



W&M ScholarWorks

VIMS Articles

3-1975

Enewetak (Eniwetok) Atoll- Aspects of Nitrogen Cycle on a Coral Reef

K. L. Webb

Virginia Institute of Marine Science

William D. DuPaul

Virginia Institute of Marine Science

W. Wiebe

W. Sottile

R. E. Johannes

Follow this and additional works at: <https://scholarworks.wm.edu/vimsarticles>

 Part of the [Environmental Sciences Commons](#)

Recommended Citation

Webb, K. L.; DuPaul, William D.; Wiebe, W.; Sottile, W.; and Johannes, R. E., "Enewetak (Eniwetok) Atoll- Aspects of Nitrogen Cycle on a Coral Reef" (1975). *VIMS Articles*. 690.

<https://scholarworks.wm.edu/vimsarticles/690>

This Article is brought to you for free and open access by W&M ScholarWorks. It has been accepted for inclusion in VIMS Articles by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.

Enewetak (Eniwetok) Atoll: Aspects of the nitrogen cycle on a coral reef¹

K. L. Webb and W. D. DuPaul

Virginia Institute of Marine Science, Gloucester Point 23062

W. Wiebe and W. Sottile

Department of Microbiology, University of Georgia, Athens 30602

R. E. Johannes

Department of Zoology, University of Georgia

Abstract

Changes in the concentrations of dissolved and particulate nitrogen in seawater crossing two interisland reef transects on Enewetak Atoll were measured. The upstream half of transect II consisted largely of algal pavement; the downstream half was visually dominated by corals. The other community, transect III, consisted of benthic algal covered pavement in its entirety, with only scattered small corals downstream. Both transects showed a significant net export of combined nitrogen, implying a large input of nitrogen into the reef system from a source other than the combined nitrogen in overlying waters. High rates of gaseous nitrogen fixation were found.

There was a net uptake of nitrate by the community dominated by algae and a net export of nitrate from the transect dominated jointly by algae and corals. Although nitrification is of central importance in the marine nitrogen cycle, this coral-algal community at Enewetak appears to have been the first marine site at which this process has been measured directly.

Nitrogen is believed to be the element that most often limits primary production in characteristically nutrient-poor tropical Pacific water (e.g. Thomas 1969, 1970a,b; Thomas and Owen 1971). Coral reef communities bathed by these waters are nevertheless among the most productive natural communities known. Whereas daily gross photosynthetic carbon fixation is of the order of 0.06 to 0.5 g C m⁻² day⁻¹ in the adjacent open-ocean plankton community (c.g. Helfrich and Townsley 1963), reef communities fix from 4 to 12 g C m⁻² day⁻¹ in much shallower water columns (e.g. Smith and Marsh 1973). The ultimate source of essential elements for photosynthesis in both

communities is presumably the same—the impoverished ocean water. The paradox of high productivity in the face of low nitrogen levels is the subject of this paper. Many of the data were collected during the RV *Alpha Helix* "Symbios" Expedition to the Marshall Islands, where about 25 scientists collaborated to study the productivity and flux of nutrients in several coral reef communities

Shallow windward interisland reef communities which are situated in a unidirectional flow of water are amenable to study as single metabolic units (e.g. Odum and Odum 1955; Johannes et al. 1972). We repeatedly measured the changes in levels of various components of the nitrogen pool in the water before, while, and after it crossed two such reef communities. This enabled us to calculate net inputs and outputs of these components and to establish nitrogen budgets for the communities. In so doing, we discovered one mechanism by which this coral reef maintains its high biological productivity in the presence of low levels of dissolved combined nitrogen.

¹Contribution No. 641 from the Virginia Institute of Marine Science. Contribution of the Mid-Pacific Marine Laboratory (formerly Eniwetok Marine Biological Laboratory). This work was supported in part by National Science Foundation grant GB 24816 to the Scripps Institution of Oceanography for the operation of the *Alpha Helix* research program, by Atomic Energy Commission contract (20-2)-226, by the Janss Foundation, and by National Science Foundation grants GA 35866 and GA 35806.

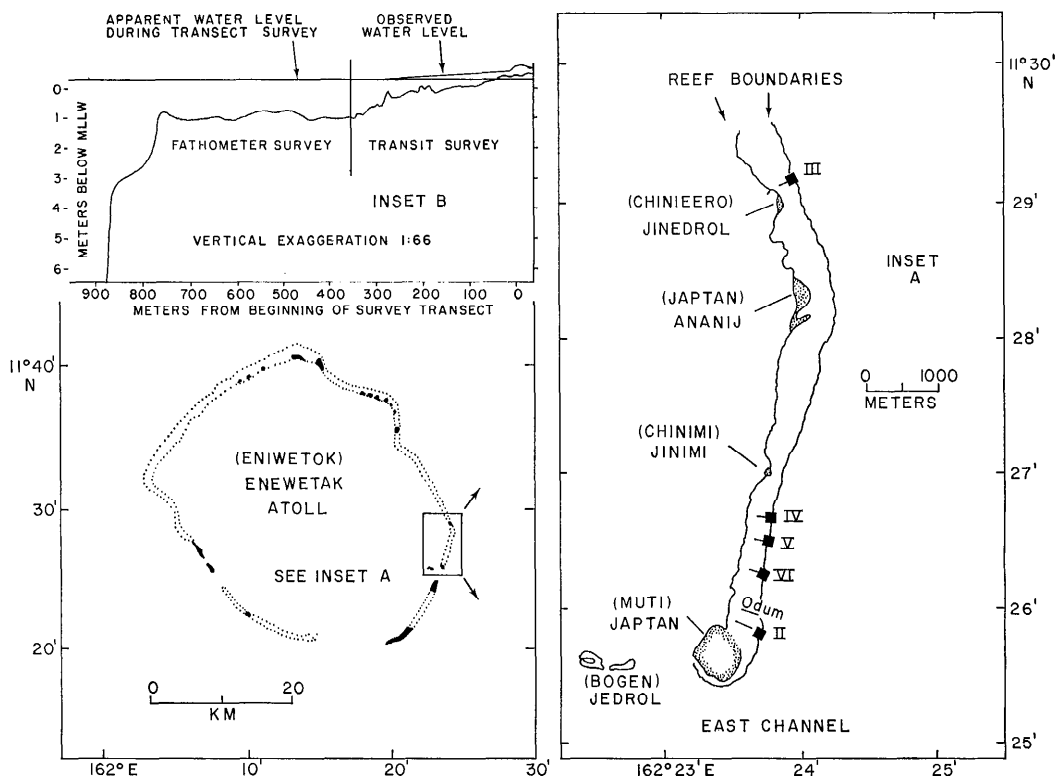


Fig. 1. Index map of Enewetak Atoll. Inset A shows locations of transects (—■) sampled as well as the Odum and Odum (1955) transect. For convenience, both naming schemes are shown. (See text for explanation.) Inset B shows a cross section of the atoll from the 0-m station on tr II to the lagoon. Note that the water breaks over the algal ridge crest and runs downhill across the transect.

