

Journal of Experimental Marine Biology and Ecology, 226 (1998) 293-316

Effects of unidirectional and oscillatory water flow on nitrogen fixation (acetylene reduction) in coral reef algal turfs, Kaneohe Bay, Hawaii

Susan L. Williams^{a,*}, Robert C. Carpenter^b

^aDepartment of Biology, San Diego State University, San Diego, CA 92182-4614, USA ^bDepartment of Biology, California State University, Northridge, CA 91330-8303, USA

Received 15 May 1997; received in revised form 17 September 1997; accepted 28 October 1997

Abstract

Rates of acetylene reduction (nitrogenase activity) by algal turf communities from Kaneohe Bay, Oahu, Hawaii, were measured as a function of increasing water flow speeds under unidirectional and oscillatory flow regimes in an enclosed incubation chamber. Water flow speeds, shear stress, and turbulence intensities were measured with thermistor probes in the chamber and over the turfs in the field. The thickness of the boundary layer varied significantly and linearly with bulk water flow speeds in the field. Although the boundary layer in the chamber also decreased with increasing flow speeds, turbulence intensity and flow speeds in the chamber were mostly lower than those typically measured in the field. Rates of acetylene reduction were positively related to water flow speed. Oscillatory water flow, which increased turbulence intensity five times, resulted in a significant increase in acetylene reduction compared to unidirectional flow. Even at the lowest mean flow speeds measured in the field ($< 0.1 \text{ m s}^{-1}$), mean rates of acetylene reduction (27 nmol ethylene cm⁻² h⁻¹±11 SD) were high under oscillatory flow. Equivalent high rates under unidirectional flow were not achieved until flow speed was more than doubled. The slope of log-log linear regressions of acetylene reduction versus flow speed was 0.5 for both oscillatory and unidirectional regimes. This result suggests that acetylene reduction rates in the chamber were controlled by mass transfer of a rate-controlling solute, such as acetylene or oxygen (inhibitory to nitrogenase), through a laminar diffusion boundary layer. Because coral reefs exist in areas of very low nitrogen availability, nitrogen fixation is fundamentally important for coral reef primary production and biogeochemistry. Yet, current understanding of nitrogen fixation on coral reefs has been derived primarily from measurements made under unnatural conditions of no or low water flow. This study lends support to the importance of water flow as a major control of the metabolism of organisms occupying coral reefs. © 1998 Elsevier Science B.V.

Keywords: Algal turfs; Coral reefs; Hydrodynamics; Nitrogen fixation

0022-0981/98/\$19.00 © 1998 Elsevier Science B.V. All rights reserved. PII: S0022-0981(97)00252-9

^{*}Corresponding author. Tel.: +1 619 594 2738; fax: +1 619 594 5676; e-mail: swilliam@sunstroke.sdsu.edu

1. Introduction

Coral reefs thrive in areas of high water flow (Hamner and Wolanski, 1988). Although the importance of water flow to corals has received considerable attention (e.g., Jokiel, 1978; Andrews et al., 1988; Patterson and Sebens, 1989; Patterson et al., 1991; Sebens and Johnson, 1991; Helmuth and Sebens, 1993; Genin et al., 1994; Lesser et al., 1994; Fabricius et al., 1995), effects of water flow on other dominant reef organisms and the reef community is not well known. In this study, we focus on water flow effects on nitrogen fixation in algal turfs. Algal turfs are inconspicuous diverse communities that occupy considerable areas of 'barren' substrata and dead corals on all coral reefs. They are the dominant autotrophic community on coral reefs (Kinsey, 1985; Hatcher, 1988) and have high rates of nitrogen fixation (Wilkinson et al., 1984; Larkum et al., 1988; Shashar et al., 1994b). Algal turfs comprise densely-packed unicellular and small filamentous algal species from at least four Divisions plus many kinds of cyanobacteria and bacteria (Hackney et al., 1989; Carpenter, 1990; Williams and Carpenter, 1997). This diversity distinguishes algal turfs from other nitrogen fixing communities on coral reefs, for example intertidal mats of largely single species of cyanobacteria (Wiebe et al., 1975).

Algal turfs extend only a few mm above the substratum, where water flow speeds are reduced considerably relative to the bulk flow (Carpenter and Williams, 1993). Not surprisingly, increased water flow results in significantly higher rates of photosynthesis and nitrogen fixation in Caribbean algal turfs, suggesting the importance of water flow effects on algal turf metabolism (Carpenter et al., 1991).

Nitrogen fixation is an important biogeochemical process on coral reefs (Capone, 1988; Howarth et al., 1988). The significance of hydrodynamic processes to biogeochemical cycles on coral reefs has been recognized previously (Smith, 1984; Adey and Steneck, 1985; Hatcher and Frith, 1985; Hatcher et al., 1987) and experimentally confirmed for phosphorus uptake (Atkinson and Bilger, 1992; Bilger and Atkinson, 1992). In contrast, empirical studies of hydrodynamic influences on the nitrogen cycle of coral reefs are limited (Thomas and Atkinson, 1997). Rates of nitrogen fixation on coral reefs have been measured almost universally under relatively stagnant and/or anaerobic conditions (Mague and Holm-Hansen, 1975; Wiebe et al., 1975; Burris, 1976; Wilkinson and Sammarco, 1983; Wilkinson et al., 1984; Larkum et al., 1988; Carpenter et al., 1991; Shashar et al., 1994a,b), which are not typical in algal turfs found universally on the submerged dead corals and rocks within the reef matrix. How nitrogen fixation in algal turfs is influenced by water flow and oxygen, and how these conditions vary in the field at the macro-scale and within the turfs (Williams and Carpenter, 1990; Carpenter and Williams, 1993), are poorly known but undoubtedly important for understanding algal turf physiology and their contribution to biogeochemical cycles on coral reefs (Webb et al., 1975; Smith, 1984, 1988; Hatcher and Frith, 1985; Charpy-Roubaud et al., 1990).

We measured nitrogen fixation (acetylene reduction) in algal turf communities from Kaneohe Bay, Hawaii, as a function of unidirectional and oscillatory water flow regimes in an experimental chamber. We also measured water flow over algal turf communities growing in the field to describe natural conditions and to compare to conditions in the chamber. We treated algal turfs as communities, partly because time for identification of the primarily microscopic algal species was limited. The algal turfs in Kaneohe Bay, however, were typical in that coccoid, non-heterocystous filamentous, and cyanobacteria with either basal or intercalary heterocysts were common among potential nitrogen fixers (Williams and Carpenter, 1997). The contributions of individual nitrogen fixers or species to community acetylene reduction will depend on their intrinsic metabolic rates, their relative abundance, and their distribution within the turfs with respect to microscale physical and chemical regimes (Williams and Carpenter, 1990; Paerl et al., 1995). A glance under the microscope at the complexity of, and diversity in, coral reef algal turfs is a convincing demonstration that partitioning water flow effects within the community will be a daunting task.

