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RNA/DNA ratios of scleractinian corals suggest acclimatisation/adaptation in relation to light gradients and turbidity regimes

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ABSTRACT: RNA/DNA ratios are used in many organisms as an indicator of growth, biomass or metabolic functioning. We hypothesised that hermatypic corals, as they depend for growth largely on energy transferred from their algal symbionts, may show an effect in the RNA/DNA ratio with factors influencing irradiance. This was tested along 2 environmental gradients: in relation to depth (decreasing irradiance) and in relation to environmental degradation (increased turbidity). RNA/DNA ratios were determined by HPLC in coral samples collected over depth gradients in 3 islands off the coast of north-west Java, Indonesia. The reefs range from shallow reefs (depth <3 m) in very turbid water, which are close to eroded shores, to intermediate reefs (depth <6 m) further from the shore with less turbid water, to offshore clear water control reefs (depth <20 m). RNA/DNA ratio was shown to be negatively correlated with depth in all but the most turbid conditions, suggesting it is an expression of metabolic acclimatisation of the coral host-symbiont community with decreasing irradiance (i.e. photoadaptation). Comparing the RNA/DNA ratio in clear and turbid waters, the decrease with depth tended to be steeper over a shorter depth range under turbid conditions, analogous with the more rapid extinction of light in these conditions. The relationship between the RNA/DNA ratio and light was consistently higher under turbid conditions ($p = 0.0037$), indicating that RNA/DNA ratio had shifted to a higher level, possibly indicating a genetic adaptation in the metabolic functioning of corals in the turbid environment. The positive relation between light and RNA/DNA ratio would suggest the opposite, i.e. lower ratio under turbid conditions. The RNA/DNA ratio may provide a relatively reliable method to determine the metabolic functioning (health) of individual coral colonies.

KEY WORDS: RNA/DNA ratios · Coral health · Environmental stress · Sediment · Turbidity · Photoadaptation · Growth · Bio-indicator

INTRODUCTION

Much work has been devoted in marine biology to applications of nucleic acid measurements, especially to the RNA/DNA ratio (Dell'anno et al. 1998, Stoeck et al. 1998, Buckley et al. 1999). The RNA/DNA ratio has been used as a biochemical growth-rate indicator, providing an estimate of growth rates and metabolic status in a wide variety of marine organisms such as larval fish (Bulow 1987, Buckley et al. 1999, Kawakami et al. 1999, McNamara et al. 1999), phytoplankton (Dortch et al. 1983), copepods (Nakata et al. 1994, Wagner et al. 1998, Biegala et al. 1999), marine invertebrates (Wright & Hetzel 1985, Frantzis et al. 1992, Pierce et al. 1999, Wo et al. 1999), and bacteria and microbial communities (Kerkhof & Ward 1993, Dell'anno et al. 1998, Stoeck et al. 1998, Kerkhof & Kemp 1999, Yu & Mohn 1999). Some studies have found only poor or even no correlation between growth rate and the RNA/DNA ratio (e.g. Clarke et al. 1989, Anger & Hirche 1990, Frantzis et al. 1992).

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The reasoning behind its use is that because RNA is an essential component of protein synthesis its concentration in tissues or samples often reflects the rate of protein synthesis. A relationship with organismal growth was established early on (Sutcliffe 1965, 1970). The RNA/DNA ratio provides an index of protein synthetic capacity per cell, since the amount of DNA per cell is assumed not to vary with condition or with growth rate. The RNA content of tissue is related to growth rate, food density, and temperature and may also be affected by gametogenesis and developmental stage.

Coral reefs worldwide are in decline as a result of increased use of the coastal zone and the related environmental pressures (Ginsburg & Glynn 1994), possibly aggravated by indirect effects from global warming (Glynn 1991). Recent unprecedented degradation of reef areas as a consequence of bleaching (e.g. Hoegh-Guldberg 1999) has demonstrated the immediate need for better indicators of coral metabolic functioning or 'health'. How can we measure whether coral colonies are performing under less than optimal conditions? Parameters that have been suggested include stress proteins (Miller et al. 1992), tissue regeneration rate (Meesters & Bak 1994), pulse amplitude modulated (PAM) fluorometry (Jones et al. 1999), coral lipids (Harriott 1993), ATP (Fang et al. 1987) mitotic indices (Brown & Zamani 1992), coral calcification rates (Davies 1990), spectral analysis (Holden & LeDrew 1998) and population structure (Bak & Meesters 1999, Meesters et al. 2001). Here we want to present the RNA/DNA ratio as a measure of health in corals.

