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Biomonitoring of Coral Bleaching - A Glimpse on Biomarkers for the Early Detection of Oxidative Damages in Corals

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Abstract

Corals live in a symbiotic life with single-celled algae, zooxanthelle. Anthropogenic threats and natural threat-mediated stress destabilize the photosynthetic electron transport chain resulting in an increased production of reactive oxygen species (ROS) in symbiont algae and causes coral bleaching. In this review, the early warning system and biomarkers for oxidative damages in corals are explained. The review also discusses (1) the mechanism of coral bleaching, (2) the uses of biomarkers to detect the early signs of bleaching, and (3) laboratory and field studies that are carried out on biomarkers and coral bleaching.

Keywords: Antioxidant enzymes, oxyradicals, coral bleaching, sym32gene, cytochromeP450

1. Introduction

Coral reefs are among the most productive and diverse ecosystem on earth and support myriad of fish and invertebrate species. The importance of their productivity has prompted the world conservation strategy (UNEP/WWF) to recognize coral reefs as the most essential life support system for food production, health, and other aspects of human survival and sustainable development [1,2]. Coral reefs provide a wide array of food organisms such as fish, mollusks, crabs, shrimps, and algae that are consumed by humans. The destruction of these coral reefs would definitely lead to substantial reduction in supply of animal protein in the diet of coastal population.



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In general, the major hazardous threat to coral reefs can be categorized into anthropogenic and natural origin [1]. Bryant et al. [3] developed a risk index based on the impact of anthropogenic threats to the health of coral reef system, namely, coastal development and marine pollution. Under the natural threats, mortality of corals as a result of increased sea temperature is a relatively recent phenomenon that has resulted in the dramatic decline in the number of healthy reefs around the world [4]. Although various numbers of factors are proposed as a threat to coral reefs, the important toxic consequences is oxidative stress, which leads to coral bleaching [5].

The suitable way to assess sub lethal effects of oxidative stress or early detection of coral bleaching is to quantify the physiological and biochemical responses of corals as a biomarker in response to natural and anthropogenic disturbing agents. The measurement of biochemical responses (antioxidant enzymes, oxyradicals, cytochrome P450 isoforms, heat shock protein, and symbiosis-specific genes) in reefs with response to oxidative stress caused by various factors (temperature, UV radiation, and contaminants) will serve as a good biomarker for the early detection of bleaching.

2. Coral bleaching

The major reason for global degradation of corals by bleaching is a process whereby corals lose their algal symbiont or the symbionts photosynthetic pigments degrade [6]. The existence of corals is dependent on a mutualistic symbiont relationship between the individual coral polyp and a photosynthetic dinoflagellate such as zooxanthella. Zooxanthellae are intracellular residents of the tissues of coral and provide the coral with energy produced by its photosynthetic activities. In return, the coral effectively fertilizes the zooxanthella, providing nutrients in the form of ammonia and phosphates [1]. The successfully proposed model concerning a possible mechanism of coral bleaching is based on the response to oxidative stress by both components of the symbiont relationship [7]. However, understanding the structure of coral tissues could facilitate readers to know about the mechanism of coral bleaching. Corals are formed of two layers of cells known as epidermis and gastrodermis. Both these layers were covered by mucus layer and connected with porous calcium carbonate skeleton. Tissues of corals contain large populations of eukaryotic algae, bacteria, and archaea as well as numerous viruses. In the beginning of 1883, it has been reported that hard corals were associated with intracellular microscopic algae [8], and further it was identified as dinoflagellates, Symbiodinium [9]. Symbiodinium supply a large part of the energy requirements of the corals by transferring photosynthetically fixed carbon to the coral [10]. In addition, during photosynthesis, algae produce large amounts of molecular oxygen for the respiration of corals.