2. Methods

The study was conducted at Kaneohe Bay, Oahu, Hawaii. The field sites were located along the seaward portion of the reef flat of the barrier reef enclosing the bay. Dissolved ammonium and nitrate concentrations flowing over the reef flat were $< 1 \mu$ M. Algal turfs cover most of the substratum, which is predominately small (median size 7–8 cm long) pieces of coral rubble (Cheroske, 1997). Larger coral 'bommies' are scattered throughout the reef flat. The experiments were conducted at the Hawaii Institute of Marine Biology, University of Hawaii.

2.1. Incubation chamber characteristics

The design of the incubation chamber was constrained by the sensitivity of the acetylene reduction assay, the predicted rate of acetylene reduction based on previous data from Caribbean algal turfs (Williams and Carpenter, 1997), and an optimal incubation length. Short incubations were desired to avoid substrate limitations and to complete a flow speed series within a single day to minimize the time between collection of algal turfs and experiments. The design compromise was a donut-shaped ultra-violet transparent acrylic chamber (10.9121 volume) with a 60 cm outer diameter and 36 cm inner diameter (Fig. 1). The design eliminated a water return section to maximize the air-water exchange surface and exposure of turfs to water. The working cross-section of the flume was 7 cm high by 10.5 cm wide. Two wells for algal-covered plates (see below) were smoothly cut into the floor of the flume, one on each side of a propeller (four blades, each 2 cm wide). O-ring sealed transparent lids to provide access to plate wells were cut near but not over the plate wells and flush with the flume top. Small holes for serum septa were drilled in the top of the flume away from the plate wells for gas injection and sampling. The propeller shaft was attached with a pin hub bevel gear (M48N-IS, W.M. Berg, Inc. East Rockaway, NY) to a shaft driven by a direct current motor (1/18 horsepower, 1800 rpm, Model #4Z141, Dayton Electric Manufacturing Co., Niles, IL), which was housed on top of the chamber. The propeller shaft was located 2 cm above the flume floor. The shaft entry to the flume was made gas-tight with a standard oil seal that was replaced frequently. The motor speed and reversal were controlled with an adjustable relay circuit. The period of the oscillation was 16 s. This



Fig. 1. Diagram of the acrylic incubation chamber viewed from the top (to scale). Algal turfs growing on plates were placed flush with the bottom in the plate well. Gas injection and sampling were through septa in the top of the chamber. Access ports were positioned to avoid shading algal turfs. Flow probe ports on each side of the chamber were drilled at three heights above the floor and fitted with septa for positioning the probe across the plates in the wells.

period was reasonable for ocean swells and any shorter period increased cavitation at the propeller upon flow reversal. Two types of flow straighteners made with plastic drinking straws were placed on both sides of the propeller. One set was cut at an angle toward the plate well with longer (6 cm) straws at the outer wall of the flume and progressively shorter straws (1 cm long at the inner wall) in an attempt to minimize the flow differential across the flume (referred to hereafter as 'diagonal' flow straighteners). In addition, a strip of flow straighteners 1 cm wide was placed 1 cm behind each diagonal flow straightener to minimize a jet from the propeller (R. Bilger, personal communication). A 1 mm diameter stainless steel 'trip' wire was placed 5 cm (curvilinear distance) downstream from the short strip of straighteners and 17 cm before the leading edges of the plates.

Water flow in the flume was visualized using fluorescein, and calibrated in unidirectional and oscillatory flow with hot film thermistor probes. 'Ruggedized' hot film thermistor probes (Model 1269W, TSI, Inc., St. Paul, MN) were inserted into septa in ports positioned for cross-stream measurements over both plates midway downstream from their leading edges. The ports were 0.8, 3, and 5 cm above the flume floor. At a given motor speed and position within the flume, flow was sampled at 10 Hz over a 2 min period and averaged for the mean flow speed (Carpenter and Williams, 1993).

296

Turbulence intensity was calculated as the root mean square/mean speed (Denny, 1988). A total of 650 motor speed-position measurements were completed successfully to characterize flow after preliminary measurements and modifications were made in the flume. Probes were calibrated initially and checked daily to weekly in the middle of the working section (10 cm high \times 11 cm wide \times 110 cm long) of a standard flume (27.81) independently calibrated by video analysis of brine shrimp cyst movements. Calibration was conducted at \pm 1°C of the temperature used for measurements (25°C). Probes were cleaned after use with ethanol and distilled water.

Unidirectional flow in the chamber also was calibrated by video analysis of brine shrimp cyst movement. The camera was placed normal to the chamber walls and the edge of the plate. Light from a fiber optic source was directed from the top of the chamber through a 1 mm wide slit cut parallel to the curvature of the chamber walls. The camera was focused at 1 cm before the leading edge of the plate. A 2-min interval was filmed and analyzed using NIH Image 1.60 software. To characterize the boundary layer and the shear velocity (U_*) near the bottom of the chamber just before the leading edges of the plates, the slope of flow speed U (m s⁻¹) versus ln *S*, where *S* is the height (m) above the bottom of the chamber, was determined. Flow speeds were determined from brine shrimp cyst movements in the middle of the cross-sectional area (= middle of plate) in unidirectional flow.

2.2. Water flow over algal turfs in the field

A total of 25 field flow profiles were made over algal turfs growing on coral rubble at six sites on the reef flat using hot film thermistors and the methods of Carpenter and Williams (1993). The probe was positioned at S = 300, 200, 100, 50, 30, 20, 15, 10, 7, 5, 4, 3, 2, 1 mm above the substratum. Mean algal turf canopy height (<math>n = 5) was measured in the vicinity of the probe. Freestream flow speed (U_{∞}) versus S relationships were used to characterize the shear velocity (U_*) near the substratum. U_{∞} represented the average of mean flow speeds at S = 50-300 mm. Turbulence intensity (TI) was calculated as above. We did not empirically differentiate between the thinner momentum boundary layer wherein flow is affected by the turfs and the benthic boundary layer wherein flow speed reaches 99% of U_{∞} (discussed in Shashar et al., 1996).

Longer term mean flow speeds were measured with an electromagnetic current meter (InterOcean, Model S4, InterOcean Systems, Inc., San Diego, CA) deployed 0.5 m above the substratum during times when the profiles over algal turfs were made. The meter sampled at 2 Hz for 20 min every 6 h for 3 days.

2.3. Measurement of nitrogenase activity as acetylene reduction

Algal turfs were grown on $7.5 \times 8.5 \times 1$ cm plates (63.75 cm²) cut from fossil *Porites lobata* removed from a quarry and cleaned so algal turf inoculum and soluble nutrients were not available. Plates were placed in ≤ 2 m water depth in the coral rubble field on the seaward edge of the Kaneohe Bay reef flat, just behind the reef proper (Site A, 21°28′06″N, 157°47′35″W). Plates were attached to PVC pipes cut in half and secured to coral 'bommies'. Algal turfs developed on the plates for over 1 yr before data were

collected. Beginning 1 month before the experiments commenced, plates were lightly scraped with a putty knife once a week to prevent buildup of sediments and uneven growth of algal turfs. Plates were selected randomly and placed in a running seawater table for ≤ 24 h before the experiments.