Although the RNA/DNA ratio is used as a growth/health index in many organisms and communities, it has not been applied to scleractinian corals; although Gates & Edmunds (1999) have given some preliminary results. We speculated that reduced light, through its effect on the energy metabolism of the endosymbiotic zooxanthellae (for reviews see Barnes & Chalker 1990, Falkowski et al. 1990, Brown 1997), is likely to have a direct effect on the RNA/DNA ratio of the coral host-symbiont association and we tested this in 2 ways. Firstly, the ratio was assessed in corals over a natural depth interval from 0.2 to 18 m. Secondly, we compared ratios between colonies from 3 sites along a gradient in turbidity due to different sediment loading of the water column.

MATERIALS AND METHODS

Study area and sampling. We sampled reefs at 3 islands off the north-western coast of Java, Indonesia, from 10 to 17 April 1999 (Fig. 1). The 2 islands Gosong Dadapan and Pulau Kubur are situated in the turbid Bay of Banten. Water clarity in Banten Bay is very low, probably due to strong resuspension of a former river delta in the eastern part of the bay causing a strong turbidity gradient (E. H. Meesters & R. P. M. Bak unpubl. data). The third island, Pulau Tunda, is in the relatively clear water of the open Java Sea, approximately 17 km north of Banten Bay. Gosong Dadapan is the most inshore, close to a rapidly eroding muddy shore. Corals occur only over a very narrow depth range (0.5 to 3.1 m). The island is now submerged, emerging partly at low tide. Pulau Kubur is farther away from the shore but still inside the muddy bay. Reefs here occur to a depth of 6 m. At Pulau Tunda, corals grow in relatively clear water to depths of approximately 20 m. The islands lie along an inshore to offshore turbidity gradient. This difference in turbidity between the islands was consistently observed during SCUBA dives. Light measurements were taken at high tide (variation tide level <30 cm, A. J. F. Hoitink & P. Hoekstra unpubl.) during the period February 1998 until April 1999 using an IL 1400A light meter (International Light) equipped with a cosine corrected, underwater sensor (400 to 700 nm). Mean extinction values at the sampling stations were 0.78 (SD = 0.28, n = 14 profiles) off Gosong Dadapan, 0.42 (SD = 0.19, n = 16) around Pulau Kubur and 0.26 (SD = 0.031, n = 2) at Pulau Tunda. Due to the small sample size at Pulau Tunda, differences between Pulau Tunda and Pulau Kubur were not statistically significant, but data such as long-term sedimentation patterns (^{210}Pb) from gravity cores, short-term sedimentation patterns from sediment traps and optical backscatter measurements (A. J. F. Hoitink & G. D. van den Berg unpubl.) confirm the observed pattern in turbidity. Separate suspended sediment (SPM) readings during the whole period of study (January 1998 to April 1999) could be well above 100 mg l^{-1}, but average concentrations were around 10 mg l^{-1} in the bay and 1.4 mg l^{-1} in the Java Sea.

Colonies of massive *Porites* species were sampled at the north-western sides of Pulau Kubur and Gosong Dadapan and at the northern side of Pulau Tunda. Due to extremely large variation in skeletal micro-characteristics of colonies, we were not able to determine species names. In terms of traditional taxonomy, sampled colonies were either *Porites lutea* or *P. lobata*. Large and overlapping variation in skeletal characteristics both within and between colonies and between colonies in different environments has now been acknowledged, preventing confident identification in the genus *Porites* (Veron 1995). For the purpose of our study, it is relevant to realise that variation in DNA and RNA is likely to be minimal or non-detectable between congenerics G.C.A.D. pers. obs.). From each colony, we took a small (1 cm diameter) core from the top of the colony with a pneumatic drill. Each core was cut

