study (Brown and Holley 1982) found no apparent metals bioaccumulation by corals on an impacted reef flat where other organisms showed significant metal accumulation in tissues. Those authors suggest that one reason for the apparent discrepancy involves differences in the bioavailability of metals to the corals and other organisms; trace metals in solution in seawater undoubtedly have different uptake routes than metals in particulate form. Even in those studies where corals did accumulate metals, an important complication is the finding that different species of coral from the same site demonstrate different uptake rates of trace metals (e.g., Hanna and Muir 1990). This finding suggests an active metabolic role of corals in the uptake of contaminants as opposed to simple passive uptake at ambient concentrations. Given this, it seems altogether possible that different individuals of the same species, living in different ambient conditions (with regard to depth, wave exposure, etc.) may also demonstrate different uptake rates of contaminants.

**Research recommendation:** While this bioaccumulation assay shows promise for reef water quality monitoring, it is apparent that further research is needed for a thorough understanding of the process of contaminant uptake by coral skeletons (including differences in the uptake of soluble and particulate fractions) and subsequent calibration of the skeletal signal to ambient water concentrations of the contaminant in question.

*Non-coral indicators***Pollutant: Mixture****Attribute:** Benthic filter feeders (sponges and ascidians\_**Region tested:** Strait of Gibraltar (Southern Spain)**Region applicable:** Global**References:** Carballo and Naranjo 2002

**Protocol/Endpoint:** The structural composition of sponge and ascidian assemblages was used to discriminate between disturbed and undisturbed areas, establishing environmental health categories, from moderately to strongly disturbed areas and in ascertaining the extension of the area of each health category.

The joint presence of the sponge *Cliona vastifica* and the tunicate *Policitor adriaticum* seemed to always indicate a minimally impaired situation.

**Research recommendation:** The use of sponges and ascidians in monitoring is very promising and is only limited by knowledge about biotic and abiotic factors affecting the structure of these communities (Carballo et al. 1994, 1996; Naranjo et al. 1996).

**Pollutant: Mixture**

**Attribute:** Butterflyfish

**Region tested:** Pacific, Red Sea

**Region applicable:** Global

**References:** Reese 1981, Hourigan et al. 1988, Nash 1989, White 1989, Reese 1994, Crosby and Reese 1996, Bell and Galzin 1984, Bouchon-Navaro et al. 1985, White 1989; Roberts and Ormond 1987, Roberts et al. 1988, Brown 1988, Jones and Kaly 1996, Erdmann 1997a, Erdmann and Caldwell 1997

**Protocol/Endpoint:** Undeniably, the most widely-discussed (and often misunderstood) bioindicators of environmental stress on coral reefs are the chaetodontids or butterflyfish, which have now been incorporated into a number of reef monitoring programs in the Indo-Pacific (Nash 1989, White 1989, Crosby and Reese 1996). Reese (1981) first gave a detailed definition of the butterflyfish bioindicator hypothesis, which has been re stated again in Hourigan et al. (1988), Reese (1994), and Crosby and Reese (1996). In summary, this hypothesis states that for those species of butterflyfish which are obligate corallivores, a decline in the condition of a coral reef, manifested by decreasing food quality of the stressed coral polyps, will result in a decrease in the abundance and diversity of these species and an increase in territory size, feeding rate and agonistic encounters as mated pairs attempt to maintain their nutritional intake by expanding their territories to include more coral colonies. After a time, feeding rates may actually decrease as more time is spent defending territories from neighboring pairs.

Since the hypothesis was first published, a number of studies have shown a positive correlation between chaetodontid diversity and abundance and percent live coral cover (but not decreasing food quality of the stressed coral polyps) or coral species richness (e.g., Bell and Galzin 1984, Bouchon-Navaro et al. 1985, White 1989; but see Roberts and Ormond (1987) for conflicting evidence).

The often misunderstood aspect of the technique (which is not elucidated in Crosby and Reese 1996) is that: “the early warning function of the butterflyfish bioassay was never intended to be a direct indication of specific measured environmental stresses such as specific toxins” (Crosby, personal communication).

As such, the technique as presently outlined (Crosby and Reese 1996), is useful as a preliminary screening mechanism that could trigger more detailed studies to determine the specific cause of the decreasing food quality of the stressed coral polyps.

On a precautionary note, butterflyfish populations are sometimes subject to intensive human exploitation; not only are they favorite targets of marine aquarium collectors, they are often sought as food-fish in many developing countries (Erdmann 1997a).

For this reason alone they are included in the Reef Check monitoring protocol - not as bioindicators of reef health, but as indicators of aquarium-collecting pressures (Hodgson 1997). Mechanisms must be incorporated into monitoring programs to insure that butterflyfishes are adequately protected from harvest and exploitation.

**Research recommendation:** To date, no research has yet quantitatively shown effects on butterflyfish abundance, diversity, feeding rate, territory size or aggressive encounters as a result of a specific chronic, sub-lethal stressor on hard corals. This and other concerns have led a large number of workers to question the relevance and utility of the butterflyfish bioassay (Roberts and Ormond 1987, Roberts et al. 1988, Brown 1988, Jones and Kaly 1996, Erdmann 1997a, Erdmann and Caldwell 1997).

To develop the butterflyfish bioassay into a response specific technique (i.e. take it beyond the preliminary screening tool phase) we recommend the following.