The chamber was filled with 8.91 of 20 μ m cartridge-filtered seawater (see controls below). The water depth in the chamber was 6 cm, leaving 1 cm for a headspace. One plate was placed in each plate well and secured flush with the chamber floor using non-toxic putty (Sculpey III, Polyform Products, Schiller Park, IL). The chamber was placed in a white trough filled with freshwater circulated through a refrigeration unit to maintain chamber temperatures within 2°C of ambient seawater temperatures (27–29°C). With the motor running, 300 ml of C₂H₂, freshly generated from calcium carbide, was added to the 21 headspace (13% C₂H₂; aerobic incubations). A balloon attached to a septum stopper in the top of the chamber provided a gas sampling reservoir. A collapsing balloon indicated a leak in the chamber.

Each experiment consisted of incubating a set of two plates at each of five flow speeds under either a unidirectional or oscillatory flow regime. A new incubation commenced at each flow speed using the same plates. Flow speeds were run in random order, and if possible the first speed was re-run at the end of the day to check for early morning lag periods. At most six incubations could be completed in a day. A total of 13 experiments under oscillatory and 17 under unidirectional flow regimes were performed in July– August 1995. Incubations proceeded for 60 min, with sampling every 10 min. Experiments were performed during the day when light was at saturation levels for algal turf photosynthesis (800 μ mol photons m⁻² s⁻¹, unpubl. data) between 0900 and 1600 local time. Light was monitored with a cosine sensor and data logger (LI-COR, Inc., Lincoln, NE). A neutral density mesh screen was placed over the chamber to reduce light to 800–1800 μ mol photons m⁻² s⁻¹ if it exceeded 2300 μ mol photons m⁻² s⁻¹. The initial and final temperature of the chamber water was measured.

Acetylene and ethylene concentrations in the head space were analyzed in a Shimadzu mini-II gas chromatograph with dual Poropak R columns. Calibration curves were generated in the morning using five concentrations of ethylene (n = 5 for each concentration) and checked periodically during the day. Ethylene production was corrected for solubility (Flett et al., 1976) and headspace volume and estimated from the regression of corrected concentration versus time.

The following controls were performed. Although Hanson and Gundersen (1977) found no planktonic nitrogen fixation in waters of Kaneohe Bay, rates of acetylene reduction in the chamber without algal turfs were measured as controls for planktonic acetylene reduction. The chamber was filled with (1) unfiltered, (2) 0.2 μ m Millipore-filtered seawater freshly-collected from the reef flat, and (3) seawater from the HIMB seawater inflow line filtered to nominal 20 μ m with a pleated cellulose cartridge. Incubations proceeded at 0.10–0.14 m s⁻¹ flow speed for 90–120 min. Acetylene reduction in the absence of algal turfs was negligible compared to that for algal turfs (see Results): 0.00074 nmol chamber⁻¹ min⁻¹ for unfiltered water and 0.00039 and 0.00053 for the respective filtered waters. No ethylene was produced by algal turfs in the chamber (oscillatory mean flow speed 0.1 m s⁻¹) in the absence of acetylene nor did ethylene concentration change when added instead of acetylene over a 70 min long incubation.

In preliminary experiments (n = 7), the pH of the water in the chamber with algal turfs photosynthesizing at saturating photon flux density increased at most 0.1 unit from initial values (8.1–8.4) over 3 h; thus, carbon should not have been limiting during the

2.4. Algal biomass

experiments.

At the end of the experiments, algal turfs were removed for estimation of biomass parameters. Using a sampling grid divided into $1 \text{ cm} \times 1 \text{ cm}$ squares and placed over each plate, five random squares were selected. The height of the tallest filaments in each square was recorded. Algae were scraped 1 mm into the substratum in each square for chlorophyll *a* concentration (Carpenter, 1986). Five additional replicates were scraped for ash-free biomass (AFDM, combusted at 550°C for 8 h). Replicates from both plates (*n* = 10) were averaged.

3. Results

3.1. Characteristics of water flow in the chamber

Water flow in the chamber was faster along the outer wall of the chamber and in one direction of the propeller driven by the reversing motor (pushing vs. pulling water). Flow was characterized by a jet from the propeller at ~0.5 cm from the chamber floor. The jet was most evident at the middle of the plate on the 'fast' (i.e., closer to the propeller) side of the chamber. Video analysis revealed that the water flow was fastest from 0.25 to 0.67 cm above the chamber bottom, and speeds of particles moving within this range were used to calculate the average freestream flow speeds (Fig. 2). On both the fast and slow sides of the chamber, average water flow speeds increased by up to ~0.6 m s⁻¹ from the inner to outer plate edge across the chamber, particularly at higher speeds (Fig. 3).

To characterize the magnitude of turbulence (U_*) near the bottom of the chamber just before the leading edges of the plates, flow speeds were determined from brine shrimp cyst movements in the middle of the cross-sectional area (= middle of plate) in unidirectional flow (Fig. 4). U_* was 0.003, 0.006, 0.005 m s⁻¹ at relative speed settings 1–3, respectively. Mean U_* for these respective motor settings were 0.047, 0.063, and 0.078 m s⁻¹. At these heights, the values of U_* were $< 0.1U_{\infty}$, the order considered typical for water flow in boundary layers over rough bottoms (Denny, 1988).

Turbulence intensities (TI) estimated from flow speeds measured with thermistor probes in oscillatory flow were roughly five times higher than in unidirectional flow (Table 1). For example, at 0.8 cm above the chamber floor (height designated for freestream flow for thermistor data), TI ranged from 0.11 to 0.28 for unidirectional flow and 0.53 to 1.10 for oscillatory flow.

Because of the inequality in speeds in the two directions of propeller revolution, oscillatory mean flow conditions were slower than unidirectional flow at the same motor rpm (Fig. 5).



Fig. 2. Results of video analysis of particle movement speeds versus height above chamber floor under unidirectional flow at relative speeds 1-3. Camera focused over the middle of the plate cross-section (= middle of chamber cross-section) at 1 cm before leading edge of plate.

3.2. Flow speed profiles over algal turfs in the field

The field flow speed profiles were made under a variety of sea and wind conditions (Fig. 6). If swells or bulk water flow changed noticeably during the measurements, which lasted ~1 h, the profile was not used. Minor changes in conditions contributed to some of the poorer fitting regressions of mean flow speeds versus ln *S* (Table 2). There was a four-fold difference in canopy heights (0.9–3.8 mm) of the turfs measured and an order of magnitude difference in U_{∞} and in U_* (Table 2). In general, U_* near the bottom was close to the typical order $(0.1U_{\infty})$ expected in boundary layers over rough bottoms (Denny, 1988) (Fig. 7). Only two of 25 values were lower by more than 50%. As expected, the thickness of the boundary layer, where flow is reduced as momentum is extracted by friction, varied significantly and linearly with bulk water flow speeds in the field (Fig. 8).