As mentioned in the introductory section, anthropogenic and natural threat-mediated stress can destabilize the photosynthetic electron transport chain resulting in an increased production rate of reactive oxygen species (ROS) in symbiont algae. In addition, it is worth to note that the generation of ROS occurs in the choloroplast by various mechanisms associated with an electron transfer catalyzed with photosystem I and photosystem II [11,12]. According to Mehler reactions, hydrogen peroxide (H_2O_2) is generated by oxygen evolving complex, and these oxyradicals can easily diffuse from the algal symbiont in to the coral cytoplasm. When it happens above the threshold level, ROS will cause oxidative damage and bleaching to corals (Figure 1). Bleaching leads to high mortality and is considered as a serious threat to the health of reef ecosystem [13]. Supporting with earlier works, it is suggested that oxidative stress plays a major role in coral bleaching [1,5,14]. Although cellular-based mechanistic models concerning oxidative stress and coral bleaching are not well established, an increasing number of works have been carried out on coral symbiotic oxidative damage in relation to free radicals generated by disturbance of symbionts photosystem [5,14,15]. Hence, an effective management of the health state of coral reefs requires an early detection or biomonitoring of the oxidative stress. The suitable way to assess sublethal effects of oxidative stress or early detection of coral bleaching is to quantify the physiological and biochemical responses of corals in response to natural and anthropogenic disturbing agents.



3. Biomarkers

The conditions and health state of reefs are unknown since majority of them occur in remote locations [3]. It is very difficult to make a decision about the sustainable use of their resources without having an appropriate data/evidence on their health status. Hence, increased monitoring of reefs is urgently needed. We hope that biochemical responses (antioxidant enzymes, oxyradicals, fluorescent proteins, Cyp 450 isoforms, HSP, and symbiosis-specific genes) on

reefs in response to oxidative stress caused by various factors (temperature, UV radiation, and contaminants) will serve as a good biomarker for the early detection of bleaching.

3.1. Oxyradicals and antioxidant enzymes

In summer, the elevation of water temperature may affect the cnidarians symbiotic life by generating oxyradicals. Ultraviolet (UV) radiation has also been shown to cause bleaching either alone or by acting synergistically with elevated temperature, wherein they produce active forms of oxygen in the zooxanthellae of corals [16]. The absorption of excitation energy in the presence of oxygen leads to the production of reactive oxygen species, ROS (O_2^- , H_2O_2), etc.

$$O_2 + e^- \rightarrow O_2^-$$
$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

ROS will further lead to the photoinhibition of photosynthesis in algae and causes bleaching in symbiotic cnidarians. Superoxide dismutase inactivates the superoxide anion by transforming it into hydrogen peroxide (H_2O_2) . Hydrogen peroxide is then quickly altered by catalase and peroxidases into dioxygen (O_2) and water (H_2O) . Different studies have confirmed that the production of H₂O₂ under the action of SOD is the triggering factor in the natural antioxidant defense mechanisms. SOD therefore seems to be the key enzyme in the natural defense against free radicals. Thus, antioxidant enzyme superoxide dismutase (SOD ; $2O_2^- + 2H^+ \rightarrow$ $H_2O_2 + O_2$, catalase (CAT; $2H_2O_2 \rightarrow 2H_2O + O_2$), glutathione peroxidase (GSH-Px; 2GSH + ROOH \rightarrow GSSG + ROH + H₂O), and ascorbate peroxidase are demonstrated to inactivate the oxyradicals such as O_2^- and H_2O_2 (Figure 2). In 2004, Mydlarz and Jacobs [17] revealed that H_2O_2 production occurred as an oxidative burst in a physically injured *Symbiodinium* sp. that was isolated from *Pseudopterogorgia elisabethae*. Since H₂O₂ acts as an important signaling molecule between Symbiodinium, i.e., zooxanthellae, and their symbiotic host, it is believed that H₂O₂ is the most important ROS associated with coral bleaching [18]. Ross et al. [19] reported that micromolar concentrations of H₂O₂ (>10 µM) induced cell death in the cyanobacterium Microcystis aeruginosa. In 2009, Higuchi et al. [20] studied the response of antioxidant enzymes in the coral Galaxea fascicularis against elevated level of H₂O₂. During short-term H₂O₂ exposure experiment, SOD and CAT activities in zooxanthellae were not significantly altered even at higher H₂O₂ concentration. Similarly, coral bleaching was not observed when exposed to H₂O₂ concentration for a period of 5 days. Both SOD and CAT activities in coral tissue and zooxanthellae were increased with high seawater temperature. It denotes that both.O₂⁻ and H_2O_2 were formed within the cell by the increased temperature. Further, they opined that high seawater temperature had a greater impact on the SOD and CAT activities of the coral. Anithajothi et al. [21] analyzed antioxidant enzymes (SOD, CAT, and GSH-Px) in selected scleractinian corals such as Acropora formosa, Echinopora lamellosa, Favia favus, Favites halicora, Porites sp., and Anacropora forbesi. They concluded that the assay of these enzymes can be used as biomarkers for identifying the susceptibility of corals towards coral bleaching. Regoli et al. [22] characterized the antioxidant efficiency of *Petrosia ficiformis* on a monthly basis by combining an analysis of the main antioxidants (superoxide dismutase, catalase, glutathione S-transferases, glutathione reductase, and glutathione peroxidases) with measurements of the total oxyradical scavenging capacity (TOSC). In summer season, significantly increased levels of catalase and TOSC were observed. The greater production of H_2O_2 in the symbioses during this period supports the hypothesis that seawater temperature can significantly modulate the pro-oxidant and antioxidant status in Mediterranean symbioses.