The work reported here took place at what has been known as Eniwetok Atoll in the Marshall Islands. There is a certain amount of difficulty associated with determining place names for the islands comprising the atoll, and the atoll itself, which lies at about 11°30'N and 162°15'E. The Marshallese prefer the English spelling, Enewetak, rather than the German spelling, Eniwetok. Individual islands have various names resulting from the presence of Marshallese, Germans, Japanese, and the U.S. military authorities. The names used in previous papers from the Symbios Expedition were the "official names" approved by the Pacific Science Information Center for the Trust Territory of the Pacific in 1971.

According to a report by J. A. Tobin, (Community Development Advisor, Ma-

juro, Marshall Islands) Muti Island (Fig. 1) is more appropriately called Japtan, Chinimi is more appropriately spelled Jinimi, the island labeled Japtan on the map (Fig. 1) becomes Ananij, and transect III (tr III) is just north of Jinedrol rather than Chinieero. The island to the west of the deep channel is Jedrol rather than Bogen. In deference to the Enjebi and Enewetak People, we will henceforth use the names they prefer.

We thank our colleagues of the Symbios Expedition for their ideas and physical assistance. Special thanks are extended to S. V. Smith for reviewing the manuscript, to T. Tsuda for identifying algae, to E. L. Schmidt for examining slides by immunofluorescence, and to P. Helfrich for his extensive help in logistics.

Methods

Locations of transects (tr) are shown in Fig. 1, inset A. The principal sampling area for water chemistry, tr II, was adjacent to the Odum and Odum (1955) transect. Tr II was 340 m long; stations are identified according to the number of meters downstream from the 0-m station. The 0-m station was about 40 m downstream from the crest of the algal ridge where the water consistently flowed lagoonward (*see Fig. 1, inset B, transect survey*). Tr II contained the same sequence of biotic zones as described by Odum and Odum (1955), including an upstream region dominated by benthic algal and containing few corals and a downstream region dominated visually by corals. Another transect (tr III) was established about 6 km north near the island of Jinedrol. This transect in its entirety resembled the upstream portions of tr II in the near absence of coral and the visual dominance of benthic algal. Smith (1973) has provided a more extensive description of these transects. Twenty-six samplings, which extended collectively over all hours of day and night, were carried out on tr II between 9 May and 24 June 1971. Twenty-one of these samples were taken at relatively low tides (volume transports of 0.036 – $0.23 \text{ m}^3 \text{ sec}^{-1} \text{ m}^{-1}$ of reef front) in an effort to maximize concentration changes of measured constituents in the water crossing the reef and to minimize the physical difficulty of taking the samples. The five remaining samples represent volume transports of between 0.50 and $0.88 \text{ m}^3 \text{ sec}^{-1} \text{ m}^{-1}$ and were taken at mid- to high tide. Additional samples were taken from the open ocean in front of tr II, from the lagoon behind tr II, on several transects lateral to tr II (tr IV, V, VI), and from within coral heads and tide pools.

Samples were taken in 4-liter glass bottles. Tr II was sampled by two sampling teams. One team waded across the reef flat from Japtan Island to the 100-m station on the permanent transect line. There they measured water depth and current velocity. This group then proceeded to the 0-m station and collected water samples. Occa-

sionally they also sampled the water at the 150-m station at the beginning of the zone of small coral heads (Odum and Odum 1955). A second team sampled the downstream water from a small skiff at a 340-m station, the end of the zone of large coral heads. This team sampled the water just beneath the surface and just above the bottom. Suitable precautions were taken with sampling and timing between stations to avoid contamination of the water by the samplers. All mid- to high-tide sampling was done from skiffs.

Flux rates were calculated using the difference in concentration of a constituent X between two stations of a transect ($\Delta X \text{ m}^{-3}$), the volume transport past a meter of reef front normal to the direction of flow ($\text{m}^3 \text{ sec}^{-1} \text{ m}^{-1}$) and the length of the transect (m). The following formula was used:

$$\begin{aligned} \text{Flux of } X \text{ sec}^{-1}\text{m}^{-2} \\ = \Delta X \text{ m}^{-3}(\text{m}^3 \text{ sec}^{-1}\text{m}^{-1})\text{m}^{-1}. \end{aligned}$$

To monitor the import or export of macroscopic particulate material and plankton by the coral community, we anchored plankton collectors at two stations 200 m apart on tr II. One collector was situated just upstream of that position on tr II visually dominated by corals, the other just downstream of it. Each plankton collector consisted of three plankton nets of progressively smaller mesh size interlocked in tandem on a single frame; it retained particles larger than about $60 \mu\text{m}$. The nets were placed with the mouth openings facing upstream and were left in place for 12-hr periods. Continuous records of current velocity and water depth during the sampling periods (carried out by R. Clutter and J. Maragos), plus measurements of net filtration efficiency made with a plankton meter, enabled us to construct a balance sheet for nitrogen contained in drifting particulate macroscopic materials entering and leaving the coral community. Some results of the net plankton work have been described by Johannes and Gerber (in press).

Nitrate was analyzed by the method of Wood *et al.* (1967), ammonia by the method of Solórzano (1969). About 4 liters of water were filtered through a Flotronics silver

membrane (pore size $0.45 \mu\text{m}$). Total suspended particulate organic nitrogen and carbon were determined by combusting the silver filters in a F & M model 185 CHN analyzer using the two-temperature method to avoid carbonate interference (Telck and Marshall 1974). The material obtained with the plankton samplers was dried, ground, and thoroughly mixed before aliquots were analyzed on the CHN analyzer. Dissolved organic nitrogen was determined on 25 ml of filtrate by Kjeldahl digestion, as described by Strickland and Parsons (1968) with subsequent ammonia determination by the method of Solórzano (1969). The Kjeldahl digest was neutralized with NaOH and diluted to 50 ml at a pH of 8–8.5. The precipitate which developed on pH adjustment was removed by centrifugation after color development. Low ammonia water was obtained by passing deionized water through a cation exchange column.

