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An Experimental Study of the Response of the Gorgonian Coral *Subergorgia suberosa* to Polluted Seawater from a Former Coastal Mining Site in Taiwan

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Isani Chan, Li-Chun Tseng, Samba Kâ, Ching-Fong Chang, and Jiang-Shiou Hwang (2012) An experimental study of the response of the gorgonian coral *Subergorgia suberosa* to polluted seawater from a former coastal mining site in Taiwan. *Zoological Studies* **51**(1): 27-37. The response of the gorgonian coral *Subergorgia suberosa* to heavy metal-contaminated seawater in the Yin-Yang Sea of Liang-Dong Bay, a former mining site in northeastern Taiwan, was investigated. *Subergorgia suberosa* bioaccumulation and tissue injury were recorded and examined throughout the study period. Heavy-metal concentrations in tissues of the corals showed a significantly increasing trend with incubation time. Cu, Zn, and Cd each showed characteristic bioaccumulation in this soft coral. Metallic Zn accumulated, but rapidly dissipated. In contrast, Cu easily accumulated, but was slow to dissipate, and Cd was only slowly absorbed and dissipated. Our results indicate that significant bioaccumulation of heavy metals occurred in *S. suberosa* coral. Histopathological and scanning electron microscopic results identified polyp necrosis, mucus secretion, tissue expansion, and increased mortality in *S. suberosa* corals exposed to water polluted with heavy metals.
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Key words: *Subergorgia suberosa*, Heavy metals, Bioaccumulation, Histological examination.

Coral reefs play vital roles in marine ecosystems, by serving as home to 25% of all marine fish species and other fauna important to the marine food chain (Bryant et al. 1998, Selkoe et al. 2008, Almany et al. 2009). Worldwide, coral reef ecosystems have recently experienced increasing direct and indirect threats from anthropogenic pressures, including pollutants (Bruno et al. 2007, Jones et al. 2009, Haas et al. 2010, Mendonca et al. 2010, Niggl et al. 2010, Tseng et al. 2011). Consequently, coral reefs are being degraded and damaged by land- and sea-based human activities within the Pacific region (Bryant et al. 1998, Chanmethakul et al. 2010, Tseng et al. 2011).

Previous studies showed that environmental pollutants can accumulate in marine organisms, such as polynuclear aromatic hydrocarbons (PAHs) in copepods and polychaetes (Chandler et al. 1997, Cailleaud et al. 2007a), tributyltin (TBT) in bivalve mussels (Huang and Wang 1995), polychlorinated biphenyls (PCBs) in copepods, corals, fish, crabs, and lobsters (Miao et al. 2000, Cailleaud et al. 2007a, Chang et al. 2008), and nonylphenols (NPs) Polyethoxylates (NPEs) in copepods (Cailleaud et al. 2007b). However, there are few reports of corals being used as marine bioindicators of environmental pollution to monitor the presence of trace metals (Howard and

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Brown 1986, Brown 1987, Shen and Boyle 1987). Corals can incorporate trace metals by a variety of mechanisms: dissolved trace metal species can migrate into crystal lattices, and trace metal particulates may become trapped within skeletal cavities by the uptake of trace metals from coral tissue or through feeding (Howard and Brown 1984). Different coral species were shown to be good tracers of pollutants in marine environments (Pai et al. 1990a, Guzman and Jimenez 1992, Bastidas and Garcia 1999). Hence, continual pollution inputs can result in the accumulation of trace metals and other pollutants in marine organisms (Reichelt-Brushett and Harrison 1998). Coral reefs are widely used as environmental indicators, because they are known to experience such threats (Guzman and Jimenez 1992, Reichelt-Brushett and Harrison 2000, Guzman and Garcia 2002, Kawahata et al. 2004, Yap 2007). However, the accumulation of heavy metals in soft corals has rarely been discussed.

In Liang-Dong Bay, off the northeastern coast of Taiwan, there is a mixture of blue normal and brown abnormal seawater locally known as the Yin-Yang Sea (YYS). The body of water is considered polluted as a result of copper refining by the Taiwan Metal Mining Corporation (TMMC) (Yang and Yeh 1990). Although the factory was closed in 1987, the YYS still exists in the estuary of Liang-Dong Creek where abandoned pits are located. It was suggested that the iron and aluminum dissolved in the effluent of the abandoned pits form flakes of ferric and aluminous hydroxides when they come into contact with the near-neutral pH waters of the estuary (Pai et al. 1990b, Yang and Yeh 1990). These flakes adsorb heavy metals from suspended particles of clay and are transported by estuarine waters to form the YYS (Yang and Yeh 1990).

In this study, we investigated responses of the soft coral *Subergorgia suberosa* to exposure to this polluted seawater from the YYS. The aims of the present study were (i) to examine the bioaccumulation of trace metals by the soft coral species *S. suberosa*, and (ii) to determine the extent and form of tissue damage resulting from exposure to the heavy metal-polluted water of the YYS.

MATERIALS AND METHODS

Coral colonies and water collection

Subergorgia suberosa coral samples were collected from the coastal area of Heping I. (Fig. 1) by scuba divers at a depth of 26 m. Coral colonies were cut into 17-cm lengths using scissors, and specimens were rapidly placed in an icebox containing clean ozone-treated seawater, and supplied with air bubbles during transportation to the laboratory. Experimental water for the bioaccumulation study was collected from the YYS at Liang-Dong Bay (Fig. 1), and water for the control group experiments was collected from the Aquaculture Department of National Taiwan Ocean Univ., Keelung, Taiwan.

Experimental procedures

Eighteen *S. suberosa* colonies were distributed equally among 6 tanks, and these were divided into 3 treatments containing water as a control (control group), and 3 treatments containing YYS sample water (treatment group). The YYS treatment water was not renewed during the experimentation. At weeks 1, 4, 5, 6, and 7 of the experiment, 5-cm lengths of coral (skeleton with tissue attached) were collected from the control and experimental tanks for trace metal analyses. The water tanks were maintained within a salinity range of 34.5-35.0 psu at 24-26°C, with a seawater flow rate of 7-9 cm/s. The water tanks were illuminated from 06:00 to 18:00 with natural daylight, and were kept in darkness in the period 18:00 to 06:00. Fresh *Artemia franciscana* nauplii were provided twice daily as food, during the morning at 10:00 and in the evening at 23:00.

