Coral Reefs and Carbon Dioxide

Hajime Kayanne *et al.* (*1*) investigate diurnal changes in CO_2 partial pressure (P_{CO_2}) and community metabolism on Shiraho reef (Ishigaki Island, Japan) and conclude that "reefs might serve as a sink, not a source, for atmospheric CO_2" because of high net organic production relative to net calcification (Table 1). This conclusion is at odds with recent reports (*2*, *3*, *4*) and data (*5*) suggesting that reefs are net sources of atmospheric CO_2, albeit of limited importance in the current carbon budget. Furthermore, it is well established that the value of gross primary production (P_g) is close to that of respiration (R) in most coral reef flats, and that the net production (P_n) is around zero (*6*). One would expect some reefs to depart from this general trend, but consideration of currents and community distributions suggests that the data in the report by Kayanne *et al.* (*1*) represent neither the whole Shiraho reef nor all coral reefs.

The reef flat at Shiraho comprises five different benthic communities and is under strong tidal influence (*1*, *7*). Because Kayanne *et al.* measured P_{CO_2} at a single station over several days, the water masses at hand had different origins and crossed different communities depending on the direction of the current. Therefore, the observed changes in P_{CO_2} likely did not result from the metabolic activity of the entire reef flat but, rather, from a variable combination of the communities located around the study site.

Also, Kayanne *et al.* calculated mean daytime and nighttime reef water P_{CO_2} with the use of a predictive regression equation

$$P_{CO_2} = 352 - 0.13\ (I)$$

where I is light intensity, $N = 13$, and $r^2 = 0.41$. Our computation indicates that the local oceanic P_{CO_2} (322 μatm) lies within the 95% confidence intervals for both values (daytime = 279 ± 103 μatm and nighttime = 352 ± 97 μatm), which prevents us from drawing any conclusion with regard to source versus sink.

Kayanne *et al.* measured community metabolism during slack-water periods. Kinsey (*8*, p. 439) pointed out that this technique provides metabolic parameters which are "related to a very precisely defined community because of the limited mixing which occurs with water from adjacent zones." Suzuki *et al.* (*9*) also used this technique and found $P_g/R = 1.4$ at Shiraho reef (Table 1), a value much higher than those commonly found on reef flats [$P_g/R = 1.0 \pm 0.1$, that is, $P_n \approx 0$ (*6*)]. The study site (*1*) seems to be located in an area dominated by algal turf and brown algae, which may explain the relatively high value for P_n. Nakamori *et al.* (*7*) investigated the metabolism of various communities on the same reef and estimated production and calcification for the whole system. They found P_n to be much less (×0.217) than the value reported by Kayanne *et al.* (Table 1). The localized observations of Kayanne *et al.* (*1*) confirm well-known spatial and temporal variations of metabolic processes within reef ecosystems. Calculations based on a recent model (*4*), and estimates of the larger community metabolism from Nakamori *et al.* (*7*), predict that the Shiraho reef was, at the time of measurement, a net source of CO_2 for the atmosphere (with an evasion of 12 mmol CO_2 m^{-2} d^{-1}).

Table 1. Available data on community metabolism of the Shiraho coral reef (Ishigaki Island, Japan). Gross primary production (P_g), respiration (R) and net production (P_n) in mmol CO_2 m^{-2} d^{-1}; net calcification (G) in mmol $CaCO_3$ m^{-2} d^{-1}.

Source	P_g	R	P_n	G
T. Nakamori *et al.*, 1992 (*7*)	302	278	24	60
H. Kayanne *et al.*, 1995 (*1*)	—	—	110	100
A. Suzuki *et al.*, in press (*9*)	460	320	130	160

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Kayanne *et al.* (*1*) interpret their observations as evidence that coral reefs may be net sinks for atmospheric CO_2, but specific and general evidence counter this interpretation.

Kühlmann (*2*, p. 19) described the Ishigaki Island reefs as "greatly stressed by human activity" and affected by siltation and agricultural chemicals. Anthropogenic stress increases the ratio of organic production to calcification, and the behavior of stressed or transitional reef communities is not representative of normal coral reef metabolism (*3*, *4*).