Compared to field conditions, oscillatory flow in the chamber was slow, although the fastest mean unidirectional speed was in the range of mean field values. In general, the chamber had lower values of U_* compared to the field, although U_* at low flow speeds in the chamber were comparable to the lowest values measured in the field. A limitation of this comparison is that field measurements were made under oscillatory conditions, while the chamber was run under unidirectional conditions for video analysis of



Fig. 3. Mean flow speeds across the chamber at two heights above the bottom and at two relative motor speeds (slow, fast). Measurements were taken over a plate mid-way downstream from the leading edge on the fast side of the chamber. The plate extended from 2.0 to 9.5 cm across the chamber (5.5 cm position is approximately midplate). Mean \pm SD of two measurements (each averaging 2 min of sampling at 10 Hz) made with a hot film thermistor probe after repositioning the probe. Most of the SD error bars are too small to see. Flow was oscillatory with a 32 s period.



Fig. 4. Characterization of the momentum boundary layer in the chamber under three relative speeds of unidirectional flow. Each point represents the speed of a brine shrimp cyst. S = height above chamber floor over the middle of the plate cross-section on the fast side of the chamber. r^2 = coefficient of determination.

Table 1

Turbulence intensities in the flume. Turbulence intensity = root mean square flow speed/average flow speed. Flow speeds were measured with thermistor probes with a 10 Hz sampling rate for 2 min. S = height above flume floor. For brevity, only three relative speeds (motor settings) are presented. 'Fast' and 'slow' indicate plate closer to and farther from propeller when moving water most efficiently, respectively. Position refers to height above flume floor (= S) and position across plates from side closest to inner wall of flume to side closest to outer wall of flume. Oscillatory period 32 s

Side of plume: Relative speed: Position	Unidirectional Fast			Oscillatory Fast			Unidirectional Slow			Oscillatory Slow		
	1	3	5	1	3	5	1	3	5	1	3	5
S = 0.8 cm												
Inner	0.23	0.20	0.21	0.53	0.57	0.59	0.16	0.24	0.25	0.73	0.92	0.77
Middle	0.15	0.19	0.23	0.76	0.75	0.87	0.13	0.20	0.20	0.77	1.00	1.10
Outer	0.28	0.13	0.16	0.62	0.71	0.78	0.11	0.12	0.13	0.56	0.63	0.93
S = 3.0 cm												
Inner	0.34	0.18	0.21	0.39	0.58	0.55	0.11	0.21	0.56	0.63	1.41	0.84
Middle	0.18	0.18	0.38	0.38	0.42	0.70	0.20	0.22	0.30	0.52	0.92	0.93
Outer	0.16	0.15	0.15	0.55	0.85	1.00	0.15	0.14	0.25	0.93	0.73	0.90
S = 5.0 cm												
Inner	0.24	0.28	0.28	0.39	0.72	0.77	0.16	0.22	0.40	0.59	1.11	0.82
Middle	0.17	0.19	0.34	0.49	0.45	0.56	0.15	0.23	0.35	0.55	0.56	0.71
Outer	0.10	0.17	0.21	0.76	0.77	0.92	0.27	0.16	0.30	0.54	0.65	0.49

near-bottom turbulence. TI in the chamber under unidirectional flow was lower than over algal turfs in the field (5 cm above substratum), but TI under oscillatory flow was comparable to that in the field (Tables 1 and 2).

The longer term average water flow speeds obtained from the current meter ranged from < 0.1 to 0.7 m s⁻¹, with speeds from 0.1 to 0.3 m s⁻¹ occurring over 50% of the time logged (Fig. 9).

3.3. Nitrogenase activity in algal turfs

Nitrogen fixation in algal turfs, estimated as the areal rate of ethylene production, was linear over the 60 min incubations (Fig. 10) and longer (Fig. 11), except for a short (<20 min) lag at the slowest flow speed (and occasionally the next slowest, Fig. 10). Rates of ethylene production were calculated only from linear portions of time courses, which had $r^2 \ge 0.95$ and typically ≥ 0.98 . The disappearance of a time lag with increased water flow suggests that the lag was due primarily to advection and diffusion of substrate or oxygen, which is inhibitory to nitrogenase.

For each experiment, the relationship of ethylene production rate versus water flow speed was described with a power function (Table 3). The mean of the exponents was not significantly higher for oscillatory flow (independent *t*-test, P = 0.120, df = 28) but the mean of the coefficients was significantly greater for oscillatory flow (independent *t*-test, P = 0.014, df = 28). On average, the oscillatory flow regime resulted in a steeper increase in ethylene production rates with increasing flow speeds (Fig. 12). Under



Fig. 5. Unidirectional and oscillatory (period 32 s) flow speeds over the middle of the plate cross-section at 0.8 cm above chamber floor. Means (n = 2, SD) of average flow speed from plates on fast and slow sides of motor (n = 4). Data from thermistor probes.

unidirectional flow, the maximum rates of ethylene production were achieved at the highest flow speed ($\sim 0.25 \text{ m s}^{-1}$) and ranged from 7.75 to 47.2 nmol/µg chl *a*/h and from 1.87 to 10.3 nmol/mg AFDM/h (see tables and figures for the same rates expressed per cm²). Maximum rates (6.27 nmol/µg chl *a*/h and 12.8 nmol/mg AFDM/h) under oscillatory flow were equivalent to those under unidirectional flow and likewise were achieved at the highest flow speed possible under oscillatory flow ($\sim 0.9 \text{ m s}^{-1}$). The fastest oscillatory flow speed was $\sim 0.16 \text{ m s}^{-1}$ lower than the highest flow speed under unidirectional flow (due to inefficiency of the propeller when rotating in the reverse direction). Nitrogenase activity was about double at equivalent mean flow speeds with oscillation and the concomitant five-fold increase in turbulence intensity in the chamber. Because of the variation in water flow speed across the chamber and the difference between speeds over plates on the fast and slow sides of the propeller, the flow speeds should be considered as relative rather than absolute.

The slope of log–log linear regressions of metabolic rates versus water flow speed can be related to theoretical expectations for the scaling of mass transfer of a limiting substrate to the organism (White, 1988; Patterson et al., 1991). The slopes of the regressions through the mean values for oscillatory and unidirectional flow regimes were ~0.5 (Fig. 12). This is the expected value for scaling of a metabolic rate to water flow



Fig. 6. Flow speed profiles over algal turfs in Kaneohe Bay (see Table 2 for site coordinates). Data from thermistor probes. Height of probe above coral rubble substratum. Flow speed was averaged over 2 min. $r^2 = \text{coefficient of determination}$.

speed if mass transfer is controlled by a laminar diffusion boundary layer (White, 1988; Patterson et al., 1991). An analysis of covariance (ANCOVA) was performed with flow speed as the covariate because flow speeds were not the same under oscillatory versus unidirectional flow. We used the arbitrary cut off of r^2 values < 0.500 (log–log regressions) to delete experiment results too equivocal (n = 4) to be reasonably described by the mechanistic model. The ANCOVA assumptions were met; plots of residuals indicated no problems with normality and the slopes of ethylene production on the covariate flow speed were homogenous (P = 0.431, df = 1, 124). The effect of oscillatory versus unidirectional flow was significant (P = 0.004, df = 1, 125) when adjusted for the covariate flow speed.

