Long, S. Humphries, P. G. Falkowski *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 633 (1994).
13. A primary source of new iron in the South Pacific is atmospheric deposition, with one of the lowest annual budgets of Fe on the globe at ≤ 1 mg m^{-2} $year^{-1}$ (particulate plus dissolved). Aeolian deposition of Fe in the Atlantic ranges from 10 to 100 mg m^{-2} $year^{-1}$ [R. A. Duce and N. W. Tindale, *Limnol. Oceanogr.* **36**, 1715 (1991)].
14. J. F. Allen, *Biochim. Biophys. Acta* **1098**, 275 (1992); J. Allen, J. Bennett, K. E. Steinback, C. J. Arntzen, *Nature* **291**, 25 (1981).
15. C. W. Mullineaux, M. J. Tobin, G. Jones, *Nature* **390**, 421 (1997).
16. T. Burger-Wiersma and A. F. Post, *Plant Physiol.* **91**, 770 (1989).
17. O. Salehian and D. Bruce, *J. Luminesc.* **51**, 91 (1992).
18. M. Aoki and S. Katoh, *Biochim. Biophys. Acta* **682**, 307 (1982); C. W. Mullineaux and J. F. Allen, *FEBS Lett.* **205**, 155 (1986); S. Scherer, A. Helmar, P. Böger, *Photosynth. Res.* **15**, 95 (1988).
19. In eukaryotic photoautotrophs, photosynthesis and general cell metabolism occur separately in the chloroplast and mitochondria. Thus, under normal conditions, eukaryotic plants remain in state 1 at night. However, R. Belkhodja *et al.* [*Photosynth. Res.* **56**, 265 (1998)] reported a nocturnal reduction of the PQ pool under iron-limiting conditions in terrestrial plants and eukaryotic algae (presumably by chlororespiration), which caused increased background fluorescence.
20. *Synechococcus* was grown at 25°C under a 12-hour light–12-hour dark cycle at 250 μmol of quanta per square meter per second. State 2 transitions were studied during the first ≈55 min of darkness after the 12-hour photoperiod using the FRRf (*7, 8*). State 1 transitions were induced immediately thereafter by exposing cells to PSI-specific far-red light [light-emitting diode (LED) source with Schott RG-695 long-pass filter]. State transition experiments were conducted during the first few days after the onset of nitrate or iron starvation. PSI and PSII concentrations were determined according to methods described by Dubinsky *et al.* [Z. Dubinsky, P. G. Falkowski, K. Wyman, *Plant Cell Physiol.* **27**, 1335 (1986)].
21. In cyanobacteria, decreases in σ_{PSII} are observed after state 2 transitions when fluorescence excitation spectra include green or orange wavelengths, as in our field studies (*7*). When measured with a blue excitation spectrum, no change in σ_{PSII} is observed (D. Bruce, personal communication). The mechanism responsible for this discrepancy is unknown. During our laboratory studies, the primary fluorescence excitation source was blue-light LEDs. Consequently, no changes in σ_{PSII} (mean 702 ± 29 A^2 per photon) were observed despite the clear induction of state 2 transitions and their reversal upon exposure to far-red light (Fig. 4).
22. H. Dau, *Photochem. Photobiol.* **60**, 1 (1994)
23. Greene *et al.* [R. M. Greene, Z. S. Kolber, D. G. Swift, N. W. Tindale, P. G. Falkowski *Limnol. Oceanogr.* **39**, 1061 (1994)] did not observe the large nocturnal decreases in F_v/F_m in the equatorial Pacific illustrated in Fig. 1B. Based on the kinetic rates we observed, this discrepancy resulted from inadvertent induction of a state 2 transition during the prolonged dark adaptations (>20 min) during the previous study.
24. J. A. Guikema and L. A. Sherman, *Plant Physiol.* **73**, 250 (1983); G. Sandmann, *Photosynth. Res.* **6**, 261 (1985); N. A. Straus, in *The Molecular Biology of Cyanobacteria*, D. A. Bryant, Ed. (Kluwer Academic, Boston, 1994), pp. 731–750; I. R. Vassiliev *et al.*, *Plant Physiol.* **109**, 963 (1995); R. M. Greene, R. J. Geider, Z. Kolber, P. G. Falkowski, *ibid.* **100**, 565 (1992).
25. Supported by NASA grants NAG5-7437 and UPN161-35-05-08. We thank A. Bale, K. Coale, and K. Johnson for assistance during IronExII; S. Laney and J. Aiken for AMT-1 data; D. Vaulot, B. Coste, and the crew of *L'Atalante* for assistance during OliPac; K. Wyman for laboratory assistance; and especially P. Falkowski, L. Sherman, D. Bruce, C. Mullineaux, R. Geider, R. Barber, D. Mauzerall, S. Seitzinger, and O. Prasil for helpful suggestions and encouragement.