Estimates of unstressed source-sink relationships can be derived from reef sediment characteristics. A carbon sink relevant to climate would have to take up atmospheric CO_2 and sequester it from atmospheric exchange for centuries or millennia. Reef sediments provide an integrated view of the stored products of past reef metabolism. In a "pure" coral reef environment where organic-inorganic fractionation and nonreef contributions to sediments are negligible, a reef that is an atmospheric CO_2 sink would have to deposit sediments that average more than 12 weight % organic matter or more than 6 weight % organic C [if one assumes 0.6 mol of CO_2 evolved per mole of $CaCO_3$ precipitated (*5*); one mole of CO_2 consumed per mole of CH_2O photosynthesized; and organic matter dry weight ≈50% organic C]. However, organic C typically constitutes less than 1% of the dry weight of carbonate-dominated reef sediments (*6*). Biogenic carbonate skeletons normally contain only a few percent organic matter by weight (*7*).

Reef sediments thus fall far short of the composition required for a CO_2 sink, which indicates that normal reef metabolism has probably been a net CO_2 source during the recent geologic past. A reservoir of high-organic reef sediments that contained enough organic C to transform the system into a sink would have to be unrealistically rich in organic material in order to make up for the low organic C content of most sediment. Export of dissolved as opposed to particulate organic C (DOC/POC) from reef to ocean or export by downslope sediment transport would not provide a credible mechanism for differential sequestration of organic C that could account for the discrepancy.

Loss of coral reef habitat is often associated with transitions to benthic communities dominated by noncalcareous benthos (*4*, *8*). The results of Kayanne *et al.* (*1*) reveal metabolic measures to be sensitive indicators of reef health (*9*), but the findings more likely reflect the condition of the local sampling site than the normal behavior of healthy reef communities.

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Response: We are grateful for the comments we have received about our report (*1*). They bring up points that we did not fully discuss in the report.

Gattuso *et al.* stressed that our measured values of productions are remote from the common reef production rate. His reference of "standard performance" of $P_g/R = 1.0 \pm 0.1$ (*2*). The standard performance of coral reefs are a first-order estimation; it is not a precise indicator with regard to the CO_2 sink versus source problem. The threshold between CO_2 sink or source is very sensitive to precise organic and calcium carbonate production rates, and thus direct measurement of P_{CO_2} changes provides better information. We should obtain more direct measurements of P_{CO_2} changes to better address the reef sink versus source problem. Moreover, the idea that P_g/R is close to 1 is partly based on the fact that the tropical ocean is depleted in nutrients for supporting net organic production. However, as we stated in our report, recent studies have revealed the importance of nitrogen fixation in coral reefs, which provides new nutrients to coral reefs.

As we stated in our report, different reef zones might act differently as to CO_2 fluxes. The whole-reef production values in the review by Kinsey (*3*) were mainly estimated from lagoon water chemistry. In our report, we presented an example that shows how a reef flat might act as a sink of CO_2, and suggested that reefs, in general, may not be sources of CO_2.

On the basis of our earlier investigations, together with those by many other reef scientists on community structure of corals and other benthic organisms, we chose Shiraho reef to represent the common features of fringing coral reef. If one considers the pattern of community distribution and flow regime on Shiraho reef, then it is clear that our measurement of P_{CO_2} changes shows average reef metabolism on this reef.

Buddemeier questions the health of our study site by referring to Kühlmann's review of Ishigaki Island reefs (*4*). Kühlmann surveyed Ishigaki Island as a whole, but focused on the worst part of it in this review (*4*). In his original paper (*5*), however, he showed stepped stages of human impact and found that living coral coverage of our study site [north of Shiraho in table 1 in (*5*)] was 70%, which represents the highest value of all the sites he investigated. He classified Shiraho reef as one of the "reefs with no signs of deterioration." Planck *et al.* investigated our study site thoroughly and concluded that the Shiraho coral reef ecosystem is "world-class with respect to its diversity of corals and fishes" (*6*). Veron identified 359 species in Ishigaki and nearby islands (Yaeyama Islands), almost the same number as that found in the Philippine Islands. He furthermore ranked North Shiraho reef as preserving a high diversity of corals (*7*).