The mean concentration of chl a/cm^2 in the algal turfs was 8.70 ± 4.11 (\pm SD, n = 30) and ranged from 3.77 to 20.31. Mean ash-free algal biomass was 7.71 mg cm⁻² ±2.99 (\pm SD, n = 30) and ranged from 3.25 to 13.9. Mean algal canopy height was 2.1 ±0.8 (\pm SD, n = 30) and ranged from 0.9 to 3.4. The biomass and canopy heights of algal turfs growing on plates were similar to turfs growing on natural coral rubble on the reef

Table 2

Summary of field flow characteristics over algal turfs on the reef flat of Kaneohe Bay, Hawaii. U_{∞} is the freestream flow (average of mean values for heights ≥ 5 cm above the substratum). U_* is the slope of the line relating flow speed to ln *S*, multiplied by von Karman's constant (0.4). The coefficient of determination is for the flow speed versus ln *S* regression. TI is turbulence intensity (rms/mean flow speed) at S = 5 cm. The algal turf canopy height is the mean of five measurements made under and near the flow probe. nd = no data

Date	Site	$U_{\infty} \ ({\rm m\ s}^{-1})$	$U_{*} ({ m m \ s}^{-1})$	r^2	TI	Canopy height (mm)
1/19/96	I-1 ^a	0.274	0.014	0.77	0.903	2.4
1/24/96	I-2	0.292	0.040	0.83	0.817	1.4
1/26/96	I-3	0.280	0.028	0.79	0.658	1.4
1/29/96	I-4	0.370	0.018	0.78	0.984	2.2
2/5/96	I-5	0.240	0.017	0.88	1.110	1.4
2/8/96	$H-7^{a}$	0.230	0.017	0.75	0.700	1.8
2/27/96	H-8	0.410	0.040	0.88	0.993	1.0
3/4/96	H-9	0.460	0.042	0.98	0.810	2.2
3/9/96	H-10	0.320	0.004	0.26	0.772	1.4
3/12/96	$G-1^{a}$	0.180	0.017	0.66	0.810	2.4
3/27/96	G-2	0.493	0.004	0.59	0.801	2.0
3/30/96	G-3	0.369	0.035	0.64	0.864	1.4
4/25/96	G-4	0.250	0.036	0.74	0.725	2.2
7/10/96	F-1 ^a	0.015	0.012	0.76	nd	1.4
7/10/96	F-2	0.230	0.015	0.63	nd	1.8
7/11/96	F-3	0.120	0.010	0.53	0.687	1.6
7/11/96	F-4	0.210	0.026	0.90	0.761	3.6
7/16/96	A-1 ^a	0.430	0.037	0.39	0.852	2.6
7/16/96	A-2	0.630	0.065	0.87	0.882	3.8
7/18/96	A-3	0.580	0.077	0.87	0.744	3.4
7/18/96	A-4	0.400	0.056	0.90	0.802	2.2
7/23/96	C-1 ^a	0.210	0.031	0.86	0.624	2.4
7/23/96	C-2	0.230	0.030	0.88	0.796	2.0
7/25/96	C-3	0.180	0.026	0.88	0.683	3.0
7/25/96	C-4	0.230	0.024	0.96	0.796	2.6
Mean $(n = 25)$		0.303	0.028		0.807	2.1
SD		0.135	0.018		0.115	0.7

^aGeographic coordinates derived from a global positioning system: A (21°28′06″N, 157°47′35″W), C (21°27′54″N, 157°47′42″W), F (21°27′55″N, 157°47′26″W), G (21°27′50″N, 157°47′30″W), H (21°28′22″N, 157°47′55″W), I (21°28′20″N, 157°48′20″W).

flat (Cheroske, 1997, Table 2). The variation in algal biomass in part contributed to the variation in acetylene reduction rates.

4. Discussion

Acetylene reduction by coral reef algal turfs is positively related to water flow speed (Carpenter et al., 1991; this study). Although the experiments presented here were constrained by the design of the chamber used, they demonstrated the relative importance of oscillatory flow to nitrogenase activity in algal turfs. Although algal turfs grow well within the boundary layer (Fig. 6), they exhibit high rates of nitrogenase



Fig. 7. Characterization of the momentum boundary layer over algal turfs in Kaneohe Bay (see Fig. 5). Data from thermistor probes. S = height above coral rubble substratum. Flow speed was averaged over 2 min, sampling at 10 Hz.

activity under oscillatory flow even at very low mean bulk water flow speeds $(<0.1 \text{ m s}^{-1})$. The natural oscillatory flow regime thus will be very important when mean flow speeds are $<0.1 \text{ m s}^{-1}$, conditions which occur $\sim 10\%$ of the time on the Kaneohe Bay reef flat (Fig. 9). One limitation of the chamber was that oscillatory flow speeds were low compared to natural conditions on the reef flat, and thus the measured rates might underestimate nitrogen fixation on the reef flat, perhaps as in previous studies reporting rates within the range reported here (summarized in Capone, 1988).



Fig. 8. Relationship between the momentum boundary layer as characterized by U_* and freestream flow speed. U_* is the slope of the relationship U versus ln S multiplied by von Karman's constant (see Fig. 6). Freestream flow speed is the mean of the average flow speeds at heights 5, 10, 20, and 30 cm above the substratum. $r^2 = \text{coefficient}$ of determination; P = probability level.

The experiments also provided evidence that low water flow can lead to reduced advection of substrate to, and/or oxygen from, algal turfs, contributing to time lags in acetylene reduction (Figs. 10 and 11). There was no evidence that nitrogenase activity required more than 10 min to be induced. Long time lags in acetylene reduction have been reported in coral reef studies (e.g., Wiebe et al., 1975; Hanson and Gundersen, 1977; Williams et al., 1987; Shashar et al., 1994b) and attributed in general to the advection of substrate, nitrogenase induction, and temporary oxygen inhibition (Taylor, 1983; Shieh and Lin, 1992; Shashar et al., 1994b). We suggest time lags are experimental artifacts that could be removed if more natural water flow regimes were used.

The physiological mechanism for enhanced nitrogen fixation with increased water flow speeds, oscillation, and turbulence is a subject for further research. We suggest that the rate of nitrogen fixation in algal turf communities in photosynthesis-saturating light is controlled by the mass transfer of a limiting solute to the surface of the algae or of oxygen (produced during photosynthesis) away from the surface. The decrease in the boundary layer at higher flow speeds in the chamber under unidirectional flow (Fig. 4) and in the field under oscillatory flow (Fig. 7) is related to a decreasing thickness of the diffusion boundary layer (DBL) (Vogel, 1983; Patterson et al., 1991; Lesser et al., 1994; Shashar et al., 1996). The DBL is the portion of the boundary layer closest to the



Fig. 9. Flow speed histograms at two sites on the Kaneohe Bay reef flat. Flow speeds were measured with an electromagnetic current meter deployed 0.5 m above the substratum with a sampling frequency of 2 Hz. Sampling occurred over the times thermistor measurements were made (Figs. 6–8). Refer to Table 2 for site coordinates.