1. The "early warning" function of the butterflyfish bioassay needs to be substantiated. Although the butterflyfish bioindicator hypothesis suggests that sublethal degradation of coral reefs (manifested as decreasing food quality of the stressed coral polyps) can be detected by changes in the behavior and abundance of obligate corallivorous chaetodonts, available published data shows only correlations between chaetodont abundance and percentage live coral cover. To be of use to reef management programs, the bioassay must be able to detect such sublethal deterioration before a reduction in live coral cover occurs. If butterflyfish provide no early warning function before reductions in live coral cover occur, then one might as well directly monitor live coral cover.
2. The response specificity of the butterflyfish bioassay must be calibrated. Presently, if butterflyfish are simply responding to a reduction in live coral cover or food quality of coral polyps, monitoring their populations provides no insight into the specific stress causing these changes.
3. Following the above two points, the butterflyfish bioassay will also need a framework (statistical or numeric index-based) for interpreting the results of monitoring.

**Pollutant: Mixture**

**Attribute:** Parasites as indicators of pollution

**Region tested:** Pacific

**Region applicable:** Global

**References:** Esch et al. 1975, Gray 1989, Evans et al. 1995, Erdmann 1997b, McVikar et al. 1988, Erdmann (1997b), Diamant et al. (1999), MacKenzie (1999)

**Protocol/Endpoint:** Evans et al. (1995) further suggest that measurement of the incidence of parasitism on coral reef fishes can provide an indirect measure of water quality conditions surrounding coral reefs. Previous authors have suggested that incidence of parasitism and/or disease may increase in "stressed" organismal populations (Esch et al. 1975, Gray 1989).

In a study on the incidence of the isopod ectoparasite *Renocila* sp. on the coral reef fish *Abudefduf saxatilis* under varying pollution regimes, Evans et al. (1995) found weak evidence that parasite load was higher at heavily polluted sites than at less polluted sites.

Erdmann (1997b), however, examined the incidence of the gastropod ectoparasite *Caledoniella montrouzieri* on reef flat stomatopod assemblages in Indonesia and found no significant differences in parasite load between stomatopod assemblages at heavily polluted sites and relatively pristine sites. He suggested that some parasites, especially those with direct host transmission, may require high population densities of their host organisms for successful transmission. Host organisms which are sensitive to pollution and demonstrate reduced abundance under polluted conditions would therefore be unlikely to show increased incidence of parasitism with increasing pollution.

Other authors studying fish disease in polluted marine areas (e.g., McVikar et al. 1988) likewise suggest no clear correlation between pollution and incidence of disease/parasitism, and both Esch et al. (1975) and Gray (1989) conclude that the evidence for such a connection is equivocal at best.

Diamant et al. (1999) combined parasitological investigations with physiological assessment of rabbitfish in the Red Sea and the Mediterranean to demonstrate the potential for using fish parasites as bioindicators.

MacKenzie (1999) proposed that parasites in marine organisms are a promising indicator of the effects of pollution. Since the free-living transmission stages of such internal parasites as helminths are highly sensitive to environmental condition, the incidence of helminth endoparasites tends to decrease as water quality declines. On the other hand, marine ectoparasites tend to be highly tolerant of pollutants, thereby tending to increase in abundance as water quality declines.

**Research recommendation:** Research has not been extensive and further investigation may be fruitful.

**Pollutant: Mixture**

**Attribute:** Larval assemblages of fish and other reef taxa

**Region tested:** Pacific, Caribbean

**Region applicable:** Global

**References:** Gajbhiye et al. 1987, Herrnkind et al. 1988, Garrity and Levings 1990, Doherty 1991, Erdmann 1997b

**Protocol/Endpoint:** One result of the stomatopod bioindicator work which appears to be common to similar studies on other reef organisms is the apparent extreme sensitivity of the larval and postlarval stages to water quality deterioration (Erdmann 1997b). This result has been reported by other workers for stomatopods (Gajbhiye et al. 1987), spiny lobsters (Herrnkind et al. 1988), and reef-flat gastropods (Garrity and Levings 1990). Likewise, Doherty (1991) proposes that the environmental sensitivity of larval coral reef fish assemblages makes them ideal candidates for reef biomonitoring studies.

**Research recommendation:** This suggestion obviously requires substantial additional research before larval fish assemblages can be used in an effective bioassay, but the broader implication here is that biomonitoring of a wide variety of reef organism larval and postlarval stages may prove an extremely sensitive method of detecting water quality deterioration. Future research efforts on reef bioindicators should certainly address this potential. An important obstacle to larval bioassays is the difficulty of reliably and quantitatively sampling larval assemblages (Erdmann 1997b). Doherty (1991) overcomes this problem by using expensive automated light traps, but these may well be outside the scope of most monitoring programs' budgets.

**Pollutant: Mixture (PCB's, dioxins)**

**Attribute:** Organic contaminants and the development of fishes

**Region tested:** Pacific

**Region applicable:** Global

**References:** Kerr 1995, Kerr et al. 1997, Kerr 1999, Kerr Lobel 2004

**Protocol/Endpoint:** Ongoing work on Johnston Atoll by Lisa Kerr (personal communication) involves quantifying the effects of organic contaminants on the development of fishes. Kerr is trying to develop the use of the occurrence of developmental defects in a demersal spawning fish as a bioindicator of pollution effects. She has been studying colonies of the damselfish *Abudefduf sordidus* in areas contaminated with PCBs and will also be looking in areas contaminated with dioxins. Kerr's preliminary data suggests that with increasing sediment PCB concentration there is an increase in the occurrence of developmental defects. Her current study will track effects in the offspring of individual fish and then relate the level of effects in individuals to the individual's contaminant body burden.