Figure 2. Oxyradicalsand antioxidant enzymes in Coral Sp.

It is very important to study the stimulation of oxyradical production in corals *in vivo* by water temperature and to what extent the oxyradicals overcomes antioxidant defenses to cause oxidative damage. No detailed study have been carried out so far on the direct measurement of oxyradicals generation *in vivo*, but an indication of such process can be obtained by detecting/analyzing the lipid peroxidation products and carbonyl proteins in heat stress exposed corals. The formation of carbonyl groups on amino acid residues as a result of free radical-initiated reactions is well documented [23]. Carbonyl formation is increased by oxidative stress and is a good marker of protein degradation [24,25]. An increased number of works have been carried out on carbonyls [26] and lipid peroxidation products [27], which shows that it could serve as a biomarker for contaminant stimulated oxidative damage. Downs et al. [7] demonstrated that heat stress causes oxidative damage in corals, which is exacerbated by exposure to light. Later in 2002 [5], they made an attempt to test whether oxidative damage is associated with coral bleaching and examined the levels of protein carbonyl and lipid peroxidation (LPO). Carbonyl protein concentration differed significantly with the exact combination of sampling date and depth and was positively correlated with ocean tempera-

ture. Lipid peroxide (LPO) also showed a similar pattern. The levels of oxidative damage products increased with water temperature and preceded coral bleaching.

3.2. Fluorescent proteins

Corals produce fluorescent proteins (FPs) that are similar to the green fluorescent protein (GFP) of jellyfish. Fluorescent protein absorbs high-energy light and protects corals. These proteins are predominantly found in scleractinian corals and constitute up to 14% of the total protein content [28]. These highly conserved molecules contain 238 amino acids that comprise 11 beta sheets and fold to form a cylinder like shape with three amino acids: serine, glycine, and tyrosine forming a posttranslationally modified fluorescent. Although the function of FPs in corals remains unclear, it is believed that it is involved in photoprotection and also acts as an antioxidant [29,30]. Blue light significantly affects corals and their symbionts. Blue light photoreceptors of corals, which are known as cryptochromes, are thought to play a role in coral bleaching during the elevation of seawater temperature. Blue light primarily damages photo system II directly and secondarily inhibits the repair of photo system II through the production of ROS [31]. The GFP of corals maximally absorbs high-energy blue light and provides photoprotection on corals. In 2009, Palmer CV and coworkers [32] found that scleractinian's fluorescent protein scavenges H₂O₂ and revealed that FPs also act as antioxidant. Carolyn Smith-Keune and Sophie Dove [33] explained that gene expression of host-specific genes such as GFP homologs may act as highly sensitive indicators for the onset of thermal stress within host coral cells. Thus, in future studies, fluorescent protein could be used as a biomarker for the early detection of thermal stress in coral reef, and based on this indication, necessary prevention steps could be taken to prevent coral bleaching.