With regard to nutrient input which may alter reef community structure, we have measured inorganic nitrogen concentrations on the reef flat water of Shiraho reef (*8*). The concentrations of nitrate, nitrite, and ammonium were less than 0.4, 0.08, and 0.45 μM, respectively, which contradicts Buddemeier's statement that Shiraho reef might be strongly eutrophicated. On the basis of these studies, we think that Shiraho reef shows the typical community structure of healthy fringing coral reefs.

The other point Buddemeier raises is that the small amount of organic material preserved in reef sediments refutes the idea that reefs might act as a CO_2 sink. However, the fate of organic products in coral reefs is not only sediment burial, but also transport to the outer ocean and biomass increase. We have measured a positive net primary production on the reef flat, which includes consumption and respiration. We think that most of the net organic products are washed out into the outer ocean. However, the fate of organic materials in the ocean is beyond the scope of our report. Its purpose was to point out that the reef flat at Shiraho—the most productive zone in reefs—acts as a sink of CO_2. However, we cannot comment on the statement "export of DOC/POC from reef to ocean or export by downslope sediment transport would not provide a credible mechanism for defferential sequestration" without conducting actual measurement of these fluxes.

Gattuso *et al.* also question the representativeness of our study site. We agree that our report did not provide adequate explanation of which part of the reef community metabolism our measurements represented in relation to the flow regime. During high tides, our current measurements showed that water comes steadily from north-northeast with a current speed over 5 cm s^{-1} [figure 2 in our report (*1*)]. This water comes from the outer ocean over the reef crest north of the study site and runs over turf algae and living coral communities [figure 1B in (*1*)]. During low tide stagnant periods, we observed a slow current with an average of 3 to 4 cm s^{-1}, which mixed well the water around the study site surrounded by living coral patches. Therefore, our measurements likely show the metabolism of corals and algae on the reef flat at Shiraho. We are carrying out further measurements of currents at other points to clarify the flow regime.

Gattuso *et al.* also point out that errors of the mean daytime and nighttime P_{CO_2} values overlapped with the offshore value. The purpose of this estimation was to convert the visually conspicuous relations of P_{CO_2} and light intensity into a quantified discussion, but the data were too small to make a statistically rigorous conclusion; we agree that we should obtain more data in the future. However, we would like to point out that the light intensity in March, when we made the observations and estimations, is relatively low compared to other months in this island (mean light intensities are 552 and 864 μmol m^{-2} s^{-1} in March and in August, respectively). Productions depend primarily on light intensity, and we have obtained higher productions in August.

Gattuso *et al.* point out the discrepancy between our estimates of production and those of Nakamori *et al.* (*9*). Their estimates of organic production and calcium carbonate production, however, depend only on one daytime (4-hour) set and one nighttime (3.5-hour) set of measurements of pH and alkalinity changes. From these data, they estimated the whole day net production on the basis of the tentative assumption that the daytime length is 7.5 hours. After the publication of their study, we have accumulated numerous measurements on organic production and calcium carbonate production of Shiraho reef and have related them with actual change in light intensity. In addition to the conventional pH-alkalinity method to estimate the productions, we computed them from more measured alkalinity and P_{CO_2} changes, six times through P_{CO_2} observation.

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Self-Fertilization, Linkage Disequilibrium, and Strain in *Plasmodium falciparum*

We are impressed by the elegant data presented by R. E. L. Paul *et al.* (*1*) on the high rate of self-fertilization in *Plasmodium falciparum*, the agent of the most malignant form of malaria. This study contributes significantly to our knowledge on this pathogen's basic biology. Nevertheless, we find that there is a major logical gap in the conclusions arrived at by Paul *et al.* They appropriately argue that frequent selfing in *P. falciparum* is a medically relevant feature, for it should favor the maintenance of "multi-locus phenotype associations," in particular those governing virulence, drug resistance, or variant surface antigen polymorphism. This is quite logical: Self-fertilization, by inhibiting genetic recombination, leads to a situation of actual clonality (*2*) (offspring genotypes that are identical to the parental cells), which should help stabilize those multi-locus associations that are elsewhere favored by natural selection. Then they state that "there was sufficient outbreeding to disrupt any linkage disequilibria" (linkage disequilibrium is the nonrandom association between genotypes scored at different loci). These two proposals taken separately are conceivable, but they are incompatible to each other.