**Research recommendation:** Contingent upon ongoing research results.

**Pollutant: Mixture**

**Attribute:** Bioaccumulation in molluscs and macrophytes

**Region tested:** Pacific

**Region applicable:** Global

**References:** Bryan and Hummerstone 1973, Goldberg et al. 1978, Hungspreugs and Yuangthong 1984, Brown and Holly 1982, Phillips 1994

**Protocol/Endpoint:** Bioaccumulation of trace metals and phosphorus by hard coral skeletons has been previously discussed, but several non-coral organisms have also been proposed as bioaccumulators of marine pollutants impinging on coral reefs. Specifically, Brown and Holly (1982) examined metals bioaccumulation by a macrophytic alga (*Padina tenuis*) and several molluscs, including the bivalves *Saccostrea cucullata* and *Isognomon isognomon* and the gastropod *Nerita chamaeleon*, on a reef flat affected by tin dredging and smelting. Their results showed that specimens of both bivalve species from the affected reef had elevated metals levels in their tissues relative to specimens from control sites, whereas the alga and the gastropod tissues showed no such clear pattern.

As discussed above, these authors suggest that differences in bioavailability of the metals (mostly in particulate form in this study) account for the differences between organisms: the filter-feeding bivalves consumed the metallic particulates, whereas the alga (and hence the herbivorous algal-feeding gastropod) were unaffected. Though additional studies of bioaccumulators are rare in the coral reef literature, temperate analogs of each of these organisms have been used extensively in bioassays worldwide (Bryan and Hummerstone 1973, Goldberg et al. 1978, Hungspreugs and Yuangthong 1984, Phillips 1994).

Two further issues involve the expense and relevancy of these bioassays. Chemical analysis of tissues for metals concentrations requires substantial money and equipment, two resources which small-scale monitoring programs may find in short supply. Furthermore, there is always the question of whether the pollutants being accumulated are even considered detrimental to reef health. Obviously, bioaccumulation assays should be limited to monitoring those pollutants with known impacts on coral reefs.

**Research recommendation:** While the same issues of calibration, bioavailability and differences in uptake mentioned above for coral bioaccumulators apply here, with further development these bioassays may be useful in some reef monitoring contexts as well.

**Pollutant: Mixture**

**Attribute:** Sessile reef organisms (sponges, gorgonians)

**Region tested:** Caribbean

**Region applicable:** Global

**References:** Shannon and Weaver 1949, Pielou 1966, Green and Vascotto 1978, Alcolado et al. 1994

**Protocol/Endpoint:** Alcolado et al. (1994) have suggested taxonomically-expanded surveys of the sessile reef community as an effective means of monitoring environmental conditions on reefs. These authors propose the use of two well-known diversity indices,  $H'$  (Shannon-Weaver heterogeneity index; Shannon and Weaver 1949) and  $J'$  (Pielou's evenness index; Pielou 1966), to evaluate environmental stresses on three groups of sessile reef taxa (scleractinians, gorgonians, and sponges). Specifically, they propose that calculation and comparison of  $H'$  and  $J'$  for each of these three taxonomic groups allows a rough classification of the environmental conditions faced by organisms on a particular reef. The environmental classification scheme proposed ranges from "favorable and predictable" (high values of both  $H'$  and  $J'$ ) to "unpredictably severe" (low values of both  $H'$  and  $J'$ ). Using sponge communities in Cuba, the authors have developed and calibrated a numerical index for interpreting the various values of these diversity indices which they claim reliably segregates polluted reef stations. Unfortunately, the details of these investigations are reported in Cuban journals which were unavailable to the authors, preventing a detailed review of this technique.

**Research recommendation:** Although several workers (e.g., Green and Vascotto 1978) have argued against the use of diversity indices in water quality assessment, this technique appears worthy of further investigation. Potential issues regarding its appropriateness include questions about the early-warning function and response specificity of the bioassay, as well as problems of taxonomic resolution (especially in the hyper-diverse Indo-Pacific).

**Pollutant: Mixture**

**Attribute:** Heterotrophic macroinvertebrates

**Region tested:** Caribbean, Pacific

**Region applicable:** Global

**References:** Tomascik and Sander 1987a, Kinsey 1988, Tomascik et al. 1994, Risk et al. 1994, Dahl and Lamberts 1977, Dahl 1981, Dustan and Halas 1987, Kinsey 1988, Tomascik et al. 1994, Risk et al. 1994, Vail in press

**Protocol/Endpoint:** Another promising, but largely undeveloped, set of indicators of reef condition have been proposed based upon the well-documented ecosystem shift which has occurred on many reefs in urban, polluted areas. A number of workers have described a distinctive shift in pollution and sediment-stressed reefs from those dominated by coral-algal symbionts and reef fish towards those dominated by heterotrophic macroinvertebrates, especially scavengers, filter feeders, deposit feeders and internal bioeroders (Tomascik and Sander 1987a, Kinsey 1988, Tomascik et al. 1994, Risk et al. 1994). Organisms which are reported to have increased dramatically in abundance include zoanths, sponges, barnacles, crabs, hydroids, tunicates, bioeroding (boring) sponges and bivalves, as well as a range of echinoid, holothurian and crinoid echinoderms (Dahl and Lamberts 1977, Dahl 1981, Dustan and Halas 1987, Kinsey 1988, Tomascik et al. 1994, Risk et al. 1994, Vail in press).

Abundance measures of a number of these taxonomic groups are already included in several reef monitoring programs (e.g., Dahl and Lamberts 1977, Dahl 1981, Risk et al. 1994, McManus et al. 1997, Hodgson 1997), apparently based upon the assumption that increases in abundance of these groups may indicate deteriorating environmental conditions on the surveyed reef.