3.3. Cytochrome P450 and monooxygenase system

Cyp 450 and flavoprotein reductase components of the microsomal mixed function oxidase (MFO) system are involves in the formation of ROS in the presence of contaminants

 $RH + O_2 + NADPH + H^+ \leftrightarrow ROH + H_2O + NADP^+.$

It has been clearly demonstrated that algae have an ability to bioaccumulate and metabolize (via biotransformation) xenobiotic compounds through available detoxifying system such as cytochrome P 450 [34]. Also, the presence of cytochrome P 450-dependent MFO system has been documented in sea anemone and scleractinian coral [35]. CYP–carbon monoxide difference spectra have been detected for the coral species *Favia fragum, Siderastrea siderea,* and *Montastraea faveolata* [36,37]. Ramos et al. [38] analyzed the activities of cytochrome P450 and monooxygenase enzymes (CYP450, P420, and NADPH cytochrome c reductase) in corals collected from two different sampling sites (one from least contaminated site and other from contaminated site). An increased content of CYP450, P420, and NADPH cytochrome c reductase was observed in the corals collected from the contaminated site. This difference was attributed to the difference in contamination levels between the two sampling sites. Ben-

zo(a)pyrene-induced CYP gene expression analysis in the scleractinian coral Montastraea faveolata [37] revealed that fuel oil exposure [39] induces CYP gene expression. Environmentally induced changes in CYP activity were observed in the coral Stylophora pistillata after exposure to hyposaline conditions [10] as well as in Madracis mirabilis after exposure to the photosynthesis inhibitor Irgarol [40]. Rosic et al. [41] discovered the presence of three new cytochrome P450 (CYP) genes from the reef-building coral endosymbiont Symbiodinium. Alteration in the expression of coral's CYP genes were analyzed during exposed to severe and moderate heat stress experiments. Samples of the scleractinian coral Acropora millepora were exposed to two different elevated temperatures (18-h period and 120-h period, i.e., rapid thermal stress and gradual thermal stress). The Symbiodinium CYP mRNA pool increased by 30% after 18 h of gradual heating and incubation at 26°C. An increase in the temperature above the average sea temperature (29°C after 72 h) resulted in a two- to fourfold increase in CYP expression. Both rapid thermal stress and gradual thermal stress at 32°C resulted in 50% to 90% decreases in CYP gene. The expression of CYP gene decreased under the enhanced thermal stress conditions at 32°C. These findings indicate that elevated sea temperature may affect the corals and induce the production of chemical stressors that regulate the expression of CYP genes encoding cytochrome P450 monooxygenases. This may alter the mechanism of biotransformation in corals. The studies emphasize that changes in the expression of CYP450 gene in corals could also be acted as a biomarker for the early detection of heat stress-mediated coral bleaching.

3.4. Mitochondrial integrity

Changes in environmental conditions destabilizes the symbiotic relationship between cnidarians and their dinoflagellate symbionts, Symbiodinium spp. As mentioned earlier, most of the studies have revealed that a breakdown in the symbiosis begins with increased ROS generation within the symbiont due to a decoupling of photosynthesis. Tchernov et al. [42] hypothesized a model for coral bleaching linking dysfunction of mitochondrial integrity to the mortality of the host animal. Mitochondria are known as batteries of the cell, which provides energy in the form of ATP and involves in ROS generation. During thermal stress, algal symbionts produce ROS that exceeds the level threshold. These molecules change the integrity of mitochondria and activate a caspase cascade within the host cell, which leads to the apoptosis and death of the corals. On the other hand, it is found that algal symbiont has the ability to remove or scavenge the ROS and gives protection from coral bleaching. It is noted that varied response was observed in the corals Seriatopora hystrix and S. pistillata to thermal stress. Although both the corals were bleached, the apoptotic response was elevated in *S. pistillata*, which resulted in the death of corals. On the contrary, apoptotic response was decreased in S. hystrix, which indicates that the response of corals against thermal stress is species specific and the algal symbiont of S. hystrix is strongly involved in scavenging ROS. However, in the case of S. pistillata corals, elevated ROS level induced the changes in mitochondrial integrity and further caused death (Figure 3). Dunn et al. [43] corroborate with the above said mechanism by evaluating the changes of mitochondrial integrity of host cnidarians in response to thermal stress. They assessed the overall morphology of host mitochondria associated symbionts under an experimental thermal stress using confocal and electron microscopy. It is noted that thermal

stress degraded the integrity of cnidarian host mitochondria. Further, the potential sites of host mitochondrial disruption were confirmed by measuring changes in the expression of genes associated with electron transport and ATP synthesis using quantitative RT-PCR. They believed that the primary site of degradation appeared to be downstream of complex III of the electron transport chain with a significant reduction in host cytochrome *c* and ATP synthase expression. Hence, it is believed that this reduction may affect the ability of the host to remove ROS and cellular energy supplies. This finding may give us a clue on the importance of host/ coral response to thermal stress and in symbiosis dysfunction that has significant implications for understanding how coral reefs will survive during the climate changes.