Nitrogenase activity of samples of various benthic algal communities was estimated by the acetylene reduction method (Stewart et al. 1967). Substrate scrapings or chips approximating 2 cm^2 were returned to the RV *Alpha Helix* and immediately placed in 25-ml Erlenmeyer flasks with 5 ml of seawater. Flasks were sealed with serum vial stoppers, and for experiments under helium the gas space was flushed with helium using two syringe needles through the stopper. Flasks were then injected with 3 ml of C_2H_2 and 3 ml of the atmosphere was withdrawn to equalize the pressure. Incubations were carried out for 0.5 to 2 hr in duplicate under air and under helium in ambient light in a flowing-water clear aquarium on the deck of the ship. Controls were negative for the production of C_2H_4 in the absence of C_2H_2 . Duplicate Formalin-killed samples were also incubated for 2 hr under both air and helium. The gas phase was sampled at the end of the incubation in a Vacutainer. Gas samples were returned to the home laboratory and analyzed by gas chromatography for ethylene. After Project Symbios, we used a hammer and chisel to remove blocks of

algal pavement of which $10\text{--}100 \text{ cm}^2$ was algal covered. Surface area was estimated with aluminum foil of known weight per unit area; foil was cut and folded to fit the algal covered surface and then weighed. Experimental material from the reef was also selected to fit the experimental containers without modification; containers consisted of widemouth clear glass jars, generally of either 250- or 500-ml total volume, the lid of which had been modified to accept a serum vial stopper. Experiments were set up so that about 50% of the jar was gas space and the remainder occupied by the experimental material covered with freshly collected low ammonia seawater.

Experimental incubations generally lasted for from 2 to 4 hr after the injection of C_2H_2 , equivalent to 15% of the gas phase volume. Light intensities ranged from that of in situ incubations at midday on the reef flat to dark experiments started at the end of the natural dark period and incubated in the lab. Gas samples of 100 to $150 \mu\text{l}$ were analyzed immediately after sampling, using a gas chromatograph equipped with a flame ionization detector with a 2.7-m stainless steel column packed with Poropak R operated at 53°C . Samples of the gas phase were analyzed at least six times during the experiment; after a variable lag time, C_2H_2 reduction rates were linear. Rates were calculated by regression analysis using at least four data points from the linear portion, criteria for linearity being $r \geq 0.90$ and $p \leq 0.10$. About 4% of the rate data did not meet these criteria and were rejected; most regressions gave $r > 0.99$ and $p < 0.001$. Amounts of C_2H_4 were calculated from a calibration curve determined with pure ethylene. Small variations in pipetting the gas samples were normalized by using the trace CH_4 in the C_2H_2 as an internal standard. A C_2H_4 to N molar conversion factor of 1.5 was used (Hardy et al. 1968).

Results

Concentrations of nitrogen species measured from major transect stations are reported in Table 1. All components in-

Table 1. Ammonia (NH_4^+), nitrate (NO_3^-), dissolved organic nitrogen (DON), and particulate organic nitrogen (PON) along transects at Enewetak Atoll. The values given under each heading are mean nanomoles per liter, standard error, and number of observations.

	NH_4^+	NO_3^-	DON	PON
Transect II				
Offshore	300±50(15)	20±7(15)*	3000±400(6)†	100±14(14)†
0 m	240±24(26)	100±9(27)	1800±130(25)	160±13(22)
340 m surface	280±23(26)‡	170±19(27)‡	2000±140(25)§	190±17(22)¶
340 m bottom	290±26(21)§	170±18(27)‡	2300±250(20)§	230±29(13)§
Lagoon	280±27(14)	70±11(14)*	2300±230(14)†	250±54(6)†
Transect III				
0 m	200±40(10)	300±40(8)	1700±220(12)	160±63(6)
470 m	200±40(11)	80±12(10)‡	2300±230(11)¶	180±72(6)

*Significantly different from the 0-m mean $P < 0.01$.

†Significantly different from the 0-m mean $P < 0.05$.

‡Significantly different from the 0-m mean $P < 0.01$.

§Significantly different from the 0-m mean $P < 0.05$.

¶Significantly different from the 0-m mean $P < 0.10$.

t-test for paired comparisons.

t-test for paired comparisons.

t-test for paired comparisons.

creased significantly in concentration as the water passed from the 0- to the 340-m station of tr II. Data were evaluated by a paired value *t*-test. Dissolved organic nitrogen (DON) values are about an order of magnitude greater than the particulate organic nitrogen (PON) values. Offshore and lagoon values are included but have little bearing on our subsequent discussion. The DON values are generally at the low

end of the range reported for the Pacific; Williams (1967) for example reported a range of 2.5–12.5 μM DON, and Thomas et al. (1971) 3.4–13.8 μM DON with higher concentrations of DON associated with lower values of NO_3^- . Data from tr III are similar to those from tr II, except that NO_3^- concentrations decreased significantly in water crossing this reef, while NH_4^+ concentrations did not change significantly.

Table 2. Net rates of export (+ value) or import (– value) of nitrogenous substrates, ammonia (NH_4^+), nitrate (NO_3^-), dissolved organic nitrogen (DON), particulate organic nitrogen (PON), and total combined nitrogen on transects at Enewetak Atoll. Values are mean nanomoles per square meter per second, standard error, and number of observations.

	NH_4^+	NO_3^-	DON	PON	Total
Transect II, 0–340 m					
0000–1200 hours	6±4.6(12)*	20±4.6(13)	5.5±48.8(12)*	13.6±12.4(9)	24.4±79.2(8)†
1200–2400 hours	50±22(14)	16±7.9(14)	155±77.7(13)	45.9±20.7(11)	388±103(10)
24-hr mean	30±13(26)	18±4.6(27)	83.5±48.3(25)	31.4±12.9(20)	226±76.3(18)
0–150 m	50±26(5)	–28±12.2(5)			
150–340 m	60±53(5)	60±17.5(5)			
Transect III, 0–470 m					
0000–1200 hours	5.4±4.7(5)‡	52±21.9(5)	18±75(5)	15.4±20.4(5)	95±64.4(5)
1200–2400 hours	–12.0±6.3(7)	–104±28.7(7)	248±131(7)	3.8±5.0(4)	64±80.6(4)
24-hr mean	–4.8±4.6(12)	–82±19.0(12)	152±83(12)	10.3±11.1(9)	81±44.7(9)

*Significantly different from the 1200–2400 hours value, $P < 0.10$.