Analysis of water and coral metal contents

Concentrations of Cu, Zn, and Cd in water samples taken from the YYS and in tissues of coral samples taken from Heping I. were determined by atomic absorption spectroscopy (AAS) using a Perkin-Elmer atomic absorption spectrometer (Perkin Elmer 2000 DV ICP, 710 Bridgeport Avenue Shelton, Connecticut, USA). Water samples were stored at 4°C until the AAS analysis was conducted. Coral samples (a combination of tissues and skeleton) were placed into vials, sealed, and stored in a freezer at -40°C for 24 h. Samples were uncapped and dried in a hot-air oven (model EYELA natural

oven NDO-450 OND, Tokyo, Japan) at 50°C for 48 h. Samples were pulverized and digested in aqua regia solution (3 ml HNO_3 and 1 ml HCl) for 24 h. Digested samples were then heated to 60°C until approximately 1 mL of the digested solution remained. Milli-Q water was added to bring the solution to 25 ml (Millipore, Pocklington, York, England).

Histological observations

At weeks 1 and 7, 5-cm samples of *S. suberosa* were cut from the experimental and control tanks. The samples were fixed with neutral buffered formalin (10%) and embedded in paraffin of similar density to that of the *S. suberosa* sample. Wax sections of 3-10 and 6-8 μm were removed, and then dehydrated using 70% alcohol. Xylene was used as the cleaning agent to remove alcohol trapped within the wax medium. After cleaning, samples were embedded in paraffin wax, sectioned with a microtome, and placed on microscope slides. The glass slides were then placed in a warm oven for approximately 15 min to allow the section to adhere to the slide. The embedding paraffin wax covering the tissue was removed by washing with a xylene solvent to allow the water-soluble dye to penetrate the sections. The tissue was then stained with hematein, and a glass cover slip was placed over the specimen for protection. The stained slides were repeatedly immersed in an alcohol solution to remove any water, and then in xylene, until a permanent resinous substance had formed beneath the glass coverslip covering the section (Howard and Smith 1983). Sections were studied using an Olympus SZX10 microscope and images were recorded with an Olympus Imaging Corp. model NO.E-3 camera. The software program, ACD See Pro 2.5 was used to edit the resulting photographs.

Scanning electron microscopic (SEM) observations

Subergorgia suberosa SEM samples were oven-dried for 24 h, and then attached to SEM stubs using a liquid colloidal silver paste. Samples were then sputter-coated with gold (Jeol JFC 1100 E ion-sputtering system, Tokyo, Japan) and observed with an SEM (Hitachi S-4700, Tokyo,

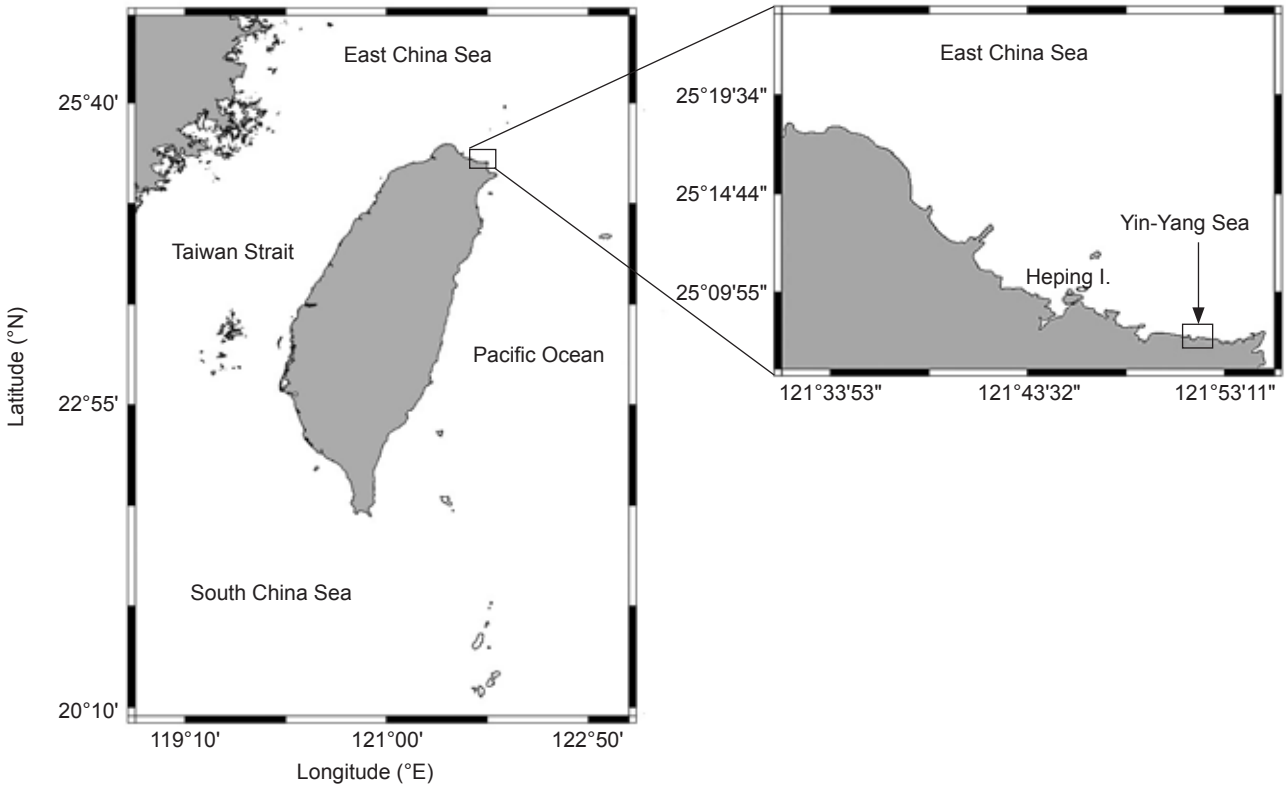

Fig. 1. Location of Heping I. and Liang-Dong Bay (Yin-Yang Sea) in northern Taiwan.

Japan) (Humphreys et al. 1974).