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The Dynamics of Zooxanthellae Populations: A Long-Term Study in the Field

I. Fagoonee,[1] H. B. Wilson,[2] M. P. Hassell,[2]* J. R. Turner[3]

Coral bleaching characterized by the expulsion of symbiotic algae (zooxanthellae) is an increasing problem worldwide. Global warming has been implicated as one cause, but the phenomenon cannot be fully comprehended without an understanding of the variability of zooxanthellae populations in field conditions. Results from a 6-year field study are presented, providing evidence of density regulation but also of large variability in the zooxanthellae population with regular episodes of very low densities. These bleaching events are likely to be part of a constant variability in zooxanthellae density caused by environmental fluctuations superimposed on a strong seasonal cycle in abundance.

Coral bleaching is normally characterized by expulsion of the endosymbiotic zooxanthellae (the unicellular dinoflagellate *Symbodinium* spp.), loss of algal pigmentation, or both. Coral bleaching events, defined here as concomitant with very low zooxanthellae density, have had serious effects on corals and reefs worldwide (*1*). Given the dependence of the coral on its symbiotic algae (*2*), it is important to determine the cause of these bleaching events. A number of explanations for coral bleaching have been proposed, including unusually high seawater temperatures (*3, 4*), high doses of ultraviolet light (*5*), bacterial infection (*6*), and changes in salinity (*7*). What is crucial to our understanding of zooxanthellae expulsion and bleaching is how the density of zooxanthellae within the coral is changing, if at all, under the prevailing range of environmental conditions. Here we present the results of a long-term field study (August 1991 to March 1997), with data collected on a weekly basis, during which the population density of zooxanthellae within the coral *Acropora formosa* (Dana 1846) in a shallow lagoon in Mauritius was monitored (*8*) and environmental variables were measured (*9*).

The time series of the zooxanthellae density over the study period is shown in Fig. 1. The mean density was 1.7×10^6 cm^{-2} (SD = 2.4×10^6 cm^{-2}), comparable to densities of about 1×10^6 to 2×10^6 cm^{-2} previously reported (*10, 11*). There is evidence of some regulatory mechanism, as the change in zooxanthellae density from one week to the next is dependent on the density during the preceding week (*12*). The time series reflects sampling over the whole coral colony and does not reflect changes in individual tips from one week to the next; the density dependence detected in the time series thus indicates trends through time within the coral colony as a whole.

In addition to the density dependence, there is considerable variation in density, with fluctuations over three orders of magnitude. Although densities from 0.5×10^6 to 5.0×10^6 cm^{-2} have been reported in different studies (*13–15*), here such variability is reported from a single coral colony over time. In particular, there was a bleaching event in the spring and summer of 1993 (density $< 0.1 \times 10^6$ cm^{-2} from 28 October to 17 December 1993; weeks 109 to 117 in

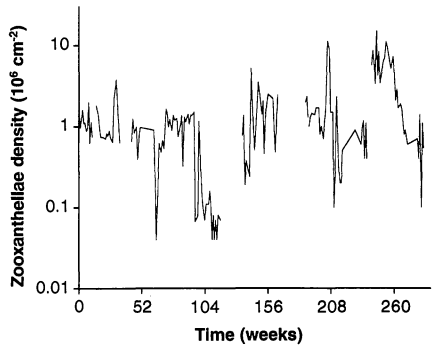

Fig. 1. Time series of zooxanthellae density from August 1991 to March 1997. The coral samples were collected on approximately a weekly basis, and density was determined by a standard methodology (*13*). At three points in the time series, there are gaps because no data were collected during these periods (for logistical reasons). The total number of data points is 159.