**Research recommendation:** While these various organisms may very well prove to be excellent bioindicators of water quality deterioration, the sensitivity of their response has not yet been fully investigated and described. Clearly, the development and calibration of these potential bioassays should be a research priority. Data collected in the Reef Check and Aquanaut programs should also provide further evidence of the value of a number of these bioindicators.

**Pollutant: Mixture****Attribute:** Stomatopod crustaceans**Region tested:** Caribbean, Pacific**Region applicable:** Global**References:** Jackson et al. 1989, Steger and Caldwell 1993, Erdmann 1997b, Erdmann and Caldwell 1997, Erdmann and Sisovann 1995, Kerans and Karr 1994**Protocol/Endpoint:** Stomatopod crustaceans were first proposed as bioindicators of marine pollution stress after a study on the effects of the 1986 Galeta, Panama oil spill indicated that these benthic reef-dwellers were highly sensitive to oil pollution (Jackson et al. 1989, Steger and Caldwell 1993). The results of that study showed that reef-flat stomatopods responded to heavy oiling by an initial, drastic decrease in abundance, followed by an extended period of reduced recruitment.

Based on these initial results, an evaluation of the bioindicator potential of Indonesian reef-flat stomatopod communities was initiated. The results of that 3-year study confirmed that stomatopod abundance, diversity and recruitment are strongly negatively correlated with sediment concentrations of petroleum hydrocarbons and selected heavy metals, and with surrogate measures of sewage and agricultural runoff (Erdmann 1997b). In general, stomatopod communities show a strong trend of decreasing abundance and diversity with increasing proximity to major human population centers (Erdmann and Caldwell 1997, Erdmann and Sisovann 1995). In addition to their demonstrated sensitivity to water quality degradation, stomatopods are abundant and ubiquitous throughout the world's reef provinces, and their taxonomy is readily taught to non-specialists. Reef-flat stomatopod assemblages in particular can be sampled quantitatively without the use of SCUBA, making them ideal candidates as inexpensive, low-tech bioindicators of reef water quality degradation.

**Research recommendation:** A further two-year project has recently been initiated with the goal of distilling the above results into a multimetric index of coral reef integrity, using as a model the successful benthic index of biotic integrity (B-IBI) developed for Tennessee Valley Authority bioassessment programs (Kerans and Karr 1994). The index will then be further calibrated based upon the results of comparative studies in 5 other regions of Indonesia.**Pollutant: Mixture****Attribute:** Nematod/copepod ratio**Region tested:** Indian**Region applicable:** Global**References:** Ansari and Ingole 2002, Montagna et al 2002**Protocol/Endpoint:** Used the nematod/copepod ratio to indicate impact or recovery of meiobenthos following an oil spill.**Research recommendation:** Needs further research to see what other pollutant stressors the ratio is sensitive to.

**Pollutant: Mixture**

**Attribute:** Amphipods

**Region tested:** Caribbean, Pacific

**Region applicable:** Global

**References:** Hart and Fuller 1979, Oakden et al. 1984, USEPA 1990b, Thomas 1993, Baker 1971, Sandberg et al. 1972, Percy 1976, Linden 1976a & b, Lee et al. 1977, Ahsanullah 1976, Swartz et al. 1985, Swartz 1987, Barnard 1958 & 1961, McLuskey 1967 & 1970, Widdowson 1971, Vobis 1973, Meijefing 1991, Jackson et al. 1989, Engle and Summers 1999, Dauvin 2000,

**Protocol/Endpoint:** Because of their ecological importance, numerical abundance, and sensitivity to a variety of toxicants and pollutants, amphipod crustaceans have long been known as sensitive environmental indicators (Hart and Fuller 1979, Thomas 1993).

Oakden et al. (1984) showed experimentally that temperate phoxocephalid amphipods actively avoided sewage and trace metal-contaminated sediments, preferring instead to burrow in "clean" sediments. Lacking a pelagic larval stage, amphipods are benthic recruiters, thereby minimizing dispersal effects. They show a high degree of habitat specificity and niche requirements and are one of the major benthic components in tropical marine ecosystems worldwide, in terms of biomass and species diversity.

The use of amphipods in environmental monitoring has been limited to the few temperate regions where long-term taxonomic and natural history investigations have been undertaken. California currently uses amphipods as primary biological monitors at sewage outfalls. Monitoring programs incorporating amphipods have been used to assess the environmental effects of oil spills in the Persian Gulf, Alaska, and Panama.

California and the Environmental Monitoring and Assessment Program (EMAP) program of EPA have designated several species of amphipods as bioassay organisms for sediment toxicity tests in soft-bottom environments (USEPA 1990b). Amphipods are so useful as bioindicators that US Government agencies now require their identification to the species level in permitting operations such as oil leases and outfalls. Their incorporation into bioassessment programs is dependent upon completion of comprehensive coastal resource inventories and taxonomic surveys (Thomas 1993).

In addition to acute and chronic sensitivities to pollutants and toxicants, amphipods exhibit a number of altered behavioral responses to sublethal levels of a variety of compounds that can cause reduction or elimination of the population (Baker 1971, Sandberg et al. 1972, Percy 1976, Linden 1976a & b, Lee et al. 1977). Amphipods are more sensitive than other species of invertebrates (decapods, polychaetes, molluscs, and asteroids) to a variety of contaminants (Ahsanullah 1976, Swartz et al. 1985, Swartz 1987). Amphipods also show responses to dredging, shoreline alteration, fishing practices, and changes in salinity and dissolved oxygen (Barnard 1958 & 1961, McLuskey 1967 & 1970, Widdowson 1971, Vobis 1973). In freshwater streams of Germany, the onset and recovery of 'stream souring' (acidification) has been documented since 1945 on the basis of distribution patterns of three species of the amphipod genus *Gammarus* (Meijefing 1991). This biological model has proved to be a more responsive and sensitive measure of environmental conditions than standard water quality protocols (Meijering 1991).