Data analysis

The effect of the treatment type and period of exposure on variations in heavy metal concentrations in the corals were analyzed using a two-way analysis of variance (ANOVA). Statistical analyses were carried out using SAS software (Cary, NC, USA). Pearson's correlations were used to correlate heavy-metal accumulation in coral tissues with time.

RESULTS

Metal levels in the water

Variations in the collected water sample metal concentrations are shown in table 1. High metal concentrations occurred in the Liang-Dong Bay (YYS) sample, with 13.67 μg/L Cu, 2.26 μg/L Zn, and 12.72 μg/L Cd. In contrast, metal concentrations were lower in Heping I. water samples, at 0.260 μg/L Cu, 0.450 μg/L Zn, and 0.124 μg/L Cd.

Metal levels in the coral

Variations in *S. suberosa* metal concentrations are shown in figure 2. Prior to the start of the experiment (week 1), corals collected from Heping I. showed high zinc concentrations (37.98 mg/kg), in contrast to Cu (5.64 mg/kg) and Cd (0.51 mg/kg) levels.

After 4 weeks of exposure to the YYS water sample, Cu concentrations in the corals were significantly greater than Zn and Cd concentrations. Metal concentrations significantly increased at 1-7 wk of the experimental treatment, showing increases of 5.64 to 95.42 mg/kg for Cu, 38.76 to 63.43 mg/kg for Zn, and 0.67 to 0.91 mg/kg for Cd. Concentrations of Cu and Zn in coral tissues were

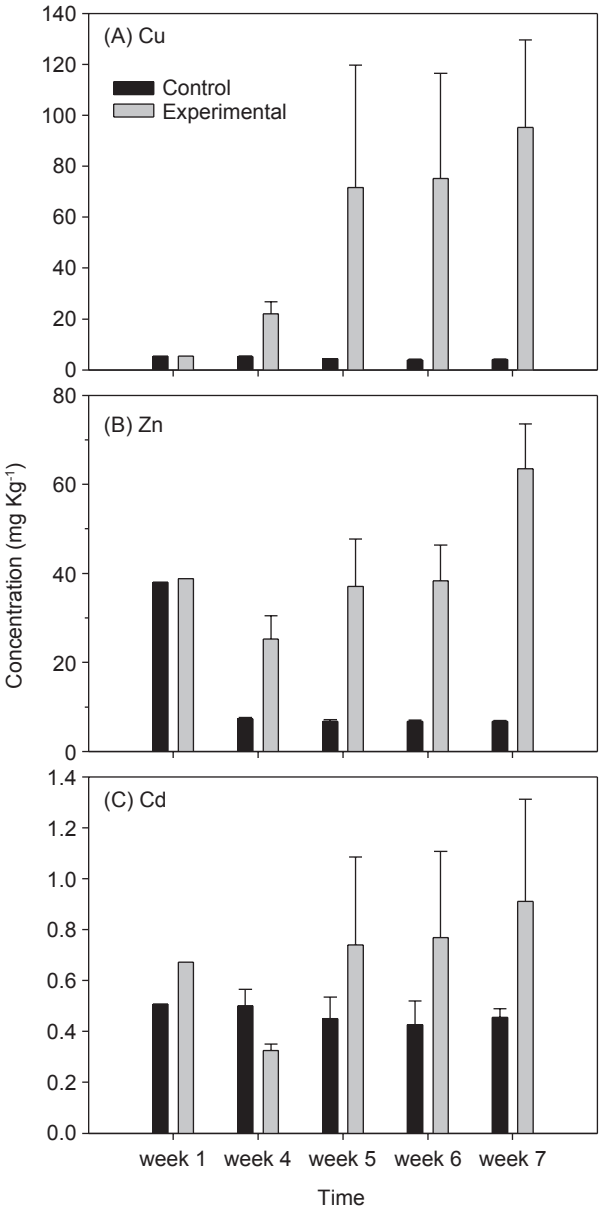

Fig. 2. Variations in the concentration of Cu, Zn, and Cd within *Subergorgia suberosa* from the control and experimental treatments during the experiment. Results are given as the mean ± standard deviation of 3 replicates.

Table 1. Concentrations of heavy metal in samples from Heping I. and the Yin-Yang Sea compared to ANZECC and ARMCANZ standards (2000). Concentrations are expressed in μg/L

Water	Cu	Zn	Cd
ANZECC and ARMCANZ	0.003-0.370	0.100-15.000	0.001-1.1
Yin-Yang Sea, Liang-Dong Bay	13.67	2.26	12.72
Heping I.	0.260	0.450	0.124

several orders of magnitude greater than in YYS water samples from the surrounding area. The bioaccumulation of Cu and Zn in the coral, after 7 wk of exposure to YYS water, was significantly greater than that for Cd.

Metal concentrations in the control treatment (normal seawater) showed a different yet significant trend. Concentrations of Cu in *S. suberosa* tissues were stable in weeks 4-7, during which time, concentrations varied 4.28-5.64 mg/kg (Fig. 2A). However, Zn concentrations dramatically decreased from weeks 1 (37.98 mg/kg) to 4 (6.76 mg/kg), then remained at a constant value in weeks 4-7 (6.57 mg/kg) (Fig. 2B). Cd concentrations in tissues of *S. suberosa* coral slightly decreased from weeks 4 to 7 from 0.51 to 0.45 mg/kg (Fig. 2C).

Results of a two-way ANOVA showed that the 3 heavy metals significantly impacted *S. suberosa* tissues ($p < 0.001$, Table 2) for all treatment groups. Zn showed significant changes in tissue concentrations following the 7-wk exposure to YYS water ($p < 0.001$, two-way ANOVA) and at week 4 ($p < 0.001$, two-way ANOVA). Interactions between *S. suberosa* and the heavy metals, Cu and Cd, were not significant for the treatment ($p = 0.118$) or period ($p = 0.125$). Coral tissue heavy-metal bioaccumulation showed a significant increasing trend with exposure time for Cu ($r = 0.623$, $p < 0.030$, Pearson's correlation), Zn ($r = 0.827$, $p < 0.001$, Pearson's correlation), and Cd ($r = 0.592$, $p < 0.042$, Pearson's correlation) (Fig. 3).