[1]Department of Biological Sciences, Faculty of Science, University of Mauritius, Reduit, Mauritius. [2]Department of Biology, Imperial College, Silwood Park, Ascot, Berks. SL5 7PY, UK. [3]School of Ocean Sciences, University of Wales, Bangor, Menai Bridge, Anglesey LL59 5EY, UK.

*To whom correspondence should be addressed. E-mail: m.hassell@ic.ac.uk

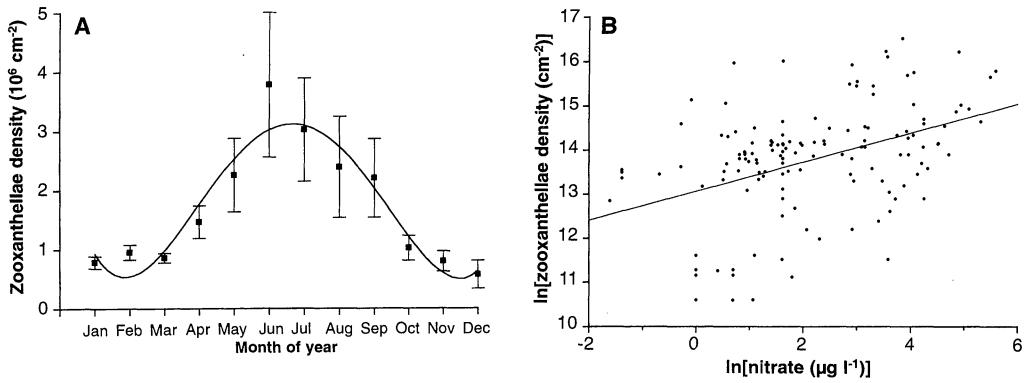

Fig. 2. (**A**) Seasonal abundance of density of zooxanthellae. For each month the mean density of zooxanthellae was calculated, and the error bars show the standard errors of the means (the number in each month varies between 7 and 19). The curve fitted is a fourth-order polynomial, the highest order polynomial that is significant. (**B**) Logarithm of zooxanthellae density versus nitrate concentration over the study period. The regression line is significant ($P < 0.0001$) and explains 16.5% of the variance ($n = 142$).

Fig. 1) and there were other episodes of very low density throughout the study period (on 11 December 1992, 2 October 1995, and 19 February 1997). Although the low density measured at these isolated times can be attributed in part to measurement error, all of these events were during spring and summer (approximately September to March). The only winter period to experience low densities was intermittently between 22 July and 16 September 1993 (weeks 96, 98, and 104 in Fig. 1), which preceded the spring-summer bleaching event in 1993.

Figure 2A shows the same data with the mean density for each month plotted. There is a strong seasonal cycle in zooxanthellae abundance: The densities in autumn and winter are three times the densities in spring and summer. This is consistent with the analysis of another long-term experimental data set from a site on the edge of the tropics (*14*) (Hawaii, 20°N). It is unknown whether such trends also occur in coral communities that are much closer to the equator and hence are exposed to less seasonal fluctuation in conditions.

Concomitant with the large fluctuations in density, there are also large fluctuations in environmental parameters over the study period (*16*). To test systematically whether there is a significant effect of the environmental parameters on the zooxanthellae density, we performed a multiple regression analysis. Table 1 shows the minimum adequate model that explains the zooxanthellae density in terms of the explanatory variables measured (time of year, temperature, dissolved oxygen concentration, and nitrate and phosphate concentrations). The full regression model accounts for 40% of the variability in density.

In the regression model, the linear and quadratic terms of time of year are both significant. The significance of the quadratic term indicates curvilinearity of the relation (Fig. 2A). Although there is a correlation between time of year and temperature (and also the amount of solar radiation as measured by the meteorological office on Mauritius), the variation in zooxanthellae density is better explained by season than by temperature (or solar radiation). This indicates that although temperature and solar radiation may be important, there may be other factors also related to season that are significant.