Ecological factors must also be considered in evaluating the potential information value of various amphipod groups. For example, in measuring the effects of an oil spill in a coral reef system, cryptofaunal and infaunal species of invertebrates may yield different patterns. Epifaunal forms could 'raft' in, while infaunal and cryptofaunal forms would have to recruit along the bottom from unaffected or minimally-impacted areas. Thus, the observed recolonization rates of the two groups, and subsequent interpretation of effects, could be quite different (Thomas 1993). In an actual oil spill on a Panama coral reef, two infaunal peracarid crustaceans (amphipods and tanaids) showed virtually no recovery after a 9-month period (Jackson et al. 1989), while other groups, including other crustaceans (brachyurans and burrowing shrimp), showed significant recovery at the same sites.

**Research recommendation:** Potential for amphipods as bioindicators exists in a wide variety of environments, especially in coral reefs, but their incorporation into such programs is dependent upon completion of taxonomic surveys and inventories.

**Pollutant: Mixture**

**Attribute:** Corallivores

**Region tested:** Pacific

**Region applicable:** Global

**References:** Birkeland and Lucas 1990, Turner 1994

**Protocol/Endpoint:** Both of the above mentioned monitoring programs also advise recording abundances of corallivores such as the crown-of-thorns starfish (*Acanthaster planci*) and *Drupella* gastropods, which in "outbreak" situations have caused severe reef destruction on many reefs throughout the Pacific (Birkeland and Lucas 1990, Turner 1994). Though the proximal causes of outbreaks of these predators are still vigorously debated, their obvious relation to "reef health" makes them a logical choice in monitoring as well.

**Research recommendation:** Further research is obviously required to develop and calibrate a bioassay involving these corallivores.

**Pollutant: Mixture**

**Attribute:** Changes in soft-bottom benthic community structure

**Region tested:**

**Region applicable:**

**References:** Pearson and Rosenberg 1978, Gray and Mirza 1979, Gray 1981 & 1989, Pearson et al. 1983, Warwick 1986, Clarke 1993, Warwick and Clarke 1993, Weston 1990, Brown 1988, Tomascik and Sander's 1987a

**Protocol/Endpoint:** Though not yet formally applied to coral reef ecosystems, a set of bioassays worthy of mention are based on a large body of work examining pollution-induced changes in macrobenthic community structure in temperate soft-bottom communities. Extensive work by Pearson, Gray, Warwick, Clarke and associates has demonstrated a number of consistent, predictable responses in soft bottom community structure to increasing pollution, including a decrease in species richness, an increase in the total number of individuals due to a "retrogression to dominance by a few

opportunistic species”, a reduction in the mean size of the average species or individual, changes in the shape of the log-normal distribution of individuals among species, and increased variability in species diversity indices such as  $H'$  (Pearson and Rosenberg 1978, Gray and Mirza 1979, Gray 1981& 1989, Pearson et al. 1983, Warwick 1986, Clarke 1993, Warwick and Clarke 1993, but see Weston 1990 for contradictory evidence).

Brown (1988) has suggested that the above models may be inappropriate for coral reefs, as these habitats are much more highly-structured than soft bottom communities and thus may respond very differently. Nonetheless, the results of Tomascik and Sander's (1987a) study on eutrophication effects on coral community structure correspond in part with this model, while a similar study by Clarke et al. (1993) demonstrates a breakdown in “seriation” (zonation pattern) in coral assemblages subject to sedimentation.

**Research recommendation:** A concerted research effort to apply the soft-bottom models to coral reef communities is clearly warranted. Furthermore, Brown (1988) suggests that even if these bioassays prove unworkable in a coral reef context, they may still be applicable to the soft-bottom communities which are often adjacent to coral reefs (lagoon bottoms and the base of reef slopes). An important consideration in applying the soft-bottom models to coral reef communities is that these models are based on community response to pollution. Coral reef studies tend to be more narrowly-focused, for example on assemblages of scleractinians or coral reef fish. Narrowing the taxonomic focus in studies of pollution effects may preclude detection of changes in the broader reef community (e.g., a decrease in coral cover with a corresponding increase in tunicate and sponge abundance). The difficulties in examining response of the entire community in highly diverse coral reef habitats may prevent the application of the soft-bottom bioassays to reef systems and is worthy of careful consideration.

**Pollutant: Mixture**

**Attribute:** FACT'97 coastal indicators

**Region tested:** Atlantic, Gulf of Mexico

**Region applicable:** Global

**References:** Bergquist et al. 1997

**Protocol/Endpoint:** FACT is structured around nine strategic issues judged to be critical to the future of Florida's coast over the next 20 years (Bergquist et al. 1997). These broad strategic issues were refined into two-to-four sub-issues or components of each issue. These sub-issues then became the final framework around which indicators were developed. The nine issues and their associated sub-issues are as follows.