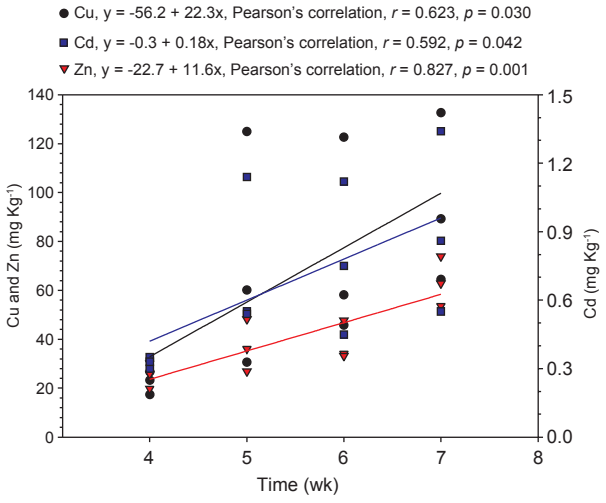

Fig. 3. Pearson's correlations between concentrations of 3 heavy metals in coral tissues and the experimental period of weeks 4-7 in the present study.

Polyp extension, mucus secretion, and tissue necrosis

Few polyp extensions were observed in the experimental tanks compared to those in the control tanks (Fig. 4A, B). Following experimental treatments (YYS water), corals exhibited mucus secretion and advanced stages of tissue necrosis (Fig. 4C, D). Fewer polyps were present in the experimental treatment tanks than were present in the controls (Fig. 4C). Corals in the YYS water exhibited a greater magnitude of tissue expansion than that of control colonies. For some colonies, tissue expansion was only observed in parts of the colony.

Histological observations

In the control tanks, coral cells remained intact with no damage to the tentacles, polyps, or outer epidermal cells (Fig. 5A, B). However, coral in the experimental tanks containing water from the YYS exhibited damaged tentacles, malformed polyps and outer epidermal cells, and increased mucus secretion, tissue expansion, and mortality of juvenile corals (Fig. 5C, D).

SEM observations

Intact tissues of *S. suberosa* were identified in the control tank (Fig. 6A). Magnification of *S. suberosa* surface tissues in the experimental tanks revealed mucus secretion, tissue necrosis, and

Table 2. Results of ANOVA testing: the effects of heavy metals on *S. suberosa*. Significance level = $p < 0.05$

Variable	*d.f.*	*F*	Significance
Cu			
Treatment	1	34.796	< 0.001**
Period	3	2.129	0.137
Treatment × period	3	2.286	0.118
Zn			
Treatment	1	180.059	< 0.001**
Period	3	9.771	0.001**
Treatment × period	3	10.194	0.001**
Cd			
Treatment	1	5.966	0.027*
Period	3	1.491	0.255
Treatment × period	3	2.225	0.125

abnormal spicule appearance, with scales ranging from 20 μm to 1 mm wide (Fig. 6B-F).

DISCUSSION

As expected, metal pollution was higher in the waters of Liang-Dong Bay (YYS) than it was at Heping I. High concentrations of metals in Liang-Dong Bay are in agreement with Chu et al.'s (1995) results for this site. According to Australian and New Zealand standards for fresh and marine water quality (ANZECC and ARMCANZ 2000, Hickey et al. 2001), levels of metal pollution in Liang-Dong Bay and Heping I. are relatively high and at noxious levels. However, Cd concentrations in the southern coastal areas of Taiwan are low (Lee et al. 1998). Our data indicate a large dispersion of cadmium and copper (Fig. 3). We propose that this situation was caused by irregular volumes of mine water discharged into the coastal area. Seawater samples from the coastal area were collected for this study. Thus, concentrations of heavy metals were diluted by mixing with seawater from offshore areas (natural seawater) or precipitation runoff from streams. Samples for this study were collected from the field, and some results showing high concentration of contaminants.

Subergorgia suberosa was exposed to water from Liang-Dong Bay for a 7-wk period. Heavy metal concentrations increased in coral tissues and reached high levels over the study period. *S. suberosa* can accumulate high concentrations of trace metals over time, and there are reports of corals' ability to accumulate metals in their tissues (Esslemont 2000, Esselmont et al. 2000,

Fig. 4. Pictures of *Subergorgia suberosa* showing (A, B) polyp expansion in the control treatments (arrow); (C) mucus secretion in the experimental tanks; (D) tissue necrosis (arrowhead). P, polyp; M, Mucus.

Rainbow 2002, and Mitchelmore et al. 2007). In contrast, some coral species do not accumulate metals (Brown and Holley 1982). Esselmont et al. (2000) reported that varying metal concentrations in tissues might result from specific selectivity for a given metal by a coral. This may explain our observations of differences in concentrations of Cu, Zn, and Cd in *S. suberosa*. However, the differences may also be linked to the concentrations of these metals (Cu, Zn, and Cd) in the YYS water.

The average concentrations obtained for trace metals in *S. suberosa* tissue samples in our study exceeded previous metal concentrations reported for coral tissues and skeletons. However, our observations concur with results of Glynn et al. (1989), who also noted high metal concentrations when analyzing coral tissue and skeletal materials. In this study, coral metal concentrations were significantly affected by the exposure time and treatment. Zn has an important role in coral biological function. At low concentrations, Zn is actively taken up by coral to meet its metabolic needs (Ferrier-Pagès et al. 2005). The extent of Zn bioaccumulation is dependent upon the duration of exposure and the seawater concentration. One group also reported a decrease in Zn in a coral skeleton resulting from tissue detoxification (Ferrier-Pagès et al. 2005). Decreases in Zn concentrations seen for coral in the control group can be explained by the slow release of bioaccumulated Zn, probably from tissues (Ferrier-Pagès et al. 2005). The low Zn concentration, compared to that of Cu, occurs by a variety of processes: substitution of dissolved metals species into the crystal lattice of