Over and above the effects of season, the zooxanthellae density is positively correlated with nitrate concentration (Fig. 2B) indicating nitrogen limitation. Although we do not know whether this particular coral species is able to take up nitrate, a number of experimental studies have shown that zooxanthellae in other corals do respond to elevated nitrate concentrations (*10, 17*). This is understandable because inorganic nitrogen from seawater is probably assimilated by the zooxanthellae. These field data thus support the earlier experimental work.

Although we have no definitive explanation for the negative correlation with dissolved oxygen concentration, it is possibly due to oxidative stress causing coral bleaching (*18*), freshwater runoff, or plankton blooms. However, there was no correlation (direct or delayed) of dissolved oxygen concentration with rainfall or with visible plankton blooms in the lagoon, which suggests that these are unlikely explanations.

To test the robustness of this model, we split the time series into two halves and fitted a multiple regression model to each half independently. In both halves, the minimum model accounted for more than 40% of the observed variability. Time of year (linear and quadratic terms) and nitrate concentration were significant in both halves, whereas dissolved oxygen concentration was significant in only the first half. Thus, the correlations with nitrates and season are robust, but the evidence for a negative correlation with dissolved oxygen may be weaker. The unexplained variability may be attributable to measurement error, coral growth at the tip (*19*), or sampling over the whole colony (*20*).

Clearly, shallow coastal lagoons such as this one experience large environmental fluctuations. Because the regression model using environmental variables accounted for 40% of the observed variability, it is clear that the environment was very variable and that this variability had an important influence on zooxanthellae density. The conditions in this lagoon (high degree of anthropogenic activity, large environmental fluctuations) are probably prevalent in lagoons in many areas of the world. Under such conditions, it seems

Table 1. Multiple regression model for zooxanthellae density. The linear and quadratic terms in all the explanatory variables (day of year, temperature, and concentrations of dissolved oxygen, nitrate, and phosphate) have been fitted. If the interaction terms between the variables are also fitted, qualitatively similar results are obtained, but the interpretation is more complex. Logarithmic transformations of the response and explanatory variables have been used, which ensures that all the error residuals are normally distributed. The full multiple regression model is fitted with all variables included, but variables that are not statistically significant are then removed (stepwise). The order of removal of the explanatory variables is based on their *t*-values (the ratio of the regression constant to its standard error). The variable with least significance (smallest *t*-value) is removed first. Explanatory variables that do not cause a significant increase in deviance are then left out. The resulting analysis of deviance table shows only the significant variables and is therefore the minimum adequate model. The full time series has 120 points (that is, 119 degrees of freedom). The R^2 value indicates that the full model explains 40% of the variability in zooxanthellae density (with the loss of only 4 degrees of freedom). The deviance associated with each variable is assessed by removal of that variable from the minimum model.

Source of variation (R^2 = 40.0%)	Regression constant (±SD)	Deviance	*F*	*P*
Quadratic term: time of year	−6.22(±2.95)	18.62	17.82	<0.0001
Quadratic term: dissolved oxygen	−0.30(±0.14)	18.20	17.42	<0.0001
Nitrate	0.23(±0.12)	15.85	15.17	<0.001
Time of year	5.34(±3.04)	12.89	12.33	<0.001

we should expect great variability in zooxanthellae density. Hence, bleaching events in corals within such lagoons may be frequent and part of the expected cycle of variability.