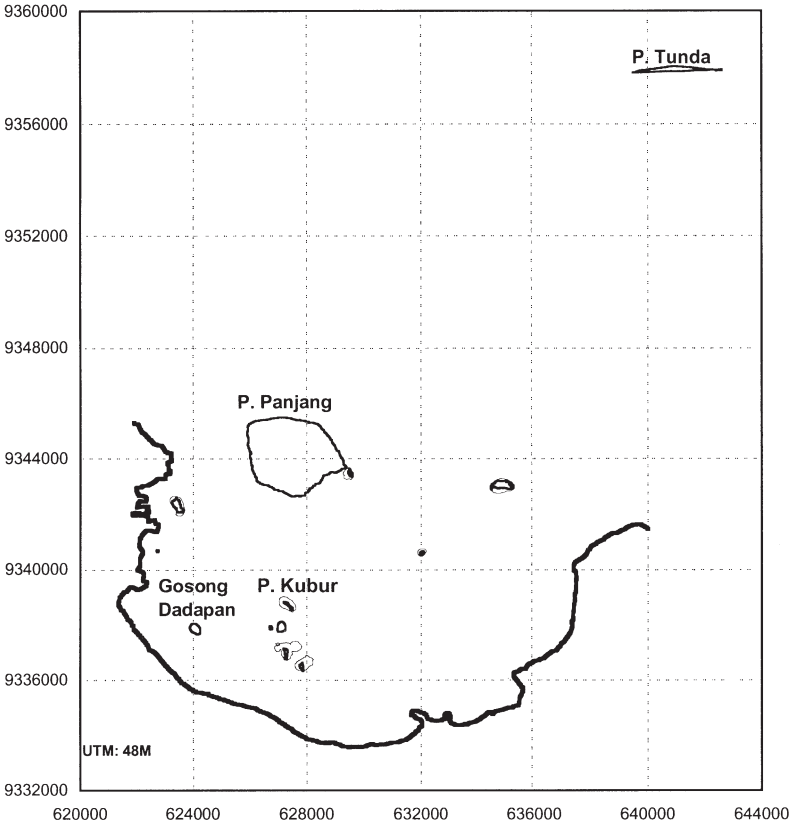

Fig. 1. Geographic map of Banten Bay, north-west Java, Indonesia. Sampling sites are Gosong Dadapan and Pulau Kubur inside Banten Bay, and Pulau Tunda outside the bay in the Java Sea. The thin lines around islands represent reef contour (ca. 3 m isobath). UTM: Universal Transvers Mercator. Scale is in m

below the coral tissue and stored in liquid nitrogen within 10 min of reaching the surface.

RNA and DNA determination. Samples were kept below −80°C and not allowed to thaw at any time. Samples were analysed with high performance liquid chromatography (HPLC) using a procedure first described by Coppela et al. (1987) and modified by A.K. (Stoeck et al. 1998). The lower detection border lies at 10 ng DNA, or RNA. Calibration curves were highly significant (r^2 of all >0.99). Recovery for both RNA and DNA was on average 97%. A sub-sample of each core was freeze-dried and digitally photographed for surface area calculation by image analysis. To minimise a possible effect of endolithic organisms, we removed as much skeleton as possible from under the coral tissue layer. However, *Porites* coral tissue is located partially inside the porous skeleton and not all skeleton was removed. Coral cores were ground including the remaining skeleton. The resulting powder was added to 1 ml of Tris-HCl buffer and sonicated 10 times (10 s with 1 min intervals). After sonication, samples were centrifuged for 5 min (3000 × *g*) and the supernatant was filtered over a 0.45 µm pore size cellulose acetate filter to remove particulate material. The filtrate was directly injected into the HPLC system, which consisted of a Waters 616 pump and Waters 600S controller unit, connected via a Nucleogen 4000-7 DEAE anion-exchange column (Macherey-Nagel, Düren) to a Waters 486 tunable absorbance detector. Peak identity in the chromatogram was confirmed by inspection of the absorbance spectrum (DNA and RNA maximum absorbance at 260 nm) in combination with either co-injection of standards (calf thymus DNA and baker's yeast RNA) or digestion of RNA with RNase. The areas of the peaks were integrated at 260 nm wavelength. Gradient flow rate and composition of eluents used for HPLC determination were as in Dell'anno et al. (1998).