Fig. 10. Time course of acetylene reduction (ethylene production) by algal turfs at five unidirectional flow speeds in the chamber in a typical experiment, which was replicated to provide data in Fig. 12.

organism wherein solute movement is controlled primarily by diffusion. A decrease in the thickness of the DBL represents a decrease in the path length over which a solute diffuses, such that the rate of diffusion will increase inversely and linearly according to Fick's first law of diffusion (Denny, 1988). Increasing the variation in the mean flow speed, i.e. adding turbulence, further increases the advective transport of solutes (Denny, 1988), as does biogenic roughness (Dade, 1993). The period of oscillation also affects the thickness of the momentum boundary layer in a complex manner (Jonsson, 1980; Cousteix, 1986). In our study, the effects of oscillation and turbulence on the boundary layer were not differentiated. The oscillation in the chamber was apparently not sufficient to create a turbulent diffusion boundary layer because the slope of log-log linear regression (Fig. 12) was less than the expected values of 0.6–0.8 (White, 1988; Patterson et al., 1991; Lesser et al., 1994). Although rates of acetylene reduction are apparently controlled by mass transfer of a rate-controlling solute through a laminar boundary layer, oscillation nonetheless must enhance the flux because the absolute rates of acetylene reduction are significantly higher than under unidirectional flow, at least at low flow speeds (Fig. 12).

Identification of the rate-controlling solute for algal turf nitrogen fixation is the subject of ongoing research (Williams and Carpenter, unpubl. data). Certainly, the substrate (acetylene or N_2) for nitrogenase, and O_2 , which is inhibitory to the enzyme (Stewart,



Fig. 11. Extended time course of acetylene reduction by algal turfs in the chamber with oscillatory flow (0.65 m s^{-1}) . The low point after 70 min was correlated with a period of low light.

1975), are contenders. Nitrogen fixation rates are predicted to increase instantaneously in response to increased water flow if nitrogenase substrates or O_2 control the nitrogen fixation rate. For other rate-controlling solutes, a lag for nitrogenase synthesis (Yates and Eady, 1980; Shah et al., 1986; Hill, 1991) should occur before rates increase in response to faster water flow. Examples of such solutes include iron and molybdenum in the cofactors for nitrogenase and nutrients such as ammonium and phosphorus (Entsch et al., 1983; Paerl et al., 1987; Capone, 1988).

Future research on mass transfer limitation of nitrogen fixation in algal turfs will benefit from an engineering scaling approach (e.g., Patterson et al., 1991; Atkinson and Bilger, 1992; Lesser et al., 1994; Thomas and Atkinson, 1997). The approach will help elucidate whether mass transfer-limited rates scale better with laminar or turbulent processes. The assumption of such an approach is that the metabolic rate is controlled by diffusion of a single substrate. This assumption works well for nutrient uptake. Photosynthesis and nitrogen fixation rates, however, are controlled not only by substrate concentration, but also by oxygen concentration, which can influence rates of dark respiration and photorespiration and nitrogenase activity. Although the theoretical effects of multiple controlling processes can be modeled with known diffusion coefficients and kinetic constants, the difficulty lies in obtaining sufficiently detailed empirical data for comparison to theory. For example, nitrogenase activity in this study was described as a Table 3

Flow regime:	Unidirectional		Oscillatory			
Experiment	Coefficient	Exponent	r^2	Coefficient	Exponent	r^2
1	60	0.649	0.544	258	0.803	0.958
2	33	0.269	0.855	791	1.143	0.884
3	26	0.428	0.494	117	0.642	0.514
4	71	0.641	0.818	652	1.052	0.473
5	134	0.765	0.765	27	0.115	0.219
6	69	0.819	0.819	52	0.590	0.516
7	213	0.699	0.699	110	0.321	0.404
8	32	0.824	0.824	77	0.452	0.950
9	65	0.978	0.978	67	0.507	0.940
10	80	0.856	0.856	98	0.475	0.935
11	39	0.880	0.880	63	0.541	0.943
12	73	0.963	0.963	94	0.480	0.757
13	53	0.830	0.83	94	0.333	0.485
Mean	73	0.739	0.794	190	0.573	0.691
SD	51	0.204	0.142	245	0.286	0.261
14	44	0.498	0.985			
15	14	0.417	0.933			
16	55	0.470	0.887			
17	68	0.604	0.693			
Mean $(n=17)$	66	0.682	0.813			
SD	47	0.209	0.140			

Parameters from power functions ($y = a^*x^b$) describing ethylene production (nmol cm⁻² h⁻¹) by algal turfs versus water flow speed (x) in flume experiments. Functions calculated based on five flow speeds

function of water flow based on only five speeds. More speeds were not possible because of the inherent analytical constraint of slow metabolic rates relative to analysis sensitivity, requiring longer, and thus fewer, incubations.

Another general challenge in applying engineering approaches to the metabolism of algal turf communities is to estimate a realistic roughness height. Although this is relatively straightforward for hard corals, algal turfs comprise many filaments of varying height, and thus represent non-uniformly distributed roughness. The degree to which flow moves through the canopy, ponds within the turf, or is deflected above it are presently unknown, but are likely to be important in controlling rates of metabolism of the algal turf community. Our measurements of shear velocity were scaled for the turf community and not for patches and individuals within the turfs, as discussed in the Introduction and in Paerl et al. (1995). The variation between experiments, however, might reflect some of the fine scale architecture of the turfs. For example, in certain experiments, the exponent of the power functions describing acetylene reduction as a function of flow speed was within the range (0.6-0.8) expected for a transition to a turbulent diffusion boundary layer. We did not investigate turbulence structure in our experiments and the smallest scale fluctuations were missed by the slow rate of sampling (10 Hz). Algal turfs grow in a well-developed benthic boundary layer (Carpenter and



Fig. 12. Log-log linear regressions of the mean rate (\pm SE, n=13 in oscillatory flow, 17 in unidirectional flow) of acetylene production by algal turfs versus average water flow speeds in the incubation chamber. $r^2 =$ coefficient of determination.

Williams, 1993; this study) but the importance of small scale turbulence to metabolism of the turf community and individual components remains to be determined.