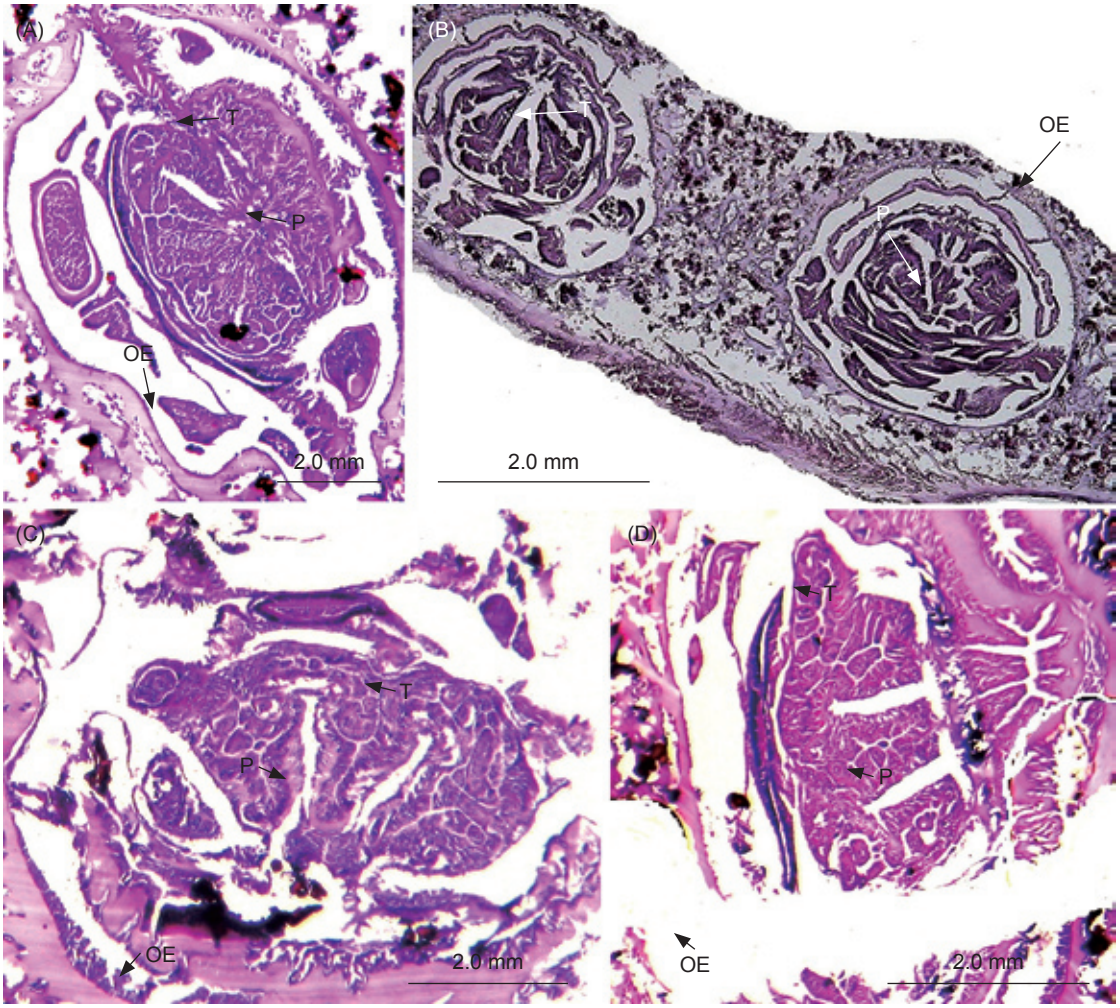

Fig. 5. Histology of *S. suberosa* corals in the control tank (A, B) showing that the tentacles (T), polyps (P), and outer epidermal cells (OE) are intact. Experimental tanks C and D show that the water with heavy-metal concentrations damaged the tentacles (T), polyps (P), and outer epidermal cells (OE) of *S. suberosa* corals.

the mineral skeleton, substitution of metals into coral tissues, trapping of particulate matter within skeletal cavities, and coral feeding (Howard and Brown 1984, Guzman and Jimenez 1992, Bastidas and Garcia 1999). Heavy-metal intake can occur during feeding by the tentacular food capture of zooplankton, which may be rich in heavy metals (Howard and Brown 1984, Anthony 2000). It was reported that metal accumulation can occur via food and water intake, with differences depending on the species, metal, and food source (Wang and Fisher 1999). Metal uptake from water and protein sources is an important route for bioaccumulation (Howard and Brown 1984). Our study produced similar results to those of previous studies into ingested food pathways, including Zn uptake via phytoplankton (Weeks and Rainbow 1993) and the uptake of Cd via zooplankton (Munger and Hare 1997) and sediments (Selck et al. 1998).

Cailleaud et al. (2007a b) showed that hydrophobic organic compounds accumulating in copepods display seasonal variations. Copepods are the major taxon of zooplankton in marine ecosystems (Hwang et al. 2004, Soussi et al. 2007,