†Significantly different from the 1200–2400 hours value, $P < 0.02$.

‡Significantly different from the 1200–2400 hours value, $P < 0.05$.

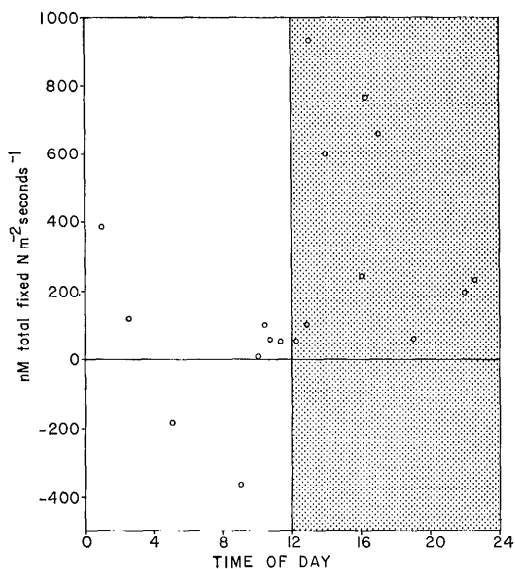


Fig. 2. Rate of flux for total combined nitrogen species vs. time of day. Data for tr II. Mean for shaded area significantly higher than for nonshaded area, $P < 0.02$.

To investigate the lateral variability of the water chemistry on the interisland reef between Japtan and Jinimi runs of tr IV, V, and VI were occasionally paired with tr II sampling. Locations are shown in Fig. 1, inset A. Nitrogen concentrations at 0 m and flux rates of components were virtually identical for the paired transects.

Flux rates of the nitrogen species for tr II and III are reported in Table 2. The mean total fluxes are the means of the totals for individual runs and not the sum of the means for each chemical species. The total export of combined N on tr II related to time of day is shown in Fig. 2. Rates appear higher in the afternoon than in the morning. To look at diel variations, data were grouped by 12-hr periods. Significant differences were not detected when fluxes between 0700 and 1900 hours (light period) were compared to the data from 1900 to 0700 hours (dark period). The 12-hr groupings chosen for inclusion in Table 2 are for midnight to noon and noon to midnight. Data thus analyzed showed significantly greater export of NH_4^+ , DON, and total nitrogen during the noon to midnight pe-

Table 3. C:N atomic ratios of particulate organic matter (POM) across reef transect tr II at Enewetak Atoll. Values are atomic ratio, standard error, and number of observations.

Station	C:N ratio
-3 to -5 km (Offshore)	14.9 ± 1.5 (4)
0 m	8.0 ± 0.62 (28)
340 m	7.0 ± 0.45 (28)
Lagoon, 900 m	6.6 ± 0.61 (16)
Midlagoon	3.6 ± 0.17 (3)

riod for tr II. The only significant diel difference on tr III was for NH_4^+ , which showed uptake from noon to midnight.

It has been assumed that the first 150 m on tr II were similar metabolically to tr III since both are dominated by similar benthic algae (Smith and Marsh 1973). On five occasions we measured NH_4^+ and NO_3^- flux separately for two portions of tr II, the benthic algal dominated zone (0-150 m) and the coral zone (150-340 m); the data are included in Table 2. Although NH_4^+ flux is similar for the two portions of tr II, there was a net removal of NO_3^- over the algal portion of tr II similar to that on tr III, but a net release of NO_3^- over the zone dominated by corals.

Atomic ratios of particulate C:N decreased progressively as water from offshore crossed the reef and entered the lagoon (Table 3). Results of upstream and downstream net plankton sampling on tr II indicated a major net import of organic nitrogen, mainly in the form of benthic algal fragments, into the coral dominated portion of the transect (Table 4). The mean input was $38.4 \text{ nM N m}^{-2} \text{ sec}^{-1}$ ($46.8 \text{ mg N m}^{-2} \text{ day}^{-1}$), which is about the rate of export of total particulate N for the entire transect.

Water from selected locations showed elevated concentrations of some nitrogenous constituents. Concentrations from inside coral pinnacles ranged upward to 400 nM NO_3^- and 800 nM NH_4^+ . Concentrations in tide pools on the south side of Japtan reached as high as $1,000 \text{ nM}$ for NO_3^- and NH_4^+ and $7,000 \text{ nM}$ for DON before

Table 4. Amount of nitrogen in the form of organic particles larger than 60 μm removed from the water flowing across the coral community of tr II. Sampling times for daytime values were between 0700 and 1900 hours local apparent noon, days mainland USA dates, values are in nanomoles nitrogen per square meter per second; night values are from 1900 to 0700 hours. Mean = $38.4 \text{ nM m}^{-2} \text{ sec}^{-1}$ ($46.8 \text{ mg N m}^{-2} \text{ day}^{-1}$).

Sample	N removed
23 June 1971, day	33.7
23,24 June, night	74.3
26,27 June, night	15.6
27,28 June, night	29.6

being flooded by rising tides. Similar concentrations were reached in the water first flooding over the adjacent algal platform.

Initially we had no idea which microorganisms were responsible for nitrogen fixation. Thus, we surveyed the fixation capability of the major visually recognizable communities. Many showed some nitrogen fixation. However, there was wide sample-to-sample variation (coefficient of variation ca. 100%), suggesting a discontinuous distribution of the nitrogen fixing organisms within the macrocommunities. The blue-green alga *Calothrix crustacea* (identified by R. Tsuda) seemed to be the major nitrogen fixing organism. Heterotrophic bacteria did not appear important, since fixation was strongly light dependent. Samples purged with helium to remove N_2 gave equivalent results to those incubated in air. The only other nitrogen fixing organisms identified were two blue-green algae, *Hormothamnion enteromorphoides* and *Rivularia* sp. (identified by R. Tsuda). The abundance of *H. enteromorphoides* was limited to localized areas of island reef flats and the nearshore lee of islands; it was not found on the transects. *Rivularia* sp. was found in the high tide and splash zone near islands and was not observed on any of the transects.

Our rate data became more precise as our techniques improved. The post-Symbios data will be reported more fully elsewhere but ranged from 0–390 nM N m^{-2}

sec^{-1} (mean of about 100) on and near the Enewetak interisland reef. Dark rates were 5–10% of the light rates.