It is difficult to simulate complex natural conditions for physiological experiments. This is particularly so for water flow (Patterson et al., 1991; Howarth et al., 1993; Paerl et al., 1995), for which few empirical data exist, at least for coral reefs (Andrews and Pickard, 1990; Carpenter and Williams, 1993; Shashar et al., 1996). Certainly our chamber was not ideal, although the data presented here guided subsequent improvements. Nonetheless, we demonstrated that acetylene reduction was sensitive to water flow and that oscillatory flow with increased turbulence changed the relationship of acetylene reduction to flow speeds in a statistically significant way. Measurements of water flow over algal turfs in the field enabled us to assess the difference in flow regimes between natural and experimental conditions and the limitations to extrapolation from chamber experiments. The water flow regime in our chamber differed from the natural environment, and was very different from the predominantly stagnant water conditions in most other studies of coral reef nitrogen fixation (see Introduction). The implication follows that the biogeochemical significance of nitrogenase activity on coral reefs might

be underestimated. Current understanding of nitrogen cycles on coral reefs rests heavily on measurements of physiological rates that have not been calibrated against the physical conditions in the natural environment (D'Elia and Wiebe, 1990).

Acknowledgements

Stacey Longmore, Rita Peachey, and Gwen Lowe assisted with field work and algal sampling. Jake Sibley performed the image analyses. We thank the staff of HIMB for facilitating our visits. We are indebted to Pat Ewanchuk for able assistance in all aspects of the project. Dr. Marlin Atkinson sponsored our 'take-over' of his laboratory and provided stimulating discussions and perspectives. Dr. Flo Thomas provided advice during the preliminary phases of the research. Our spouses tolerated long absences from home, and helped us in the field and lab during their 'vacations'. This research was supported by collaborative research grants from the National Science Foundation (OCE9317733, OCE9314470). This is contribution #284 from the Coastal & Marine Institute, San Diego State University.

References

- Adey, W.H., Steneck, R.S., 1985. Highly productive eastern Caribbean reefs: Synergistic effects of biological, chemical, physical, and geological factors. In: Reaka, M.L. (Ed.), The Ecology of Coral Reefs, Vol. 3. National Oceanic and Atmospheric Administration, Office of Undersea Research, Rockville, Maryland, pp. 163–187.
- Andrews, J.C., Gay, S., Sammarco, P.W., 1988. Influence of circulation on self-seeding patterns at Helix Reef–Great Barrier Reef. In: Proceedings of the Sixth International Coral Reef Symposium, Vol. 2, pp. 469–474.
- Andrews, J.C., Pickard, G.L., 1990. The physical oceanography of coral-reef systems. In: Dubinsky, Z. (Ed.), Coral Reefs. Elsevier, Amsterdam, pp. 11–48.
- Atkinson, M.J., Bilger, R.W., 1992. Effects of water velocity on phosphate uptake in coral reef-flat communities. Limnol. Oceanogr. 37, 273–279.
- Bilger, R.W., Atkinson, M.J., 1992. Anomalous mass transfer of phosphate on coral reef flats. Limnol. Oceanogr. 37, 261–272.
- Burris, R.H., 1976. Nitrogen fixation by blue-green algae of the Lizard Island area of the Great Barrier Reef. Austral. J. Plant Physiol. 3, 41–51.
- Capone, D.G., 1988. Benthic nitrogen fixation. In: Carpenter, E.J., Capone, D.G. (Eds.), Nitrogen Cycling in Coastal Marine Environments. Wiley, New York, pp. 85–123.
- Carpenter, R.C., 1986. Partitioning herbivory and its effect on coral reef algal communities. Ecol. Monogr. 56, 345–363.
- Carpenter, R.C., 1990. Mass mortality of *Diadema antillarum*. I. Long-term effects on sea urchin populationdynamics and coral reef algal communities. Mar. Biol. 104, 67–77.
- Carpenter, R.C., Hackney, J.M., Adey, W.H., 1991. Measurements of primary productivity and nitrogenase activity of coral reef algae in a chamber incorporating oscillatory flow. Limnol. Oceanogr. 36, 40–49.
- Carpenter, R.C., Williams, S.L., 1993. Effects of algal turf canopy height and microscale substratum topography on flow speed profiles in a coral forereef environment. Limnol. Oceanogr. 38, 687–694.
- Charpy-Roubaud, C.J., Charpy, L., Cremoux, J.L., 1990. Nutrient budget of the lagoonal waters in an open central South Pacific atoll (Tikehau Tuamotu, French Polynesia). Coral Reefs 107, 67–73.

- Cheroske, A., 1997. Effects of disturbance on algal turf biomass, canopy height, and community structure in Kaneohe Bay, Oahu, Hawaii. MS thesis, San Diego State University, San Diego.
- Cousteix, J., 1986. Three-dimensional and unsteady boundary-layer computation. Annu. Rev. Fluid Mech. 18, 173–196.
- Dade, W.B., 1993. Near-bed turbulence and hydrodynamic control of diffusional mass transfer at the sea floor. Limnol. Oceanogr. 38, 52–69.
- D'Elia, C.F., Wiebe, W.J., 1990. Biogeochemical nutrient cycles in coral-reef ecosystems. In: Dubinsky, Z. (Ed.), Coral Reefs. Elsevier, Amsterdam, pp. 49–74.
- Denny, M.W., 1988. Biology and the Mechanics of the Wave-swept Environment. Princeton University Press, Princeton.
- Entsch, B., Sim, R.G., Hatcher, B.G., 1983. Indications from photosynthetic components that iron is a limiting nutrient in primary producers on coral reefs. Mar. Biol. 73, 17–30.
- Fabricius, K.E., Genin, A., Benayahu, Y., 1995. Flow-dependent herbivory and growth in zooxanthellae-free soft corals. Limnol. Oceanogr. 40, 1290–1301.
- Flett, R.J., Hamilton, R.D., Campbell, N.E.R., 1976. Aquatic acetylene-reduction techniques: Solutions to several problems. Can. J. Microbiol. 22, 43–51.
- Genin, A., Karp, L., Miroz, A., 1994. Effects of flow on competitive superiority in scleractinian corals. Limnol. Oceanogr. 39, 913–924.
- Hackney, J.M., Carpenter, R.C., Adey, W.H., 1989. Characteristic adaptations to grazing among algal turfs on a Caribbean coral reef. Phycologia 28, 109–119.
- Hamner, W.M., Wolanski, E., 1988. Hydrodynamic forcing functions and biological processes on coral reefs: A status review. In: Proceedings of the Sixth International Coral Reef Symposium, Vol. 1, pp. 103–113.
- Hanson, R.B., Gundersen, K., 1977. Relationship between nitrogen fixation (acetylene reduction) and the C:N ratio in a polluted coral reef ecosystem, Kaneohe Bay, Hawaii. Estuarine Coastal Mar. Sci. 5, 437–444.
- Hatcher, A.I., Frith, C.A., 1985. The control of nitrate and ammonium concentrations in a coral reef lagoon. Coral Reefs 4, 101–110.
- Hatcher, B.G., 1988. Coral reef primary productivity: A beggar's banquet. Trends Ecol. Evol. 3, 106-111.
- Hatcher, B.G., Imberger, J., Smith, S.V., 1987. Scaling analysis of coral reef systems: An approach to problems of scale. Coral Reefs 5, 171–181.
- Helmuth, B., Sebens, K., 1993. The influence of colony morphology and orientation to flow on particle capture by the scleractinian coral Agaricia agaricites (Linnaeus). J. Exp. Mar. Biol. Ecol. 165, 251–278.
- Hill, S., 1991. Physiology of nitrogen fixation in free-living heterotrophs. In: Stacey, G., Burris, R.H., Evans, H.J. (Eds.), Biological Nitrogen Fixation. Chapman and Hall, New York, pp. 87–134.
- Howarth, R.W., Marino, R.J., Lane, J., Cole, J., 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems, I. Rates and importance. Limnol. Oceanogr. 33, 669–687.
- Howarth, R.W., Butler, T., Lunde, K., Swaney, D., Chu, H.J., 1993. Turbulence and planktonic nitrogen fixation: A mesocosm experiment. Limnol. Oceanogr. 38, 1696–1711.
- Jokiel, P.L., 1978. Effects of water motion on reef corals. J. Exp. Mar. Biol. Ecol. 35, 87-97.
- Jonsson, I.J., 1980. A new approach to oscillatory rough turbulent boundary layers. Ocean Eng. 7, 109–152.
- Kinsey, D.W., 1985. Metabolism, calcification and carbon production. I. System level studies. In: Proceedings of the Fifth International Coral Reef Congress, Vol. 4, pp. 505–526.
- Larkum, A.W.D., Kennedy, I.R., Muller, W.J., 1988. Nitrogen fixation on a coral reef. Mar. Biol. 98, 143-155.
- Lesser, M.P., Weiss, V.M., Patterson, M.R., Jokiel, P.L., 1994. Effects of morphology and water motion on carbon delivery and productivity in the reef coral, *Pocillopora damicornis* (Linnaeus): Diffusion barriers, inorganic carbon limitation, and biochemical plasticity. J. Exp. Mar. Biol. Ecol. 178, 153–179.
- Mague, T.H., Holm-Hansen, O., 1975. Nitrogen fixation on a coral reef. Phycologia 14, 87-92.
- Paerl, H.W., Crocker, K.M., Prufert, L.E., 1987. Limitation of N₂ fixation in coastal marine waters: Relative importance of molybdenum, iron, phosphorus, and organic matter availability. Limnol. Oceanogr. 32, 525–536.
- Paerl, H.W., Pinckney, J.L., Kucera, S.A., 1995. Clarification of the structural and functional roles of heterocysts and anoxic microzones in the control of pelagic nitrogen fixation. Limnol. Oceanogr. 403, 634–638.
- Patterson, M.R., Sebens, K.P., 1989. Forced convection modulates gas exchange in cnidarians. Proc. Natl. Acad. Sci. USA 86, 8833–8836.