- 1) Impact of Growth in the Coastal Zone
  - Impacts of Population Growth
  - Patterns of Development
  - Sufficiency of Infrastructure
  - Economic Impacts

- 2) Disruption of Coastal Physical Processes
  - Alteration of Existing Natural Systems
  - Construction of Altering Structures
- 3) Responding to Coastal Threats and Hazards
  - Coastal Hazard Mitigation
  - Incompatible Living Areas
  - Industrial Impacts
- 4) Degradation and Restoration of Coastal Ecosystems
  - Habitat Change
  - Species Population Trends
  - Water Quality Trends
- 5) Managing Fresh Water Allocation
  - Fresh Water Allocated for Ecological Maintenance
  - Fresh Water Allocated to Meet Residential Needs
  - Fresh Water Allocated to Meet Commercial/Industrial Needs
  - Fresh Water Allocated to Meet Agricultural Needs
- 6) Sustaining the Human Uses of the Coast
  - Maintenance of Recreational Value
  - Sustainable Economic Use
  - Balancing Development with Coastal Resources
- 7) Balancing Public and Private Uses of Resources
  - Private Property Issues (no indicators have been developed for this sub-issue)
  - Stewardship of Coastal Resources
- 8) Preservation of Cultural and Aesthetic Resources
  - Preservation of Archaeological and historical Resources
  - Preservation of Living Resources
  - Conservation of Coastal Open Space
- 9) Encouraging Public Awareness and Involvement
  - Public Awareness
  - Public Participation

**Research recommendation:** The change in coral reef community dynamics indicator used by FACT is the coral reef/hard bottom monitoring facet of the FKNMS water quality monitoring program. Relating other FACT indicators to coral reef ecosystem integrity will require the development of special indices and calibration.

**Pollutant: Mixture**

**Attribute:** Map-based indicators of potential threats to coral reef ecosystems

**Region tested:** Caribbean, Pacific, Global

**Region applicable:** Global

**References:** Burke et al. 1998, Burke et al. 2002

**Protocol/Endpoint:** The World Resources Institute, in collaboration with the International Center for Living Aquatic Resources Management, the World Conservation Monitoring Center and the United Nations Environment Programme and a host of other coral reef experts, has created a system of evaluating potential threats (not actual reef condition) to coral reef ecosystems using map-based indicators (Burke et al. 1998, Burke et al. 2002). Results are based on a series of distance relationships correlating mapped locations of human activity, such as ports and towns, oil wells, coastal mining activities and shipping lanes (component indicators) with predicted risk zones of likely environmental degradation. Detailed sub-national statistics on population density, size of urban areas, and land cover type were also incorporated into the analysis. Data on rainfall and topography are used to estimate potential runoff within watersheds, from inland deforestation and agriculture. While still experimental, the “Reefs at Risk” indicators flag problem areas around the world where - in the absence of good management - coral reef degradation might be expected, or predicted to occur shortly, given ongoing levels of human activity.

**Research recommendation:** To make these indicators approach reality, a time factor must be incorporated into them, otherwise there is no feeling of urgency to the threats. Some of the map-based indicator assumptions need work as they are confounded by other factors.

**Pollutant: Mixture**

**Attribute:** Rapid assessment of management parameters (RAMP) for coral reefs

**Region tested:** Pacific

**Region applicable:** Global

**References:** Pollnac, 1997

**Protocol/Endpoint:** The University of Rhode Island’s Coastal Resources Center (CRC) in collaboration with the International Center for Living Aquatic Resources Management (ICLARM) RAMP designed and field tested a set of indicators for assessing the human impacts (social, cultural and economic) on coral reefs. Indicators are organized according to proximity to the designated reef (e.g., national, regional and local), context (political, socioeconomic and cultural), reef uses (fishing, mining, tourism/recreational, etc.), and governance (institutional frameworks, knowledge bases, plans, implementation, monitoring and evaluation). A guide for information acquisition and subsequent coding for inclusion in ReefBase was also developed (Pollnac, 1997).

**Research recommendation:** Used together, RAMP and ReefBase have the potential to provide a baseline for monitoring changes in coral reef ecosystems as well as a standardized database for exploring interrelationships between the variables included. Defining and recording a standardized set of indicators is of critical importance. Presently, coastal zone and fisheries management literature is characterized by case

studies conducted by many different individuals with unknown biases and varying research methodologies and disciplinary perspectives. When sufficient cases have been entered into these data sets, with data collected and coded using the standardized techniques developed, ReefBase and RAMP indicators will enable multivariate, quantitative analysis. Independent variables can be related to important dependent variables such as reef condition or management institution status to determine the amount of variance connected to the independent variables. Results of these analyses could provide decision makers with information that can be used to select alternative courses of action which will be based on more than the currently available unsystematic, anecdotal information (Pollnac, 1997). Relating RAMP indicators to coral reef ecosystem integrity will require the development of special indices and calibration.

**Pollutant: Mixture**

**Attribute:** Bioaccumulation in sponges

**Region tested:** western Atlantic (Florida)

**Region applicable:**

**References:** D. L. Santavy, U.S. EPA Office of Research and Development, Gulf Ecology Division, pers. com.

**Protocol/Endpoint:** The efficient filter feeders and lipid rich common sponges *Chondrilla nucula* and *Aplysina fistularis* are used as coral surrogates to monitor chemical contaminants in the EPA coral disease survey in the Florida Keys National Marine Sanctuary.