RESULTS

A typical HPLC plot is given in Fig. 2. The 3 peaks from left to right were identified as tRNA, rRNA and DNA. Table 1 gives the RNA/DNA concentrations, the ratios, depths and location of each sample. The ratios vary between 0.60 and 1.04. At Pulau Tunda and Pulau Kubur, there is a significant relationship between the ratio and sample depth (Table 2). A large degree of the variation in the ratio of the different samples can be explained by depth (respectively 81 and 96%). The RNA/DNA samples from Gosong Dadapan come from a very narrow depth range (only 2.5 m) and there is no variation between samples ($p = 0.314$). The regression

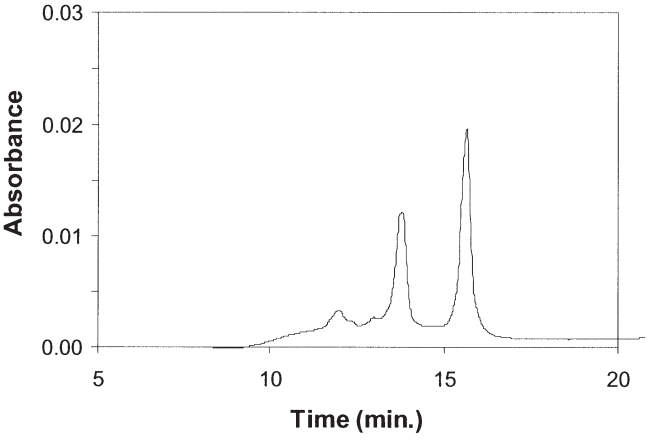

Fig. 2. Typical HPLC plot of coral sample. The 3 peaks were identified as tRNA, rRNA and DNA (from left to right)

slopes of Pulau Kubur and Pulau Tunda are significantly different from zero (Table 2). The RNA/DNA ratio appears to decrease faster with depth at Pulau Kubur, but the slopes were not significantly different (ANOVA test for differences between slopes, $F_{1,17}$ = 1.99, p = 0.18). However, when correcting for the differences in depth by analysis of covariance (ANCOVA), the ratios between Pulau Kubur and Pulau Tunda are significantly different ($F_{1,18}$ = 5.70, p = 0.028; adjusted least-squares means, respectively 0.83 and 0.77; SE 0.02 and 0.01).

Table 1. RNA/DNA concentrations and ratios of coral cores from the 3 islands. Depth is the depth at the surface of the sampled coral colony. Percentage irradiance based on average extinction coefficient (see 'Materials and methods')

Island	Depth (m)	RNA (µg cm^{-2})	DNA (µg cm^{-2})	Ratio	% irradiance
Gosong Dadapan					
	0.5	239	293	0.82	67.7
	0.6	364	443	0.82	62.6
	1.3	217	291	0.74	36.3
	2.3	156	191	0.81	16.6
	2.3	165	210	0.78	16.6
	2.3	133	166	0.80	16.6
	3.1	112	147	0.76	8.9
Pulau Kubur					
	1.2	286	309	0.93	60.4
	1.5	328	356	0.92	53.3
	2.2	98	113	0.87	39.7
	5.1	171	220	0.78	11.7
	5.6	91	127	0.72	9.5
Pulau Tunda					
	0.2	298	286	1.04	94.9
	0.3	376	415	0.91	92.5
	1	365	428	0.85	77.1
	1	361	442	0.82	77.1
	1.1	223	253	0.88	75.1
	1.3	150	172	0.87	71.3
	2.3	210	303	0.69	55.0
	2.8	272	372	0.73	48.3
	5.5	222	339	0.65	23.9
	7	188	294	0.64	16.2
	7.1	196	251	0.78	15.8
	10.1	116	173	0.67	7.2
	11.5	143	201	0.71	5.0
	12.6	113	159	0.71	3.8
	17.1	89	134	0.66	1.2
	19.7	100	166	0.60	0.6