Some unlikely reef communities exhibited high fixation rates, particularly the green alga *Dictyosphaeria cavernosa*. The fixation was due to epiphytic *C. crustacea*; when the algae were mechanically cleaned of epiphytes, nitrogen fixation ceased.

Discussion

Redfield et al. (1963) stated that marine organisms accumulate nitrogen and phosphorus in an atomic ratio of about 16 : 1 when supplies of both nutrients are adequate. Our observations, with those made simultaneously on phosphorus flux across tr II by Pilson and Betzer (1973), indicate that the atomic ratio of dissolved combined inorganic nitrogen to dissolved inorganic phosphorus in incoming water was about 2 : 1—far below the “Redfield ratio.” These data support the suggestion of Odum and Odum (1955) that nitrogen is less abundant than phosphorus, relative to community nutrient requirements, in waters upstream of the windward reefs at Enewetak. The low absolute levels of combined N upstream of transect II are also suggestive of nitrogen deficiency. It might therefore be anticipated that particulate organic matter exported by the reef community would be relatively low in nitrogen, reflecting the nitrogen levels in the overlying water from which the community obtains its nutrients. Such, however, was not the case. Whereas the particulate organic C : N ratio in oceanic waters upstream of tr II was about 15 : 1—a value characteristic for particulate matter from nitrogen deficient waters—this ratio decreased to a mean of 6.6 : 1 as the water shoaled and passed over the reef community into the lagoon. Concomitantly, there was an increase in particulate organic carbon as the water crossed the reef (Johannes et al. 1972) but at a rate proportionally lower than that of nitrogen.

It is unlikely that the net export of combined nitrogen observed is simply indicative of decreasing biomass of the commu-

nity. Using Odum and Odum's (1955) estimate of transect biomass of about 850 g m^{-2} and assuming nitrogen content to be between 2 and 6%, it can be estimated that the entire community would vanish within 2–6 months if net nitrogen export were due to decline in biomass. Clearly, some avenue of entry of nitrogen into the community not accounted for in our flow study calculations must have existed to balance the observed nitrogen export.

At Enewetak, there is a net input of nutrients into some coral communities in the form of excreta voided by fishes seeking shelter after feeding in adjacent algal communities (Chartock 1972). However, night and day observations on tr II indicated that no lateral or upstream or downstream feeding migrations of fishes occurred of a magnitude sufficient to account for a significant portion of the nitrogen required to balance the nitrogen budget.

Deep oceanic water usually contains high levels of regenerated nitrogen. Concentrations of nitrate are generally about 40,000 nM below 1,000 m in the tropical Pacific (e.g. Gundersen et al. 1972). This reserve could provide the nitrogen to balance the reef community budget if upward movement of such water occurred through porous reef structures and into the reef community at a rate of about $20 \text{ liters m}^{-2} \text{ hr}^{-1}$. This appears unlikely, however, since such water would presumably contain elevated phosphate levels as well, but there was no net export of phosphate from tr II (Pilson and Betzer 1973). Furthermore, nutrient levels are greatly reduced in water from the 1,000-m level in a seawater well at Enewetak (O. Holm-Hansen personal communication).

The most plausible unidentified avenue of input of fixed nitrogen into the community seemed, therefore, to be nitrogen fixation. Several investigators have commented on the characteristic ubiquity of blue-green algae in reef communities and the possibility that they fixed nitrogen at ecologically significant rates (e.g. Baas-Becking 1951; Odum and Odum 1955; Dawson 1966; Doty 1958; Bunt et al. 1970). The

heterocystous blue-green alga *C. crustacea* is widely and abundantly distributed in the shallow reef areas at Enewetak.

The photosynthesis : respiration ratio of 1 for tr II implies that organic matter was not being accumulated during the period of this study (Smith and Marsh 1973). Thus, the measured rate of nitrogen export, $226 \text{ nm N m}^{-2} \text{ sec}^{-1}$ ($1,000 \text{ kg ha}^{-1} \text{ yr}^{-1}$), indicates the rate of nitrogen fixation to be expected. This rate exceeds or is within the upper ranges of values reported for nitrogen fixation in managed agricultural plots (e.g. Moore 1966; Alexander 1971). Interestingly, the highest marine rates reported are for another shallow tropical community, turtle grass (*Thalassia testudinum*) beds, where Gocring and Parker (1972) report fixation rates of about $250 \text{ nm N m}^{-2} \text{ sec}^{-1}$, *Calothrix* sp., as in our study, was the major species present. Our post-Symbios data to date indicate a nitrogen fixation rate measured by the acetylene technique of about $100 \text{ nm N m}^{-2} \text{ sec}^{-1}$, based on surface area of samples. Using a ratio of 1.9 m^2 of algal covered surface per horizontal square meter of pavement and rubble zones (Dahl 1973), we get a daytime fixation rate of about $190 \text{ nm N m}^{-2} \text{ sec}^{-1}$, or the same order of magnitude as that from the nitrogen export data.

By far the greatest increase in combined nitrogen as water crossed the transects was in the dissolved organic nitrogen fraction (Table 2). It is perhaps significant that Jones and Stewart (1969a,b) found that 40% of the nitrogen fixed by *Calothrix scopulorum* was released as DON; Watanabe (1951) similarly found that about 42% of the nitrogen fixed by *Calothrix brevisima* was released as DON. It seems distinctly possible that DON released by nitrogen fixing algae was the major direct source of the DON exported by the reef.

If the light reactions of photosynthesis, nitrogen fixation, and export of fixed nitrogen from the reef were all tightly coupled, then we might expect as high a correlation between light intensity and nitrogen export as between light intensity and oxygen export. On the other hand, if the coupling were not precise there might be a time lag

or no diel rhythm in export at all. A graph of total export vs. time of day (Fig. 2) suggests that afternoon rates are higher than morning rates. We have therefore split the day at noon and midnight rather than at dawn and dusk and calculated export rates. DON, NH_4^+ , and total nitrogen export were significantly higher between noon and midnight than between midnight and noon (Table 2); light-dark differences were not significant.

Several of us (W.W., R.E.J., and K.L.W.) have subsequently studied rates of nitrogen fixation at Enewetak in greater detail. We have observed that the *Calothrix* community is variable in the length of time required in either light or dark before fixing N_2 at representative rates. Times varied from several minutes to 2 hr before rates were linear. It appears that the best analysis may be that nitrogen fixation is loosely coupled to photosynthesis (e.g. Fogg et al. 1973), depending on a buildup of ATP and reducing substrates at the beginning of the light period and continuing into the dark period until available substrates are exhausted, and that export is more directly coupled to nitrogen fixation. The export lag, however, could be due to other factors.