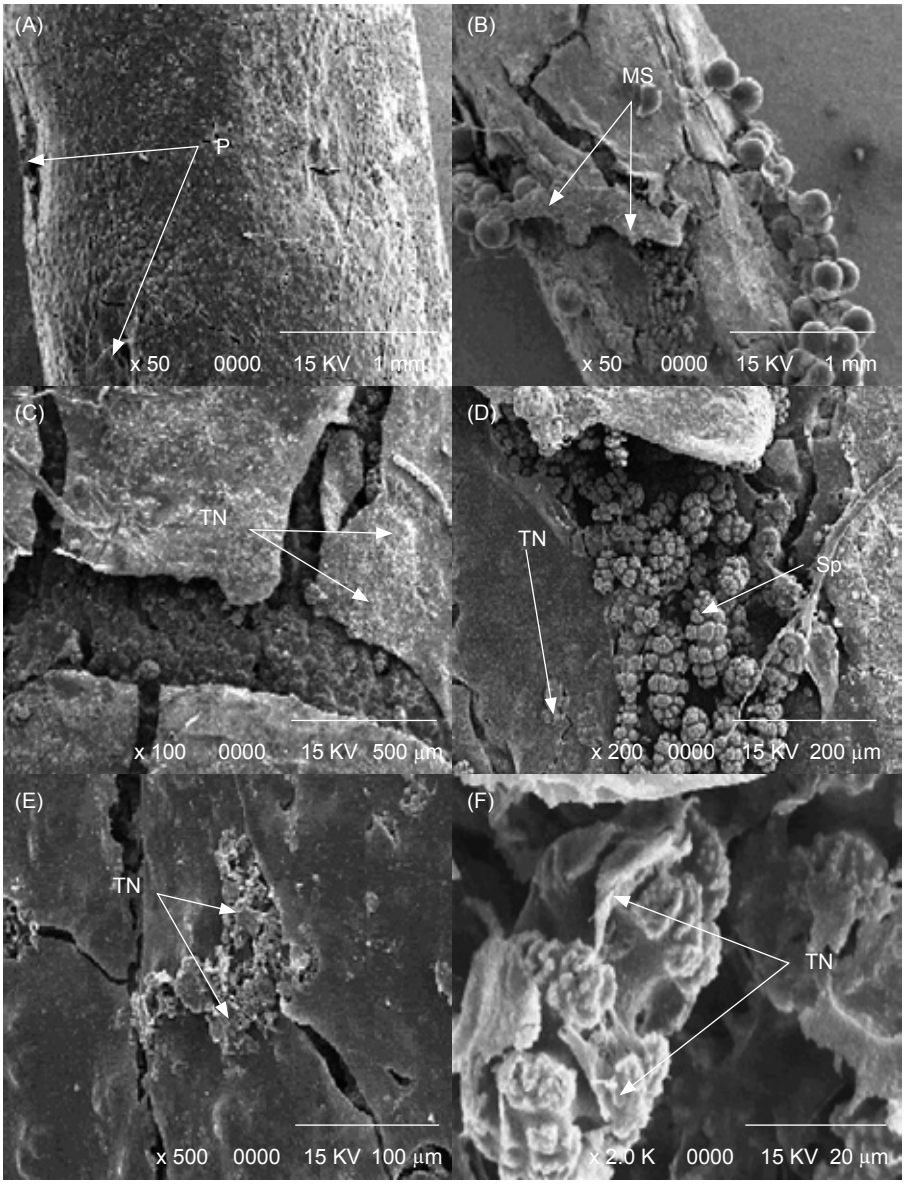

Fig. 6. Scanning electron microscopic microphotographs of *S. suberosa* corals in the control tank (A) showing intact tissues and polyps (P) induced by water with heavy-metal concentrations (B), and mucus secretion (MS) (C-F). Magnification of *S. suberosa* also shows tissue necrosis (TN) and the appearance of spicules (Sp).

Hwang and Martens 2011) and therefore play an important role in transferring materials to higher consumer levels in the ecosystem (Tseng et al. 2008 2009). Several previous studies showed that the bioaccumulation of heavy metals in copepods is diverse and significant (Fang et al. 2006, Hsiao et al. 2006 2010 2011). Our results suggest that Cu, Cd, and Zn can be transferred from the coral's prey, *Artemia*, via feeding behavior. Future studies might consider the quantity of heavy metals that are transferred from copepods to corals through the complex marine food web.

Our results confirm that benthic bioaccumulation from ambient water is progressive. *Subergorgia suberosa* exhibited mucus secretion and tissue necrosis in response to extended exposure to water samples from the YYS. This suggests that metal pollution results in an increase in mucus secretion, a stress defense mechanism and a response to environmental changes by corals (Hayes and Bush 1990, Brown and Bythell 2005). We also found that corals under stress may show symptoms of necrosis. Necrosis in corals is not widely reported. McClanahan et al. (2004) suggested that necrosis may be due to a breakdown of the function of the immune system as a consequence of external stress or disease. High concentrations of Cu and Zn present in the YYS samples may have caused the observed reduction in polyp extensions. Indeed, Reichelt-Brushett and Harrison (2005) reported that metals present in a coral reef impair the success of fertilization. Thus, metals are highly associated with the reduced numbers of polyp extensions of these corals.

The gorgonian species in this study was exposed to maximal concentrations of 13.67 μg/L Cu, 2.26 μg/L Zn, and 12.72 μg/L Cd. Mitchelmore et al. (2003) found that corals exhibited bleaching after 48 h of exposure to Cu and Cd at concentrations of 10-40 μg/L, and death without prior bleaching with exposure to 50 μg/L. Thus, corals used in this study were not exposed to high constant concentrations, and so did not completely die out; furthermore, concentrations of metal ions in the experimental tank may have decreased during the experimental period. Thus, several colonies of corals were kept in the experimental treatment tanks, and an individual organism's metabolism determined how the metals accumulated. This provides an explanation for the reduced effects exhibited by the colonies seen in our study, because surviving colonies can regenerate tissues, in contrast to observations made by Mitchelmore et al. (2003 2007).

Our results indicate that the bioaccumulation of heavy metals was significantly and positively correlated with the exposure period. Wang and Fisher (1999) suggested that the efficiency of metal assimilation is dependent on environmental conditions. Our results also confirmed that temporal factors affected the bioaccumulation of metals in this soft coral.

This study demonstrated that despite closure of the TMMC, pollution is still significant and harmful to the development of marine organisms, and particularly to corals in the YYS located in Liang-Dong Bay. High concentrations of trace metals at Heping I. gave corals from these areas a distinct signature. The high concentrations of trace metals found in *Subergorgia suberosa* coral probably arose as a result of contaminants, which were incorporated into tissue and skeletal materials during juvenile formation of the corals, and continued to accumulate over time. In summary, this study provides new insights into the bioaccumulation of trace metals by the soft coral species *S. suberosa*. Heavy-metal-contaminated water produced negative effects on *S. suberosa* corals by causing tissue necrosis, mucus secretion, tissue expansion, and increased mortality of juvenile corals. The results of this study are useful for coral reef management, with respect to marine pollution.

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REFERENCES

Almany GR, SR Connolly, DD Heath, JD Hogan, GP Jones, LJ McCook et al. 2009. Connectivity, biodiversity conservation, and the design of marine reserve networks for coral reefs. Coral Reefs **28:** 339-351.

Anthony KRN. 2000. Enhanced particle-feeding capacity of corals on turbid reef (Great Barrier, Australia). Coral Reefs **19:** 59-67.

ANZECC, ARMCANZ. 2000. Australian and New Zealand guidelines for fresh and marine water quality. October 2000. Canberra, Australia: Australian and New Zealand

Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, National Water Quality Management Strategy Paper no. 4.