References and Notes

1. P. W. Glynn, *Coral Reefs* **12**, 1 (1993).
2. L. Muscatine and J. W. Porter, *Bioscience* **27**, 454 (1977).
3. P. W. Glynn, *Trends Ecol. Evol.* **6**, 175 (1995).
4. C. B. Cook, A. Logan, J. Ward, B. Luckhurst, C. J. Berg Jr., *Coral Reefs* **9**, 45 (1990); R. D. Gates, *ibid.* **8**, 193 (1990); T. J. Goreau and R. L. Hayes, *Ambio* **23**, 176 (1994); B. E. Brown, M. D. A. Le Tissier, R. P. Dunne, *Mar. Ecol. Prog. Ser.* **105**, 209 (1994); W. K. Fitt and M. E. Warner, *Biol. Bull.* **189**, 298 (1995).
5. M. P. Lesser, W. R. Stochaj, D. W. Tapley, J. M. Shick, *Coral Reefs* **8**, 225 (1990); D. F. Gleason and G. M. Wellington, *Nature* **365**, 836 (1993).
6. A. Kushmaro, Y. Loya, M. Fine, *Nature* **380**, 396 (1996).
7. O. Hoegh-Gulberg and G. J. Smith, *Mar. Ecol. Prog. Ser.* **57**, 173 (1989).
8. The data were collected from a lagoon near a small coastal village, Trou aux Biches, in the northwest of the island of Mauritius (latitude 20°S). The lagoonal area is about 4.1×10^6 m^2 with an average depth of 2.5 m. There is a high degree of eutrophication in the lagoon with only 20% live coral cover (80 to 90% *Acropora*, and a few occurrences of *Pocillopora* and *Porites*), algal proliferation (50% *Padina*, 10% *Sargassum*, 5% *Turbinaria*, 5% *Valonia*, 5% *Galaxaura*, and 25% other), and a considerable degree of anthropogenic activity (swimming, boating, fishing, snorkeling, water-skiing). A colony was selected that lay in about 2 m of water, depending on the state of the tide. Coral samples were collected by breaking off one live coral tip (2 to 3 cm) from a randomly selected part of the same colony each week.
9. Dissolved oxygen and surface water temperature (at depths of 0.5 to 1.0 m) were taken in situ, and nitrate and phosphate concentrations were determined from water samples in the laboratory. Zooxanthellae were extracted from live coral tips according to Drew's technique (*13*) after decalcifying in 5% HCl. Aliquots of homogeneous extracts were placed on a hemocytometer (improved Neubauer counting chamber, depth 0.1 mm), and zooxanthellae cells were counted under an inverted microscope at ×400. The aluminum foil method of Marsh [J. A. Marsh, *Ecology* **55**, 255 (1970)] was used to calculate the surface area of the coral tip from which the zooxanthellae were extracted. Data on amounts of solar radiation and rainfall in the area were obtained from the Mauritius meteorological office.
10. L. Muscatine, P. G. Falkowski, Z. Dubinsky, P. A. Cook, L. R. McCloskey, *Proc. R. Soc. London Ser. B* **236**, 311 (1989).
11. P. G. Falkowski, Z. Dubinsky, L. Muscatine, L. R. McCloskey, *Bioscience* **43**, 606 (1993).
12. To test for density dependence, we assumed that the change in density was linearly related to the present density. The following model [B. Dennis and M. L. Taper, *Ecol. Monogr.* **64**, 205 (1994)] was fitted:

$$\ln(N_t/N_{t-1}) = a + bN_{t-1} + \sigma Z_t$$

where N_t is the density of zooxanthellae at time t, a and b are constants, σ is a positive constant, and Z_t has a normal distribution with a mean of 0 and a variance of 1, so that σZ_t is the term representing density-independent factors (random shocks). The time between successive points, t and $t + 1$, is 1 week. A density-dependent model has a value for b significantly different from zero. The maximum likelihood estimates for the parameter values are $a = 0.15$, $b = -0.10$, and $\sigma^2 = 0.84$; the probability of the null model being rejected is $P = 0.016$ (as calculated by parametric bootstrapping).
13. E. A. Drew, *J. Exp. Mar. Biol. Ecol.* **9**, 71 (1972).
14. J. Stimson, *ibid.* **214**, 35 (1997).
15. L. R. McCloskey, D. S. Wethey, J. W. Porter, *Monogr. Oceanogr. Methodol.* **55**, 379 (1978).
16. The sea surface temperature shows a seasonal fluctuation, with maximum temperatures in the summer period (December to April) just exceeding 30°C. The minimum temperature was 22.8°C (August 1993) and the maximum was 30.8°C (April 1994). The concentrations of dissolved oxygen (mean = 7.8 ppm, SD = 2.6 ppm), nitrate (mean = 26.9 μg liter^{-1}, SD = 45.7 μg liter^{-1}), and phosphate (mean = 20.0 μg liter^{-1}, SD = 44.1 μg liter^{-1}) all fluctuated greatly over the study period (n = 147).
17. A. M. Szmant, L. M. Ferrer, L. M. Fitzgerald, *Mar. Biol.* **104**, 119 (1990); F. Marubini and P. S. Davies, *ibid.* **127**, 319 (1996).
18. Because dissolved oxygen in the water column is continuous with water in the coelenteron, it may lead to increased oxygen concentration within the coral. High concentrations of oxygen within the coral can precipitate bleaching [M. P. Lesser, *Coral Reefs* **16**, 187 (1997)].
19. Because the data collected were from the tip of the coral and because growth is expected to be greatest at this point [E. H. Gladfelter, *Biol. Bull. (Woods Hole)* **165**, 811 (1983); F. P. Wilkerson, D. Kobayashi, L. Muscatine, *Coral Reefs* **7**, 29 (1988)], the observed variability may be attributable in part to a combination of coral growth and zooxanthellae division to exploit the newly available space [R. J. Jones and D. Yellowlees, *Philos. Trans. R. Soc. London Ser. B* **352**, 457 (1997)].
20. Some of this variability is undoubtedly because different parts of the same colony were sampled and the orientation of the coral branch to incident light is known to affect zooxanthellae density [L. R. McCloskey and L. Muscatine, *Proc. R. Soc. London Ser. B* **222**, 215 (1984); Z. Dubinsky, P. G. Falkowski, J. W. Porter, L. Muscatine, *ibid.*, p. 203]. In addition, it is possible that different strains of zooxanthellae exist in different parts of the colony [R. Rowan, N. Knowlton, A. Baker, J. Jara, *Nature* **388**, 265 (1997)]. Thus, the data collected reflect the normal degree of zooxanthellae variability expected over an entire colony. However, this cannot be the only cause of variation, as the test for density dependence specifically examined and rejected the hypothesis that the sole cause of the variability through time is just random sampling from some distribution of zooxanthellae abundance.