It should be emphasized that the transect nitrogen budget was measured during May and June and cannot be extrapolated with any confidence to mean annual conditions. There are two sources of variation that may interfere significantly with such extrapolation. It is often stated that seasonal variations in community structure and function in tropical marine environments are minimal. Relative to average conditions in higher latitudes this is often true. But seasonal variations in light, wind, temperature, and rainfall do exist. Seasonal variations in algal composition and cover and the abundance of some species of fish are quite obvious at Enewetak (personal observation, R.E.J.). Undoubtedly other community components also vary seasonally. It is reasonable, therefore, to expect community metabolism to vary seasonally as well, although the magnitude of such variation is unknown. Secondly, Doty (1971) and Stod-

dart (1971) have pointed out the importance of storms in altering reef community structure and sweeping out both detritus and biota. The magnitude of such export is also unknown. But judging by personal observations on the impact of storms on reef communities, we think it likely that such perturbations would sometimes result in the export of more nutrients in organisms and detritus in a few hours that is normally exported in weeks.

It is instructive to estimate roughly the net export of nitrogen by the entire Enewetak reef flat. The total area of the reef flat is about 64 km² (calculated from Emery et al. 1954, chart 5). Assuming that the mean rate of export of nitrogen per unit area equals the mean of the measured rates on tr II and III, we estimate a daily net export of fixed nitrogen of the order of 9 metric tons by the entire reef flat. We are ignoring the extensive coral and algal communities on the windward reef slope and on the numerous lagoon pinnacles, although we have observed heterocystous blue greens and measured nitrogen fixation in both areas. We are also making the assumption, for the sake of calculations, that nitrogen export rates are as high on the leeward reefs as the rates we measured on windward reefs. Converted to biomass, assuming a mean nitrogen content of 2% of wet weight, this would be equivalent to 450 metric tons of living material per day. Atolls such as Enewetak may consequently be sites of significant nitrogen enrichment in characteristically nitrogen-poor tropical waters. Significantly higher chlorophyll concentration and higher productivity per unit of chlorophyll have been observed in wakes downstream from Fanning and Christmas Islands by R. W. Owen (personal communication). This region of increased productivity did not extend completely to the islands. His preliminary evaluation is that either a considerable lag occurs in the response of phytoplankton to washout from islands or that perturbations of the thermocline in island wakes make nutrients available for consumption by phytoplankton.

It is generally agreed that coral reefs are

among the most mature ecosystems on earth (Margalef 1968). Such ecosystems are thought to be characterized by relatively "closed" nutrient cycles, that is, by low rates of flux of nutrient between biota and physical environment (Odum 1969). While phosphorus rigorously obeys this generalization at Enewetak (Pilson and Betzer 1973), nitrogen cycling is dramatically open-ended.

On the basis of our data, some features of the operation of the nitrogen cycle on this coral reef can be suggested. A high rate of nitrogen fixation appears to allow this mature community to be highly productive and open as far as the nitrogen cycle is concerned while being closed in terms of phosphorus. The blue-green alga *C. crustacea* seems to be the dominant nitrogen fixer. From the work of Jones and Stewart (1969a,b) on another marine representative of the same genus, we can expect about 40% of the fixed nitrogen to be released as DON. This could partially explain the high export of DON from the reef, although in the final analysis the export of DON must result from an excess of production over utilization by the numerous organisms along the transect.

For the formation of nitrate from DON it must be decaminated by some heterotroph, the resulting NH_4^+ oxidized to NO_2^- , and the NO_2^- oxidized to NO_3^- . Recent studies (Webb and Wicbe in prep.) show that autotrophic nitrification occurs in this system. These transformations, consequently, must involve at least four different organisms:

$\text{N}_2 \xrightarrow{1} \text{DON} \xrightarrow{2} \text{NH}_4^+ \xrightarrow{3} \text{NO}_2^- \xrightarrow{4} \text{NO}_3^-$. If the pathway were mainly to involve PON rather than DON, the organisms at step 2 would be fish or ammonotelic invertebrates. Since no appreciable NO_2^- is measurable over the transects, it appears that even though the cycle is open in terms of export there may be a tight and closed cycling of some components with the benthos.

The nitrifying organisms of steps 3 and 4 undoubtedly occur throughout the reef environment. In fact, there seems to be significant nitrification before the water reaches the 0-m station on the transects.

Glass slides left along the reef transect, in tide pools, and on coral pinnacles for a month bore *Nitrobacter* sp., identified and quantified by E. L. Schmidt using immunofluorescence (see Fliermans et al. 1974 for methodology). A minimal estimate of cell numbers is 1×10^6 *Nitrobacter* cells cm^{-2} (Webb and Wicbe in prep.). Excess production of NO_3^- was observed in several environments, not only the coral dominated zone of tr II. Within coral pinnacles, as within the coral dominated tr II zone, the PON pathway probably predominated, the NH_4^+ substrate in part being supplied by such fish as *Dascyllus* sp., which spend much time within coral heads. In tide pools and on the algal platform seaward from islands, the pathway is more likely through DON. Production of DON in tide pools was observed in the daytime, while utilization predominated at night. We interpret this as further evidence for light dependent nitrogen fixation as the DON source.

Utilization of NO_3^- on the transects is indicated by the decrease of NO_3^- as the water passed over tr III or the algal dominated zone of tr II (0–150 m). Dissimilatory NO_3^- reduction (denitrification) takes place only in the absence of O_2 ; since this is a highly aerobic environment, denitrification is probably of little significance, although anaerobic microhabitats undoubtedly do occur. Thus, the loss of nitrate in this system is probably through the assimilatory pathway. A minimum estimate of the rate is about 7% of the nitrogen required to support the net production on tr III (based on production data of Smith and Marsh 1973 and nitrate utilization rate in Table 2). Ammonia utilization and supply seem about in balance on tr III, unlike tr II where NH_4^+ production exceeds utilization in both the coral and algal dominated zones.

Since nitrogen is recycled with unusual efficiency by hermatypic corals (e.g. Lewis and Smith 1971), clearly corals did not dominate community metabolism. This is not surprising; Odum and Odum (1955), for example, estimated corals to be a minor energetic component of an Enewetak reef

community similar to tr II. But the point needs stressing because the literature of coral reef ecology contains frequent inferences that corals do dominate reef community metabolism; this is probably only true with regard to calcium metabolism and then only in some cases (e.g. Chave et al. 1972; Smith 1973).