Table 2. Regression of RNA/DNA ratio vs depth. Data fitted to the line: RNA/DNA = C + [k × log(Depth)], in which C is a constant, k is the regression slope and log is the logarithm base 10

Island	C	k	r^2	p
Gosong Dadapan	0.80	–0.041	0.20	0.314
Pulau Kubur	0.96	–0.295	0.96	0.003
Pulau Tunda	0.85	–0.174	0.81	<0.001

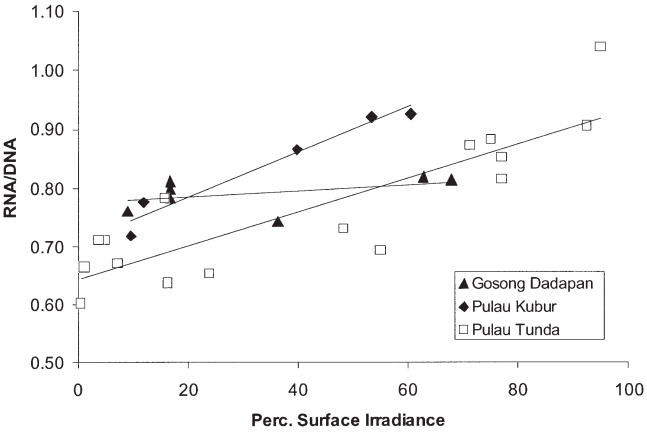

Fig. 3. RNA/DNA ratio vs estimated percentage light at the measured depth. Percentage light was estimated by using average extinction values for each site (see 'Materials and methods')

Fig. 3 shows that the range in surface irradiance of the samples from the 3 islands is very similar. If light is exclusively responsible for the variation between the different islands, the data points should lie on the same line. However, corals from Pulau Kubur appear to have higher RNA/DNA ratios than corals at Pulau Tunda. Because irradiance is derived from depth, regression results are similar to those for depth (Table 2). Corals from Gosong Dadapan do not show a significant relationship with light, while the 2 other islands do. The slopes from Pulau Tunda and Pulau Kubur are not significantly different (ANOVA test for differences between slopes, $F_{1,17}$ = 0.50, p = 0.49), but the RNA/DNA ratio in corals from Pulau Kubur is significantly higher (ANCOVA, $F_{1,18}$ = 11.1, p = 0.0037; adjusted least-squares means, respectively 0.86 and 0.76; SE 0.026 and 0.014).

DISCUSSION

RNA/DNA ratios and depth

We found a clear negative relationship between the RNA/DNA ratio and depth in 2 of the 3 locations. Because irradiance decreases exponentially with depth (Jerlov 1968), the exponential decrease of RNA and DNA with depth found in this study supports the hypothesis that light is the primary responsible factor. Metabolism and growth in scleractinian hermatypic corals depend mainly on irradiance, and many studies have shown the effect of light or depth on these processes

(e.g. Yonge & Nicholls 1931, Bak 1974, Falkowski & Dubinsky 1981, Titlyanov 1981, Chalker et al. 1983, Dubinsky et al. 1984, Falkowski et al. 1984, Muscatine et al. 1984, Bosscher & Meesters 1992). The adaptation of corals to light has been termed photoadaptation, and it involves a whole spectrum of responses ranging from changes at the cellular level to behavioural and growth form variations (Brown 1997). The decrease in RNA/DNA ratio reflects metabolic effects that are related to the dependence of the coral host-symbiont community on light and should be viewed as a phenotypic (photo) adaptation. The reduction in RNA/DNA ratio suggests that protein synthesis per cell decreases with light intensity. A similar decrease has been found for the *P*/*R* ratio, another measure of performance in relation to depth (McCloskey & Muscatine 1984).