Bastidas C, E Garcia. 1999. Metal content on the reef coral *Porites astreoides*: an evaluation of river influence and 35 years of chronology. Mar. Pollut. Bull. **38:** 899-907.

Brown BE. 1987. Heavy metals pollution on coral reefs. *In* B Salvat, eds. Human impact on coral reefs: facts and recommendation. Antene Museum E.P.H.E. French Polynesia, pp. 119-134.

Brown BE, JC Bythell. 2005. Perspectives on mucus secretion in reef corals. Mar. Ecol. **296:** 291-309.

Brown BE, MC Holley. 1982. Metal levels associated with tin dredging and smelting and their effect upon intertidal reef flats at Ko Phuket, Thailand. Coral Reefs **1:** 131-137.

Bruno JF, ER Selig, KS Casey, CA Page, BL Willis, CD Harvell et al. 2007. Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biol. **5:** 1220-1227.

Bryant D, L Burke, J McManus, M Spalding. 1998. Reefs at risk: a map based indicator of threats to the world's coral reefs. New York: World Resources Institute.

Cailleaud K, J Forget-Leray, S Souissi, D Hilde, K LeMenach, H Budzinski. 2007a. Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine estuary and their transfer to a planktonic species *Eurytemora affinis* (Calanoïda, Copepoda). Part 1: PCBs and PAHs. Chemosphere **70:** 270-280.

Cailleaud K, J Forget-Leray, S Souissi, S Lardy, S Augagneur, H Budzinski. 2007b. Seasonal variation of hydrophobic organic contaminant concentrations in the water-column of the Seine estuary and their transfer to a planktonic species *Eurytemora affinis* (Calanoïd, copepod). Part 2: Alkylphenol-polyethoxylates. Chemosphere **70:** 281-287.

Chandler GT, MR Shipp, TL Donelan. 1997. Bioaccumulation, growth and larval settlement effects of sediment-associated polynuclear aromatic hydrocarbons on the estuarine polychaete, *Streblospio benedicti* (Webster). Mar. Biol. Ecol. **213:** 95-110.

Chang MY, WN Tzeng, CH Wang, CF You. 2008. Differences in otolith elemental composition of the larval *Rhinogobius giurinus* (Perciformes: Gobiidae) among estuaries of Taiwan: implications for larval dispersal and connectance among metapopulations. Zool. Stud. **47:** 676-684.

Chanmethakul T, H Chansang, S Watanasit. 2010. Soft coral (Cnidaria: Alcyonacea) distribution pattern in Thai waters. Zool. Stud. **49:** 72-84.

Chu K, TM Pan, RJ Tseng, LH Chen. 1995. A marine pollution study of northeast coastal water off Taiwan island. Chem. Ecol. **10:** 167-180.

Esslemont G. 2000. Heavy metals in seawater, marine sediments and corals from the Townsville section, Great Barrier Reef Marine Park, Queensland. Mar. Chem. **71:** 215-231.

Esslemont G, VJ Harriott, DM McConchie. 2000. Variability of trace-metal concentrations within and between colonies of *Pocillopora damicornis*. Mar. Pollut. Bull. **40:** 637-642.

Fang TH, JS Hwang, SH Hsiao, HY Chen. 2006. Trace metals in seawater and copepods in the ocean outfall area off the northern Taiwan coast. Mar. Environ. Res. **61:** 224-243.

Ferrier-Pagès C, F Houlbrèque, E Wyse, C Richard, D Allemand, F Boisson. 2005. Bioaccumulation of zinc in the scleractinian coral *Stylophora pistillata*. Coral Reefs **24:** 636-645.

Glynn PW, AM Szmat, EF Corcoran, SV Cofer-Shabica. 1989. Condition of coral reef cnidarians form the northern Florida reef tract: pesticides, heavy metals and histopathological examination. Mar. Pollut. Bull. **20:** 568-576.

Guzman HM, EM Garcia. 2002. Mercury levels in coral reefs along the Caribbean coast of Central America. Mar. Pollut. Bull. **44:** 1415-1420.

Guzman HM, CE Jimenez. 1992. Contamination of coral reefs by heavy metals along the Caribbean coast of Central America (Costa Rica and Panama). Mar. Pollut. Bull. **24:** 554-561.

Haas A, M El-Zibdah, C Wild. 2010. Seasonal monitoring of coral-algae interactions in fringing reefs of the Gulf of Aqaba, northern Red Sea. Coral Reefs **29:** 93-103.

Hayes RL, PG Bush. 1990. Microscopic observations of recovery in the reef-building scleractinian coral, *Montastrea annularis,* after bleaching on a Cayman reef. Coral Reefs **8:** 203-209.

Hickey CW, E Pyle. 2001. Derivation of water quality guideline values for heavy metals using a risk-based methodology: an approach for New Zealand. Aust. J. Ecotoxicol. **7:** 137-156.

Howard DW, CS Smith. 1983. Histological techniques for marine bivalve mollusks. *In* NOAA Technical Memorandum NMFS-F/NEC-25. Woods Hole, US Department of Commerce.

Howard LS, BE Brown. 1984. Heavy metals and reef corals. Oceanogr. Mar. Biol. **22:** 195-210.

Howard LS, BE Brown. 1986. Metals in tissues and skeleton of *Fungia fungites* from Phuket Thailand. Mar. Pollut. Bull. **17:** 569-570.

Hsiao SH, TH Fang, JS Hwang. 2006. The bioconcentration of trace metals in dominant copepod species off the northern Taiwan coast. Crustaceana **79:** 459-474.

Hsiao SH, TH Fang, JS Hwang. 2010. The heterogeneity of the contents of trace metals in the dominant copepod species in the seawater around northern Taiwan. Crustaceana. **83:** 179-194.

Hsiao SH, TH Fang, JS Hwang. 2011. Copepod species and their trace metal contents in coastal northern Taiwan. J. Mar. Syst. **88:** 232-238.

Huang G, Y Wang. 1995. Effects of tributyltin chloride on marine bivalve mussel. Water Res. **29:** 1877-1884.