- Patterson, M.R., Sebens, K.P., Olson, R.R., 1991. In situ measurements of flow effects on primary production and dark respiration in reef corals. Limnol. Oceanogr. 36, 936–948.
- Shah, V.K., Imperial, J., Ugalde, R.A., Ludden, P.W., Brill, W.J., 1986. In vitro synthesis of the ironmolybdenum cofactor of nitrogenase. Proc. Natl. Acad. Sci. USA 83, 1636–1640.
- Shashar, N., Cohen, Y., Loya, Y., Sar, N., 1994. Nitrogen fixation (acetylene reduction) in stony corals: Evidence for coral-bacteria interactions. Mar. Ecol. Prog. Ser. 111, 259–264.
- Shashar, N., Feldstein, T., Cohen, Y., Loya, Y., 1994. Nitrogen fixation (acetylene reduction) on a coral reef. Coral Reefs 13, 171–174.
- Shashar, N., Kinane, S., Jokiel, P.L., Patterson, M.R., 1996. Hydromechanical boundary layers over a coral reef. J. Exp. Mar. Biol. Ecol. 199, 17–28.
- Sebens, K.P., Johnson, A.S., 1991. Effects of water movement on prey capture and distribution of reef corals. Hydrobiologia 226, 91–101.
- Shieh, W.Y., Lin, Y.M., 1992. Nitrogen fixation (acetylene reduction) associated with the zoanthid Palythoa tuberculosa Esper. J. Exp. Mar. Biol. Ecol. 163, 31–41.
- Smith, S.V., 1984. Phosphorus versus nitrogen limitation in the marine environment. Limnol. Oceanogr. 29, 1149–1160.
- Smith, S.V., 1988. Mass balance in coral-reef-dominated areas. In: Jansson, B.-O. (Ed.), Lecture Notes on Coastal and Estuarine Studies: Coastal-offshore Ecosystem Interactions, Vol. 22. Springer, Berlin, pp. 209–226.
- Stewart, W.D.P., 1975. Nitrogen Fixation by Free-living Microorganisms. Cambridge University Press, Cambridge.
- Taylor, B.R., 1983. Assays of microbial nitrogen transformation. In: Carpenter, E.J., Capone, D.G. (Eds.), Nitrogen in the Marine Environment. Academic Press, New York, pp. 809–838.
- Thomas, F.I.M., Atkinson, M.J., 1997. Ammonia uptake of coral reefs: Effects of surface roughness and water velocity on mass transfer. Limnol. Oceanogr. 42, 81–88.
- Vogel, S., 1983. Life in Moving Fluids. Willard-Grant Press, Boston.
- Webb, K.L., DuPaul, W.D., Wiebe, W., Sottile, W., Johannes, R.E., 1975. Enewetak (Eniwetok) Atoll: Aspects of the nitrogen cycle on a coral reef. Limnol. Oceanogr. 20, 198–210.
- White, F.M., 1988. Heat and Mass Transfer. Addison-Wesley, New York.
- Wiebe, W.J., Johannes, R.E., Webb, K.L., 1975. Nitrogen fixation in a coral reef community. Science 188, 257–259.
- Wilkinson, C.R., Sammarco, P.W., 1983. Effects of fish grazing and damselfish territoriality on coral reef algae, II. Nitrogen fixation. Mar. Ecol. Prog. Ser. 13, 15–19.
- Wilkinson, C.R., Williams, D.D.McB., Sammarco, P.W., Hogg, R.W., Trott, L.A., 1984. Rates of nitrogen fixation on coral reefs across the continental shelf of the central Great Barrier Reef. Mar. Biol. 80, 255–262.
- Williams, S.L., Carpenter, R.C., 1990. Photosynthesis versus light relationships among components of coral reef algal turfs. J. Phycol. 26, 36–40.
- Williams, S.L., Carpenter, R.C., 1997. Grazing effects on nitrogen fixation in coral reef algal turfs. Mar. Biol. (in press).
- Williams, W.M., Viner, A.B., Broughton, W.J., 1987. Nitrogen fixation (acetylene reduction) associated with the living coral Acropora variabilis. Mar. Biol. 94, 531–536.
- Yates, M.G., Eady, R.R., 1980. The physiology and regulation of nitrogen fixation. In: Subba Rao, N.S. (Ed.), Recent Advances in Biological Nitrogen Fixation. Holmes and Meier, New York, pp. 88–120.