If self-fertilization, as evidenced by studying the three loci *MSP-2*, *MSP-1*, and *GLURP*, was unable to maintain any multi-locus association between these loci (as shown by lack of linkage disequilibrium at the three loci), it is not tenable that it could significantly help in stabilizing any other multi-locus combination. Two possibilities can be entertained. First, selfing can play in itself a significant role in maintaining multi-locus association, and this should be observed with the *MSP-2*, *MSP-1*, and *GLURP* loci. Second, the natural selection has the dominant role in stabilizing those multi-locus phenotypes associated with virulence, drug resistance, or "immunologically sensitive" variant antigens (a statement that is consistent with the observation of linkage equilibrium at other loci). In the latter case of variants mainly maintained by natural selection, the role of self-fertilization would appear consequently limited.

Another concern in the approach used by Paul *et al.* lies in the difficulty of evidencing any linkage disequilibrium with their data. Each of the three loci under study exhibits considerable allelic variation. The expected frequency of each possible multi-locus combination is therefore low, which proportionally lowers any possibility of evidencing linkage, even with exact statistical tests. This situation leads to a large type II error risk (to see no significant linkage while linkage does exist). If a conservative model is taken, in which five equiprobable alleles (much less than actually recorded in these data) are segregating at each locus, the probability of any multi-locus combination does not exceed $0.2^3 = 0.008$. This renders difficult to evidence any significant linkage, unless considerable sample sizes are used, which is not the case in this study.

Although the discovery of high-rate self-fertilization in *P. falciparum* is a major breakthrough in our knowledge of the agent of malaria, its actual impact on this parasite's population structure in humans still has to be clarified by classical population genetic means that depend on linkage disequilibrium analysis. The notion of strain in microbiology relies on the existence of stable multi-locus associations (especially, of course, those combinations dealing with medically relevant characters), and if no such multi-locus associations are found in *P. falciparum*, the notion of strain has to be held in abeyance for this parasite. Should this be verified, any efforts for individualizing multi-locus genotype (that is, strain characterization) in *P. falciparum* may not be successful, for these genotypes will appear as most unstable. The only approach that remains possible in this case is the typing of individual genes.

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Response: Tibayrenc and Lal highlight an important issue in genetic analyses of organisms in which sex is obligatory, especially with respect to species of medical importance such as *P. falciparum*. As stated by us and by Tibayrenc and Lal, a high degree of self-fertilization will have important medical consequences by favoring the maintenance of multi-locus phenotypes such as virulence and drug resistance. Assessing the mating structure of populations can be achieved indirectly by measurements of association between loci (linkage disequilibrium) and directly by measurements of heterozygosity. Our direct measurement of the degree of heterozygosity in the oocyst parasite population found that the mating structure was typified by a high degree of inbreeding which was in contrast to that previously found in a region of more intense malarial transmission, Tanzania (*1*). While such a high degree of inbreeding would be expected to result in linkage disequilibrium, in this study (*2*) we found no evidence for linkage, even when using sequence data only (GENEPOP Fisher exact, $P > 0.1$) (*3*). However, linkage analysis may produce misleading results (*4*) and as indeed pointed out by Tibayrenc and Lal, large sample sizes are required to detect linkage (*5*). In this study, linkage analysis was performed for a comparison with the heterozygosity data as malariologists had previously accepted the absence of linkage disequilibrium as evidence for a panmictic population structure (*6*). Our study highlighted the relative insensitivity of linkage analysis in assessing the extent of inbreeding.

A third point raised emphasizes the need to use selectively neutral loci to establish such mating patterns. In our report we used three loci, two of which were parasite surface antigens. The fact that all three loci produced the same inbreeding coefficient would suggest that the result found is real, although there is some evidence that regions of the merozoite surface proteins, other than those amplified, may be under selection (*7*).

The comments made by Tibayrenc and