It is sometimes assumed that community productivity is limited by a single nutrient at any given time. A community containing nitrogen fixing algal and suboptimal dissolved fixed nitrogen levels is one in which total primary productivity may be limited simultaneously by at least two nutrients. Because of the very low N : P ratio in incoming Enewetak waters, it is possible that nitrogen limits the productivity of non-nitrogen fixing algae. Some other nutrient, possibly phosphorus which was present in low absolute concentrations (e.g. Pilon and Betzer 1973), may limit the productivity of the nitrogen fixers. Kinsey and Domm (in press) fertilized a small coral reef in Australia with phosphorus and nitrogen; they found primary production enhanced over that of the control and over the previous year, supporting the view that some components of the reef community are nutrient limited.

While much of the fixed nitrogen in the sea is in the form of NO_3^- , little is known about the microbial processes necessary for its generation. Watson (1965) and Watson and Waterbury (1971) have isolated NH_4^+ and NO_2^- oxidizing marine bacteria; however, in situ generation of NO_3^- by these organisms in the sea has not been established. Cell numbers estimated by extinction dilution techniques are only a few per liter, not sufficiently abundant to permit in situ examination of the rate of NO_3^- formation. Relative to the rate of NH_4^+ production, the rate of NH_4^+ oxidation to NO_3^- in the euphotic zone has been assumed to be trivial (e.g. Dugdale and Goering 1967; Yoshida and Kimata 1970). Thus, it was surprising to find high rates of nitrification on a coral reef (a result since confirmed by in vitro and in situ studies: Webb and Wiebe in

prep.). For the first time, a marine community has been located that is producing NO_3^- at a measurable rate.

Schell (in press) has subsequently measured rates of nitrification in nearshore arctic water isolated naturally by ice closure of lagoon entrances and in water enclosed in bottles and incubated in situ with various substrate enrichments. Results from his two approaches agreed well, giving natural nitrification rates in the range of 0.07–0.13 $\mu\text{g-atom NO}_3^- \text{-N liter}^{-1} \text{ day}^{-1}$. Unlike the reef situation where nitrification is benthic, the responsible organisms in his experiments are principally planktonic. Undoubtedly other marine environments will be found where natural rates of nitrification can be measured and experimentally manipulated.

References

- ALEXANDER, M. 1971. Microbial ecology. Wiley.
- BAAS-BECKING, L. G. M. 1951. Notes on some Cyanophyceae of the Pacific region. K. Ned. Akad. Wet. Proc. Ser. C 54: 3.
- BUNT, J. S., K. E. COOKSEY, M. A. HEEB, C. C. LEE, AND B. F. TAYLOR. 1970. Assay of algal nitrogen fixation in the marine subtropics by acetylene reduction. Nature (Lond.) 227: 1163–1164.
- CHARTOCK, M. A. 1972. The role of detritus in a tropical marine ecosystem: Niche separation in congeneric ophiuroids, food partitioning in cryptic invertebrates, and herbivore detritus production at Eniwetok, Marshall Islands. Ph.D. thesis, Univ. S. Calif. 177 p.
- CHAVE, K. W., S. V. SMITH, AND K. J. ROY. 1972. Carbonate production by coral reefs. Mar. Geol. 12: 123–140.
- DAHL, A. L. 1973. Surface area in ecological analysis: Quantification of benthic coral-reef algae. Mar. Biol. 23: 239–249.
- DAWSON, E. Y. 1966. Marine botany—An introduction. Holt, Rinehart and Winston.
- DOTY, M. S. 1958. An enumeration of the hypothetical roles of algae in coral atolls. Proc. Pac. Sci. Congr. (8th) 1953, Quezon City 3A: 923–928.
- . 1971. Antecedent event influence on benthic marine algal standing crops in Hawaii. J. Exp. Mar. Biol. Ecol. 6: 161–166.
- DUGDALE, R. C., AND J. J. GOERING. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. 12: 196–206.
- EMERY, K. O., J. I. TRACEY, JR., AND H. S. LADD. 1954. Geology of Bikini and nearby atolls.