Turbidity

The spectral quality/quantity of submarine light field is influenced by dissolved and suspended substances. This means that the extremely turbid conditions in the bay create a very different spectral environment for the corals compared to the environment outside of the bay. Regarding the 3 study sites, we see that samples from Gosong Dadapan did not show a decline in the RNA/DNA ratio with depth. Gosong Dadapan lies very close to the shore (Fig. 1), and consequently environmental variations in sedimentation, turbidity, salinity, and temperature are larger than at Pulau Kubur and much larger than at Pulau Tunda. There is a strong gradient in turbidity, ranging from very high near the coast to low further away into the Java Sea. Average extinction values (k') for Gosong Dadapan, Pulau Kubur, and Pulau Tunda were respectively 0.78, 0.42, and 0.26 (see 'Materials and methods'). For Gosong Dadapan and Pulau Kubur, these correspond to a sediment load of approximately 4.6 and 1.9 mg l^{-1} (using an empirically established relation for Teluk Banten between suspended particular matter (SPM) in l^{-1} and average extinction, k':k' = −0.1034 SPM − 0.1607; r^2 = 0.89. However, incidental turbidity readings were found to be in excess of 10 mg l^{-1}. Sedimentation values at Gosong Dadapan were often twice as high as at Pulau Kubur and ranged from 50 to 150 g m^{-2} d^{-1}. These extreme environmental conditions at Gosong Dadapan appear to distort the relation between the RNA/DNA ratio and depth found at the 2 other islands.

Adaptation or acclimatisation

The corals of Pulau Kubur live under relatively turbid conditions when compared to corals at Pulau Tunda. They show a similar response with light (i.e. a comparable decrease in the ratio with increasing depth), but, remarkably, the RNA/DNA ratio is consistently higher (Fig. 3). This indicates that corals at Pulau Kubur are metabolically more active than at Pulau Tunda. This is either phenotypic acclimatisation or genetical adaptation.

The relationship between the RNA/DNA ratio and light is the same at the 2 islands, but raised to a higher level at Pulau Kubur. This may indicate that corals at Pulau Kubur have become genetically adapted. However, similar phenomena (resulting from genetical adaptation or phenotypic acclimatisation) have been observed in metabolic responses of poikilotherms to temperature (e.g. Berggren & Roberts 1991). Corals at Pulau Kubur may profit in some way from the turbid waters in the bay, e.g. by digesting sediment particles (Anthony 1999). The fact that the RNA/DNA ratio runs parallel at a higher value over the whole range of the environmental variable (light) suggests that the adaptation is genetic. If sediment ingestion were responsible, the reaction of the corals would not be similar over all depths, because sediment concentrations in the bay show a constant gradient with higher concentrations at greater depths (E.H.M. unpubl.). Possibly, corals at Pulau Kubur have obtained a different strain of zooxanthellae that are more efficient at the ambient irradiance levels (Chang et al. 1983, Rowan et al. 1997), but more detailed studies are needed to resolve this enigma of better performing corals under increased sediment stress.

No previous study has reported the use of RNA/DNA ratios in corals in studying the condition of the colony. The only reference to RNA/DNA ratios in corals comes from preliminary data mentioned in Gates & Edmunds (1999). They state that ratios in 10 clonal genotypes of the coral *Madracis mirabilis* vary by a factor of 5; however, no data are shown. This would suggest that there may be large interspecific differences between species, because in our study ratio differences in a dataset containing 2 species (*Porites lobata* and *P. lutea*) are less than a factor of 2. Our study indicates that the use of RNA/DNA ratios as a measure of metabolic functioning could be an indicator for 'health' in scleractinian corals. Protein turnover should be intrinsically linked to growth, metabolism and acclimatisation potential conditions (Gates & Edmunds 1999). Because RNA synthesis is fundamental to protein metabolism, the study of RNA and DNA concentrations may provide additional insights in the responses of coral organisms to environmental stress.

Further research should include temporal (seasonal) variation in RNA/DNA concentrations, as well as interspecific and intra-colony variation. Our preliminary tests have indicated that the variation in the ratios

between colonies from the same depth are very similar (average difference 8%, n = 7 pair of colonies); ratios from different samples from the top of the same colony varied 4% (n = 5 pairs of colonies).

In conclusion, we developed an accurate and sensitive assay to measure RNA and DNA concentrations in coral samples. RNA/DNA ratios were shown to be sensitive to differences in depth, most likely reflecting differences in light regime and as such indicative of photoadaptation. The measurement of RNA and DNA concentrations may provide a sensitive measure of coral colony response to environmental change.

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