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Dependence of Human Stem Cell Engraftment and Repopulation of NOD/SCID Mice on CXCR4

Amnon Peled,[1] Isabelle Petit,[1] Orit Kollet,[1] Michal Magid,[1] Tanya Ponomaryov,[1] Tamara Byk,[1] Arnon Nagler,[3] Herzl Ben-Hur,[4] Ariel Many,[5] Leonard Shultz,[6] Ofer Lider,[1] Ronen Alon,[1] Dov Zipori,[2] Tsvee Lapidot[1]*

Stem cell homing and repopulation are not well understood. The chemokine stromal cell–derived factor-1 (SDF-1) and its receptor CXCR4 were found to be critical for murine bone marrow engraftment by human severe combined immunodeficient (SCID) repopulating stem cells. Treatment of human cells with antibodies to CXCR4 prevented engraftment. In vitro CXCR4-dependent migration to SDF-1 of CD34$^+$CD38$^{-/low}$ cells correlated with in vivo engraftment and stem cell function. Stem cell factor and interleukin-6 induced CXCR4 expression on CD34$^+$ cells, which potentiated migration to SDF-1 and engraftment in primary and secondary transplanted mice. Thus, up-regulation of CXCR4 expression may be useful for improving engraftment of repopulating stem cells in clinical transplantation.

Stem cells within the bone marrow microenvironment actively maintain continuous production of all mature blood cell lineages throughout life. These rare primitive cells are functionally defined by their ability to home to the bone marrow and to durably repopulate transplanted recipients with both myeloid and lymphoid cells (*1, 2*). Several groups have established in vivo models for engrafting human stem cells (*3–8*). We developed a functional in vivo assay for primitive human SCID repopulating cells (SRCs) based on their ability to repopulate the bone marrow of intravenously transplanted SCID or nonobese diabetic SCID (NOD/SCID) mice with high levels of both myeloid and lymphoid cells (*5, 6, 8*).

Chemokines are cytokines that are best known for their ability to selectively attract subsets of leukocytes to sites of inflammation (*9*). The role that chemokines and their receptors play in homing and repopulation of human stem cells is not fully understood. The

[1]Department of Immunology and [2]Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel. [3]Hadassah University Hospital Jerusalem, 91120, Israel. [4]Kaplan Medical Center, Rehovot 76100, Israel. [5]Sourasky Medical Center, Tel Aviv 64239, Israel. [6]The Jackson Laboratory, Bar Harbor, ME 04609, USA.

*To whom correspondence should be addressed. E-mail: litsvee@weizmann.weizmann.ac.il