Figure 3. Thermal stress mediated coral death and recovery of ROS resistant coral Sp.

3.5. Heat Shock Proteins (HSP)

Heat stress in coral reef affects both corals and their symbionts, which further lead to bleaching of corals. Coral bleaching occurs due to the dissociation of the coral–algal symbiosis [44]. The sensitivity of coral and symbiont bond to heat stress is not well understood. However, it is believed that photosynthesis system can be impaired by heat stress [45,46]. Understanding the basic mechanism of corals against heat stress is crucial in knowing the reason of coral bleaching in response to changes in sea temperature. Heat shock protein (HSPs) represents a class of molecular chaperones that are well known for their quick response to environmental stresses [47]. Thus, alterations in coral's HSPs may serve as biological marker for heat stress. Heat shock proteins are involved in the thermotolerance of oxidative phosphorylation. Several studies demonstrate that oxidative phosphorylation is correlated with the induction of HSP. It is interesting to note that inhibitors of electron transport or inhibitors of complex I act as an inducer of HSP [48]. The mitochondrial low molecular weight HSP is usually produced only in response to environmental stress [49]. It was successfully demonstrated that chloroplast

HSP protects photosynthetic electron transport during heat stress [50], which revealed that HSPs are an important adaptation to heat stress and function as a protective molecular chaperones. Smith et al. [18] found a threefold increase in the level of HSP70 protein in host coral colony at 33°C. Chow et al. [51] also demonstrated a robust transient induction of Hsp60 in response to both light and heat stress in laminar coral. So far, the works carried out on HSP of corals provided a new insight into changes occurring in coral endosymbionts under heat stress. Further research works related to the utilization of HSP as a biomarker to thermal stress is needed.

3.6. Symbiotic-specific genes

Coral bleaching, defined as loss of color in corals, occurs due to the breakdown of the symbiosis with algae. Recently, cnidarian genes that are expressed as a function of the symbiotic state have been characterized in the sea anemone for studying cnidarian algal symbiosis [52]. They found that *sym32* gene is involved in the regulation of the symbiosis by mediating cell–cell interactions. Mitchelmore et al. [53] characterized several genes responsible for the regulation of cnidarians and their symbiotic interaction. Temperate sea anemone *Anthopleura elegantissima* has been used as a model species, and a symbiosis-specific gene, *Sym32*, was identified from the host genome. RT-PCR studies also suggested that the expression of Sym32 was correlated with the presence of host algae. No changes in algal numbers were observed on the exposure of cadmium to anemones under laboratory condition. However, they observed the downregulation of *sym32* may act a biomarker of cnidarians–algal symbiosis breakdown.

3.7. Field and lab observations/applications of biomarkers

Corals generally grow well in clean water with a temperature between 20°C and 30°C. The optimum temperature for the growth of coral is 24°C. Coral reefs are found in great quantity in the Indian Ocean, Southeast Asia, Central Pacific, Southwest Pacific, and Caribbean regions. The largest coral reef is the Great Barrier Reef in Australia. The second largest coral reef can be found off the coast of Belize, in Central America. Coral reefs are also found in Hawaii, the Red Sea, and other areas in tropical oceans. The presence of corals in the ocean is depicted in Figure 4.

Corals and their algal endosymbionts cannot move from their habitats when they face unwanted environmental conditions such as increased seawater temperature and solar radiation. Hence, they have to develop molecular mechanisms to acclimatize and live in those unwanted conditions. Numbers of works have been carried out on coral bleaching that occurs around the world. According to the information provided by the World Resource Institute (WRI), about 370 observations were made on coral bleaching globally between 1980 and 1997. Interestingly, more than 3,700 observations were made between 1998 and 2010. This increased numbers of reports indicate the increase in awareness among researchers to monitor the health of corals and communicate about the bleaching events to the public. The suitable way to assess early detection of coral bleaching is to quantify the physiological and biochemical responses of corals as a biomarker. As mentioned in this review, changes in the biochemical parameters



Figure 4. The presence of coral reefs in Ocean

(antioxidant enzymes, oxyradicals, cytochrome P450 isoforms, heat shock protein, and symbiosis-specific genes) of coral reefs with response to increased seawater temperature may serve as a good biomarker for the early detection of coral bleaching. Numbers of laboratory and field studies have been carried out on theses biomarkers. Some of the works relating to coral biomarkers and field applications are given in Table 1.