**Research recommendation:** none

**Pollutant: Mixture**

**Attribute:** FORAM Index (Foraminifers in Reef Assessment Monitoring)

**Region tested:** Florida Keys

**Region applicable:** Global

**References:** Hallock and Schallger 1986; Hallock 1988, 1993, 1996; Alve 1995; Cockey et al. 1996; Hallock 2000; Hallock et al. 2003

**Protocol/Endpoint:** Foraminifera are typically important contributors to reef sediments, especially species of larger foraminifera that host algal endosymbionts. Foraminiferal assemblages in reef sediments have been widely studied since 1922 primarily for the purpose of using analogies with modern biotas to interpret fossil assemblages and paleoenvironments for petroleum exploration. They are also easy and inexpensive to collect. Cockey et al. (1996) show that published accounts of foraminiferal assemblages from sediments collected 30 or more years ago can be valuable resources in efforts to determine if biotic changes have occurred in coastal ecosystems and that family level identifications may be sufficient to detect decadal-scale changes in foraminiferal assemblages in reef sediments. Models formulated by Hallock and Schlager (1986), Birkeland (1987, 1988), and Hallock (1988) predict that community response to gradually increasing nutrient flux, whether natural or anthropogenic, should favor phytoplankton, benthic algae, and heterotrophic taxa lacking algal symbionts, rather than taxa that utilize algal symbionts for enhanced growth and calcification.

Benthic succession along a nutrification gradient is a predictable response (Pearson and Rosenberg 1978) that has been commonly observed in foraminiferal assemblages (Lidz 1966, Alve 1995, Schafer et al. 1995). Pacific benthic foraminiferal assemblages have been observed to shift from predominantly algal symbiont-bearing species to dominance by small species lacking algal symbionts in response to a limited anthropogenic nutrient source (Hirschfield et al. 1968). Cockey et al. (1996) discuss how changes in foraminiferal assemblages, from dominance by algal symbiont bearing taxa in 1959-1961 to heterotrophic taxa in 1982-1992, are consistent with predictions of benthic community response to gradually increasing nutrient flux into South Florida's near coastal waters by Szmant and Forrester (1996). The paucity of eutrophication-indicating foraminiferal taxa in sediments off Key Largo supports previous studies that show that anthropogenic nutrient influx has not caused eutrophication of reef and open-shelf environments in that area. Hallock et al. (1993a) predicted that at least a 10-fold increase in nutrients resources would be required to cause eutrophication in habitats occupied by mixed coral-algal communities in the Florida Keys.

The FORAM protocol consists of a three tiered protocol. Number of tiers used depends on the region being assessed and questions being asked.

1. Sediment constituent analysis, which can address questions of historical change and reference-site suitability.
2. Analysis of live larger foraminiferal assemblages, which can indicate the suitability of sites for organisms with algal symbionts.
3. Analysis of *Amphistegina* populations, including abundance, presence of bleaching, and other evidence of specific stressors to which these foraminifers respond similarly to corals.

The FORAM Index (Hallock et al. 2003) is based on relative abundances of three functional groups of foraminifers, larger taxa that host algal symbionts, smaller taxa that bloom when food resources increase (nutrification), and stress-tolerant taxa that dominate when water quality is seriously impacted by eutrophication, heavy metals, or other pollutants.

**Research recommendation:** Future research will focus on tolerant/intolerant response of foram taxa to specific pollutants.

**Pollutant: Mixture**

**Attribute:** Reef Check 99

**Region tested:** Indo Pacific, Red Sea, Caribbean

**Region applicable:** Global

**References:** Hodgson, 1999

**Protocol/Endpoint:** Twenty-five worldwide and regional "health indicators" were used by trained volunteer recreational divers to provide information about the effects of human activities on coral reefs. The world's oceans were divided into Indo-Pacific, Red Sea and Caribbean (special regional indicators were chosen for biogeographic margins e.g. Arabian Gulf, Hawaii and the E. Pacific). Sites believed to be least affected by human activities and having the highest percentage of seabed covered by living coral and the highest populations of indicator fish and other invertebrates were selected for monitoring.

The protocol included the collection of 4 types of data: a site description; a fish survey; an invertebrate survey and a substrate survey. The underwater surveys were made along the 3 and 10 m depth contours. The following conclusions were drawn from the study. Results showed that no reefs had high numbers of most indicator organisms, suggesting to the author that few, if any, reefs have been unaffected by fishing and gathering. The low percentage cover of pollution indicators was taken to suggest that sewage pollution is not a serious problem at most of the sites (biased toward perceived good condition). Hodgson (1999) mentions some ways the protocols could be improved (i.e., establishing sample size goals and obtaining historical baseline data).

**Research recommendation:** We suggest the protocols could also be improved by:

1. Verifying data quality with an analysis of the variation between teams in controlled studies.
2. Confirming that a dose-response change in "health indicator" value is reliable, interpretable and not swamped by natural variation;
3. Sampling across a gradient of human influence rather than relying on the perception of participants to select monitoring sites least affected by human activity;
4. Classifying sites with respect to similar environmental conditions so valid comparisons among the over 300 sites can be made;
5. Resurveying the same sites every year (G. Hodgson pers. comm.).
6. Calibrating indicators or collecting data on fishing effort or pollution to determine the causes of the degradation. Otherwise the causes of changes (degradation) are assumed;
7. Using minimally degraded reference sites to compare against degraded sites (which were biased towards a perceived less impacted condition); and by
8. Not using the Bray Curtis similarity index to examine the relationships among all sites for six worldwide indicators because this index has been shown in independent tests to fail to discriminate among sites (Cao, 1997).

**Pollutant: Mixture**

**Attribute:** Reef Check Coral Reef Health Index (CRHI)

**Region tested:** Indo Pacific, Red Sea, Caribbean

**Region applicable:** Global

**References:** Hodgson 1999

**Protocol/Endpoint:** The CRHI was calculated for six indicators (butterflyfish, Haemulidae grouper, Diadema, hard corals and lobster) for 269 sites from 3 regions. The highest mean abundance of an organism recorded at any site in the world was used as the maximum possible value to determine a lower, middle and upper third for 269 sites in 3 regions. Then, for each site, a value of 0-3 was assigned for each indicator depending on the mean abundance in comparison to the cut-off levels for each third. Means in the lower, middle and upper third were assigned a value of 1, 2 and 3 respectively while a mean of zero was assigned a zero (except for Diadema where the values were reversed as high numbers are considered to be unhealthy). The CRHI was calculated by adding the 6 values together. The maximum possible CRHI is : 6 indicators X 3 = 18. The mean CRHI values from the study were 3.8, 4.0 and 3.5 respectively for the Indo Pacific, Red Sea and Caribbean regions, out of a maximum possible CRHI of 18.