Humphreys WJ, BO Spurlock, JS Johnson. 1974. Critical point drying of ethanol-infiltrated, cryofractured biological specimens for scanning electron microscopy. *In* O Johari, J Corvin, eds. Scanning electron microscopy 1974. Chicago, IL: 7th Annual Proceedings of the IIT Research Institute, pp. 275-282.

Hwang JS, Js Ho, CT Shih, eds. 2004. Contemporary studies of Copepoda. Proceedings of the 8th International Conference on Copepoda. Zool. Stud. **43:** 165-512.

Hwang JS, K Martens, eds. 2011. Zooplankton behavior and ecology. Proceedings of the conference on Zooplankton Behavior, Ecology and Aquaculture. Hydrobiologia **666:** 179-338.

Jones GP, GR Almany, GR Russ, PF Sale, RR Steneck, MJH van Oppen, BL Willis. 2009. Larval retention and connectivity among populations of corals and reef fishes: history advances and challenges. Coral Reefs **28:** 303-305.

Kawahata H, H Ohta, M Inoue, A Suzuki. 2004. Endocrine disrupter nonylphenol and bisphenol a contamination in Okinawa and Ishigaki Islands, Japan-within coral reefs

and adjacent river mouths. Chemosphere **55:** 1519-1527.
Lee CL, MD Fang, MT Hsieh. 1998. Characterization and distribution of metals in surficial sediments in southwestern Taiwan. Mar. Pollut. Bull. **36:** 464-471.
McClanahan TR, SM McLaughlin, JE Davy, WH Wilson, EC Peters et al. 2004. Observations of a new source of coral mortality along the Kenyan coast. Hydrobiologia **530/531:** 469-479.
Mendonca VM, MM Al Jabri, I Al Ajmi, M Al Muharrami, M Al Areimi, HA Al Aghbari. 2010. Persistent and expanding population outbreaks of the corallivorous starfish *Acanthaster planci* in the northwestern Indian Ocean: Are they really a consequence of unsustainable starfish predator removal through overfishing in coral reefs, or a response to a changing environment? Zool. Stud. **49:** 108-123.
Miao XS, C Swenson, K Yanagihara, QX Li. 2000. Distribution of polychlorinated biphenyls in marine species from French Frigate Shoals, North Pacific Ocean. Sci. Tot. Environ. **257:** 17-28.
Mitchelmore CL, AH Ringwood, VM Weis. 2003. Differential accumulation of cadmium and changes in glutathione levels as a function of symbiotic state in the sea anemone *Anthopleura elegantissima*. J. Exp. Mar. Biol. Ecol. **284:** 71-85.
Mitchelmore CL, EA Verde, VM Weis. 2007. Uptake and partitioning of copper and cadmium in the coral *Pocillopora damicornis*. Aquat. Toxicol. **85:** 48-56.
Munger C, L Hare. 1997. Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus punctipennis*): implications for predicting Cd bioaccumulation in nature. Environ. Sci. Technol. **31:** 891-895.
Niggl W, A Haas, C Wild. 2010. Benthic community composition affects O_2 availability and variability in a northern Red Sea fringing reef. Hydrobiologia **644:** 401-405.
Pai SC, TC Chen, ST Liang. 1990a. Distribution of dissolved and particulate heavy metals in coastal seawater near Lian-Tong Bay, northeast of Taiwan. J. Environ. Protect. **13:** 19-37.
Pai SC, TC Chen, GTF Wong, CC Hung. 1990b. Maleic acid/ammonium hydroxide buffer system for preconcentration of trace metals from seawater. Anal. Chem. **62:** 175-189.
Rainbow PS. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. **120:** 497-507.
Reichelt-Brushett AJ, PL Harrison. 1998. The effect of copper, zinc and cadmium on fertilization success of gametes from scleractinian reef corals. Mar. Pollut. Bull. **38:** 182-187.
Reichelt-Brushett AJ, PL Harrison. 2000. The effect of copper on the settlement success of larvae from the scleratinian coral *Acropora tenuis*. Mar. Pollut. Bull. **41:** 385-391.
Reichelt-Brushett AJ, PL Harrison. 2005. The effect of selected trace metals on the fertilization success of several scleractinian coral species. Coral Reefs **24:** 524-534.
Selck H, VE Forbes, TL Forbes. 1998. Toxicity and toxicokinetics of cadmium in *Capitella* sp. 1: relative importance of water and sediment as routes of cadmium uptake. Mar. Ecol. Progr. Ser. **164:** 167-178.
Selkoe KA, BJ Halpern, RJ Toonen. 2008. Evaluating anthropogenic threats to the northwestern Hawaiian Islands. Aquat. Conserv. **18:** 1149-1165.
Shen GT, EA Boyle. 1987. Lead in corals: reconstruction of historical industrial fluxes to the surface ocean. Earth Planet. Sci. Lett. **82:** 289-304.
Souissi S, Daly Yahia MN, J-S Hwang, eds. 2007. Proceeding of the 9th International Conference on Copepoda. J. Plankt. Res. **29:** i1-i162.
Tseng LC, HU Dahms, QC Chen, JS Hwang. 2009. Copepod feeding study in the upper layer of the tropical South China Sea. Helgol. Mar. Res. **63:** 327-337.
Tseng LC, HU Dahms, NJ Hsu, JS Hwang. 2011. Effects of sedimentation on the gorgonian *Subergorgia suberosa* (Pallas, 1766). Mar. Biol. **158:** 1301-1310.
Tseng LC, R Kumar, HU Dahms, QC Chen, JS Hwang. 2008. Monsoon-driven seasonal succession of copepod assemblages in the coastal waters of the northeastern Taiwan Strait. Zool. Stud. **47:** 46-60.
Weeks JM, PS Rainbow. 1993. The relative importance of food and seawater as sources of copper and zinc to talitrid amphipods (Crustacea; Amphipoda; Talitridae). J. Appl. Ecol. **30:** 722-735.
Wang WX, NS Fisher. 1999. Delineating metal accumulation pathways for marine invertebrates. Sci. Total Environ. **238:** 459-472.
Yang CY, JT Yeh. 1990. Study on the formation of Yin-Yang Hai Sea. J. Chin. Environ. Protect. Soc. R.O.C. **13:** 2-7.
Yap HT. 2007. Coral reef resilience. Mar. Pollut. Bull. **54:** 1075-1076.