1. Geology. U.S. Geol. Surv. Prof. Pap. 260-A. 265 p.
- FLIERMANS, C. B., B. B. BOHLOOL, AND E. L. SCHMIDT. 1974. Autecological study of the chemototroph *Nitrobacter* by immunofluorescence. *Appl. Microbiol.* **27**: 124-129.
- FOGG, G. E., W. D. P. STEWART, P. FAY, AND A. E. WALSBY. 1973. The blue-green algae. Academic.
- GOERING, J. J., AND P. L. PARKER. 1972. Nitrogen fixation by epiphytes on sea grasses. *Limnol. Oceanogr.* **17**: 320-323.
- GUNDERSEN, K., C. W. MOUNTAIN, D. TAYLOR, R. OHYE, AND J. SHEN. 1972. Some chemical and microbiological observations in the Pacific Ocean off the Hawaiian Islands. *Limnol. Oceanogr.* **17**: 524-531.
- HARDY, R. W. F., R. D. HOLSTEN, E. K. JACKSON, AND R. C. BURNS. 1968. The acetylene-ethylene assay for nitrogen fixation: Laboratory and field evaluation. *Plant Physiol.* **43**: 1185-1207.
- HELFRICH, P., AND S. J. TOWNSLEY. 1963. The influence of the sea, p. 39-56. *In* F. R. Fosberg [ed.], *Man's place in the island ecosystem*. Pac. Sci. Congr., 10th, 1961.
- JOHANNES, R. E., AND R. GERBER. *In press*. Import and export of net plankton by an Enewetak coral reef community. *Proc. Int. Coral Reef Symp.*, 2nd, Brisbane 1973.
- , AND PROJECT SYMBIOSIS TEAM. 1972. The metabolism of some coral reef communities: A team study of nutrient and energy flux at Enewetak. *BioScience* **22**: 541-543.
- JONES, K., AND W. D. P. STEWART. 1969a. Nitrogen turnover in marine and brackish water habitats. 3. The production of extracellular nitrogen by *Calothrix scopulorum*. *J. Mar. Biol. Assoc. U.K.* **49**: 475-488.
- , AND ———. 1969b. Nitrogen turnover in marine and brackish habitats. 4. Uptake of the extracellular products of the nitrogen-fixing alga *Calothrix scopulorum*. *J. Mar. Biol. Assoc. U.K.* **49**: 701-716.
- KINSEY, D. W., AND A. DOMM. *In press*. Effects of fertilisation on a coral reef environment—primary production studies. *Proc. Int. Coral Reef Symp.*, 2nd, Brisbane 1973.
- LEWIS, D. H., AND D. C. SMITH. 1971. The autotrophic nutrition of symbiotic marine coelenterates with special reference to hermatypic corals. 1. Movement of photosynthetic products between symbionts. *Proc. R. Soc. Lond. Ser. B* **178**: 111-129.
- MARGALEF, R. 1968. Perspectives in ecological theory. Univ. Chicago.
- MOORE, A. W. 1966. Non-symbiotic nitrogen fixation in soil and soil-plant systems. *Soils Fert.* **29**: 113-128.
- ODUM, E. P. 1969. The strategy of ecosystem development. *Science* **164**: 262-270.
- ODUM, H. T., AND E. P. ODUM. 1955. Trophic structure and productivity of a windward coral reef community on Enewetak Atoll. *Ecol. Monogr.* **25**: 291-320.
- PILSON, M. E. Q., AND S. B. BETZER. 1973. Phosphorus flux across a coral reef. *Ecology* **54**: 581-588.
- REDFIELD, A. C., B. H. KETCHUM, AND F. A. RICHARDS. 1963. The influence of organisms on the composition of sea-water, p. 26-77. *In* M. N. Hill [ed.], *The sea*, v. 2. Interscience.
- SCHELL, D. M. *In press*. Regeneration of nitrogenous nutrients in arctic Alaskan estuarine waters. *Symp. Beaufort Sea Coastal Shelf Res.*, 1974, San Francisco. Arctic Inst. N. Am.
- SMITH, S. V. 1973. Carbon dioxide dynamics: A record of organic carbon production, respiration, and calcification in the Enewetak reef flat community. *Limnol. Oceanogr.* **18**: 106-120.
- , AND J. A. MARSH, JR. 1973. Organic carbon production on the windward reef flat of Enewetak Atoll. *Limnol. Oceanogr.* **18**: 953-961.
- SOŁÓRZANO, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnol. Oceanogr.* **14**: 799-801.
- STEWART, W. D. P., G. P. FITZGERALD, AND R. H. BURRIS. 1967. *In situ* studies on N₂ fixation using the acetylene reduction technique. *Proc. Natl. Acad. Sci. U.S.* **58**: 2071-2078.
- STODDART, D. R. 1971. Coral reefs and catastrophic storms, p. 155-197. *In* J. A. Steers [ed.], *Applied coastal geomorphology*. Mass. Inst. Technol.
- STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Can.* **167**. 311 p.
- TELEK, G., AND N. MARSHALL. 1974. Using a CHN analyzer to reduce carbonate interference in particulate organic carbon analysis. *Mar. Biol.* **24**: 219-221.
- THOMAS, W. H. 1969. Phytoplankton nutrient enrichment experiments off Baja California and in the eastern equatorial Pacific Ocean. *J. Fish. Res. Bd. Can.* **26**: 1133-1145.
- . 1970a. On nitrogen deficiency in tropical Pacific oceanic phytoplankton: Photosynthetic parameters in poor and rich waters. *Limnol. Oceanogr.* **15**: 380-385.
- . 1970b. Effect of ammonium and nitrate concentrations on chlorophyll increases in natural tropical Pacific phytoplankton populations. *Limnol. Oceanogr.* **15**: 386-394.
- , AND R. W. OWEN, JR. 1971. Estimating phytoplankton production from ammonia and chlorophyll concentrations in nutrient-poor water of the eastern tropical Pacific Ocean. *Fish. Bull.* **69**: 87-92.
- , E. H. RENGER, AND A. N. DODSON. 1971. Near-surface organic nitrogen in the eastern tropical Pacific Ocean. *Deep-Sea Res.* **18**: 65-71.

- WATANBE, A. 1951. Production in cultural solution of some amino acids by the atmospheric nitrogen-fixing blue-green alga. Arch. Biochem. Biophys. **34**: 50-55.
- WATSON, S. W. 1965. Characteristics of a marine nitrifying bacterium, *Nitrosocystis oceanus* sp.n. Limnol. Oceanogr. **10**(suppl.): R274-R289.
- , AND J. B. WATERBURY. 1971. Characteristics of two marine nitrite oxidizing bacteria, *Nitrospina gracilis* nov. gen. nov. sp. and *Nitrococcus mobilis* nov. gen. nov. sp. Arch. Mikrobiol. **77**: 203-230.
- WILLIAMS, P. M. 1967. Sea surface chemistry: Organic carbon and organic and inorganic nitrogen and phosphorus in surface films and subsurface waters. Deep-Sea Res. **14**: 791-800.
- WOOD, E. D., F. A. J. ARMSTRONG, AND F. A. RICHARDS. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. U.K. **47**: 23-31.
- YOSHIDA, Y., AND M. KIMATA. 1970. Microbiological and chemical studies in the seas of Iiuchi and Bingo. 2. On the metabolic activities of inorganic nitrogen compounds by microorganisms as a whole. J. Oceanogr. Soc. Jap. **26**: 6-10.

Submitted: 11 July 1974

Accepted: 24 October 1974

Errata

In the article by Gorham et al. ("Some relationships between algal standing crop, water chemistry, and sediment chemistry in the English Lakes," July 1974, volume 19: 601-617), the senior author neglected to revise the equations in Figs. 3, 4, 9, 10, 11, and 13 following a change in units of calcium from meq liter⁻¹ to μ eq liter⁻¹, and of algal standing crop from mg liter⁻¹ to μ g liter⁻¹. The reduced major axis in Fig. 11A was also reversed without correction

of the equation. The correct equations are: Fig. 3— $\log y = 0.00456x - 0.390$ reduced major axis, $\log y = 0.00361x - 0.258$ regression; Fig. 4— $y = 0.088 + 0.000086x$; Fig. 9— $y = 0.0949x - 10.0$; Fig. 10— $\log y = 3.99 \log x - 6.66$; Fig. 11A— $\log y = 0.381 \log x - 0.285$ reduced major axis, $\log y = 0.316 \log x - 0.103$ regression; Fig. 11B— $y = 4.15 + 0.00481x$ reduced major axis, $y = 5.01 + 0.00433x$ regression; Fig. 13— $y = 172x - 980$.