There was no significant difference among the values from the three regions and the low CRHI scores were assumed to indicate how few sites had high numbers of indicators recorded.

**Research recommendation:** The comparison among sites could be improved by classifying sites as mentioned above. Much early freshwater work to detect the influence of human actions on biological systems emphasized abundance (or population size or density) of indicator taxa, often species with commodity value or thought to be keystone species. Generally, however, population size varies too much even under natural unimpaired conditions to be a reliable indicator of biological condition. Population size changes in complex ways in response to changes in natural factors such as food supply, disease, predators, temperature, salinity and demographic lags. In studies to determine environmental impacts, the interaction between variability and the size of the potential impact (effect size) must also be taken into account, because that interaction affects statistical power (Osenberg et al. 1994). High variation in population size, even in natural environments, interacts in complex ways with changes in abundances stimulated by human actions. Thus it can be very difficult to detect and interpret the effects of human actions even with advanced experimental designs. The minimum level of sampling effort may exceed the planning, sampling, and analytical capability of many monitoring situations. By shifting the focus to better-behaved indicators such as changes in taxa richness, loss of sensitive taxa, or changes in trophic organization, it is possible to use less complex experimental designs (Karr and Chu, 1999). Using the highest mean abundance of an organism recorded at any site in the world as the maximum reference condition for sites also disregards the effects of regional, seasonal and environmental factors on species abundance and is probably setting the reference bar too high.

**Pollutant: Mixture**

**Attribute:** Reef Check Distance Population Index (DPI)

**Region tested:** Indo Pacific, Red Sea, Caribbean

**Region applicable:** Global

**References:** Hodgson, 1999

**Protocol/Endpoint:** The DPI was calculated by assigning a score for both population of nearest city and the distance to that city as follows: Population 0-10,000 = 0; 10,000-50,000 = 1; 50,000-100,000 = 2; > 100,000 = 3. Distance > 50 km = 0; 25-49 km = 1; 10-24 km = 2; 0-9 km = 3. The DPI was then calculated as the sum of the population size and distance scores. The higher the index means the site is close to a dense population. The maximum DPI is 6. The CRHI was plotted versus the DPI to show that a sizable number of sites located far from population centers had a low health index.

**Research recommendation:** See comments above regarding the applicability of the CRHI.

## IV. Conclusion

Our review shows that while there are some tools available now for the monitoring and assessment of land-based pollution of coral reefs, there is a vast universe of potential new tools that are under development or in the experimental stage. The majority of the potential new bioindicators listed are for detecting mixtures of pollutants. This fact reinforces the critical need to develop a better understanding of pollution tolerant and intolerant coral reef species (Jameson et al. 2001) and other response specific diagnostic indicators (i.e., nitrogen and carbon isotope ratio, cellular, and genetic techniques).

Most importantly, the results of the review demonstrate the need for organizing these potential new tools into an integrated approach for coral reef monitoring and assessment using a variety of stressor, exposure and response indicators such as, *Diagnostic Monitoring and Assessment* (Table 4, Jameson et al. 2001, subm a, subm b), that can be adapted to various and differing stress scenarios and that has the capability of identifying the location and cause of stress as well as reflect cumulative impacts and the condition of the entire coral reef system. Picking an effective strategic approach is also important to allow the various tools to be used in their most appropriate role and it provides the blueprint to identify gaps in needed metrics and tools for effective future research and development efforts and for setting funding priorities.

It is also important for coral reef managers to communicate their needs to scientists in this process so this new paradigm for coral reef monitoring and assessment can be effective not only on the coral reef but in a court of law.

**Table 4** Coral reef *Diagnostic Monitoring and Assessment Framework* showing the appropriate roles for indicators. All indicators can be used to manage and measure environmental progress, but only biological **Response Indicators** focus on end outcomes (from Jameson et al. subm a, subm b ).

<b>Management and monitoring sequence</b>	<b>Indicators</b>	<b>Appropriate role</b>
1. Management actions	<b>Administrative</b> (i.e., permits, enforcement, plans, grants)	Measures the results of administrative actions to improve water quality.
2. Response to management		
3. Stressor abatement	<b>Stressor</b> (i.e, changes in land use practices, pollutant discharges, effluent reduction, spills and releases, fish kills, habitat modifications)	Measures changes in human activity outputs that have the potential to degrade the marine environment.
4. Ambient conditions	<b>Exposure</b> (i.e., cellular and genetic biomarkers, water & sediment chemistry, sedimentation effects, physical habitat or flow alteration, reduced spawning success, changed nutrient dynamics, fish tissue analysis)	Measures the initial effects of stressors.
5. Exposure to effects of pollution		
6. Biological response. ↓ <b>Endpoint: “ecological health” or biological condition</b>	<b>Response</b> (i.e., Indexes of Biological Integrity (Jameson et al 1998, 2001, subm a, subm b) and other biological dose-response metrics)	Measures the cumulative effects of stress and exposure and include the more direct measures of community and population response. They are assemblage based i.e., counts of whole individuals and species on the reef and represent the condition of the “system”.

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