Location	Biomarkers	Coral host	Authors
Parque Nacional Morrocoy, Venezuela	Cytochrome-P450, Antioxidant enzymes and NADPH-C reductase	Siderastrea siderea	Ramos etal. (2011) [38]
Heron Island	Heat Shock Protein 70	Porites cylindrica and Stylophora pistillata	Fitt etal. (2009) [54]
Australia	Catalase	Acropora millepora	Krueger etal. (2015) [55]
South East Coast of India	Antioxidant enzymes	Acropora formosa, Echinopora lamellosa, Favia favus, Favites halicora	Anithajothi etal. (2014) [21]
France	Catalase	Anemoniaviridis	Merie etal. (2007) [56]
Florida	Antioxidant enzymes	Coral reef	Downs etal. (2002) [5]
USA	Fluorescence protein	Acroporayongei	Roth and Deheyn (2013) [57]
Great Barrier Reef	Green Fluorescence protein	Scleractinia and Alcyonacea corals	Palmer etal. (2010) [58]
Australia	Green Fluorescence protein	Acroporamillepora	Smith-Keune and Dove S (2008) [33]
Australia	Cytochrome P450	Acropora millepora	Rosic etal. (2010) [41]

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Location	Biomarkers	Coral host	Authors
Honolulu, USA	Cytochrome P450	Stylophora pistillata	Downs etal. (2009) [10]
Italy and Maldives	Mitochondrial HSP60	Seriatopora hystrix, Montipora monasteriata, and Acropora echinata	Seveso etal. (2014) [59]
NJ, USA	Mitochondrial integrity	Zooxanthellate corals	Tchernov etal. (2011) [42]
USA	Heat shock protein	Montastrea annularis	Hayes and King (1995) [60]
Red Sea	Heat shock protein	S. pistillata and Turbinaria reniformis	Chow etal. (2009) [51]
USA	Heat shock protein	Xestospongia muta	López-Legentil etal. (2008) [61]
USA	Heat shock protein and breakdown in symbiosis between coral and zooxanthell	Montastraea faveolata ae	DeSalvo etal. (2008) [62]
USA	Symbiosis-specific gene	Anthopleura elegantissima	Mitchelmore etal. (2002) [53]

Table 1. Corals and biomarkers

In the year of 2011, World Resource Institute furnished data on thermal stress affected coral reefs, which is represented in Figure 5. From the data, it can be understood that more than 40% of the corals were affected by thermal stress in Atlantic and Indian Ocean, which is higher when compared to the other regions. On viewing the earlier research works relating to biomonitoring of coral bleaching, it can be understood that only few research works were carried out in the Indian Ocean. Since corals available in this region are believed to face thermal stress, it is important to concentrate on avoiding coral bleaching in Indian Ocean. Similarly, a large volume of works has been done only on coral antioxidant enzymes and their response against climate change or thermal stress. However, an increased number of works are needed in the aspect of host symbiosis breakdown, coral's mitochondrial integrity, and cytochrome P450 protein as a biomarker of thermal stress. This may give us a better idea about coral bleaching and the utilization of biomarkers for early detection of oxidative damages. In recent days, the early prediction of thermal stress in Ocean has been proposed as the best biomarker for coral bleaching. It is very interesting to know that a computer-based model could assess sea temperature every week and predict the changes in sea temperature and warn us to take precautionary efforts to avoid temperature-mediated coral bleaching [63]. The National Oceanic and Atmospheric Administration's (NOAA) Coral Reef Watch (CRW) and the National Centers for Environment Prediction (NCEP) carried out an excellent research work to predict thermal stress that causes mass coral bleaching. In this regard, a statistical climate model to produce the first seasonal bleaching outlook system was released in 2008 at the 11th International Coral Reef Symposium. This kind of work is another milestone in this field.



(Source: World Resource Institute (1998-2007)

Figure 5. Data on thermal stress affected coral reefs

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