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In situ sampler-incubator for simultaneous biological rate measurements via tracers and net chemical change

Abstract—A simple device has been developed to facilitate in situ incubations. The hydraulic in situ time-series sampler (HINTS) facilitates collection and incubation of a large volume of water (16 liters) without need of manipulating the sample at the surface. Injection of isotopic tracers and subsampling can be accomplished remotely via tubing connected to the incubation chamber. HINTS consists of a rigid, clear polycarbonate vessel with a gas-impermeable plastic incubation bag inside. A two-way valve permits the incubation chamber to be filled with ambient water or to communicate with the surface via a sampling and tracer-injection line. Opening and closing the two-way valve and filling and emptying the incubation chamber are done hydraulically. The HINTS is particularly useful for comparing isotopic and chemical-change methods of measuring productivity, obtaining time series of chemical properties, physiological rates or organism abundance, and determining net community PQ and C : N assimilation ratios. Preliminary tests suggest good agreement between O_2- and ΣCO_2-based measurements of net community production. The community photosynthetic quotient averaged 1.4±0.3 (95% C.I.) in four late-fall and winter measurements.

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Studies of biological rates in aquatic systems commonly involve collecting water, filling numerous bottles, adding a stable or radioactive isotope, incubating the bottles in situ or on deck, collecting bottles at various times, and performing a filtration or chemical analysis. This approach is labor

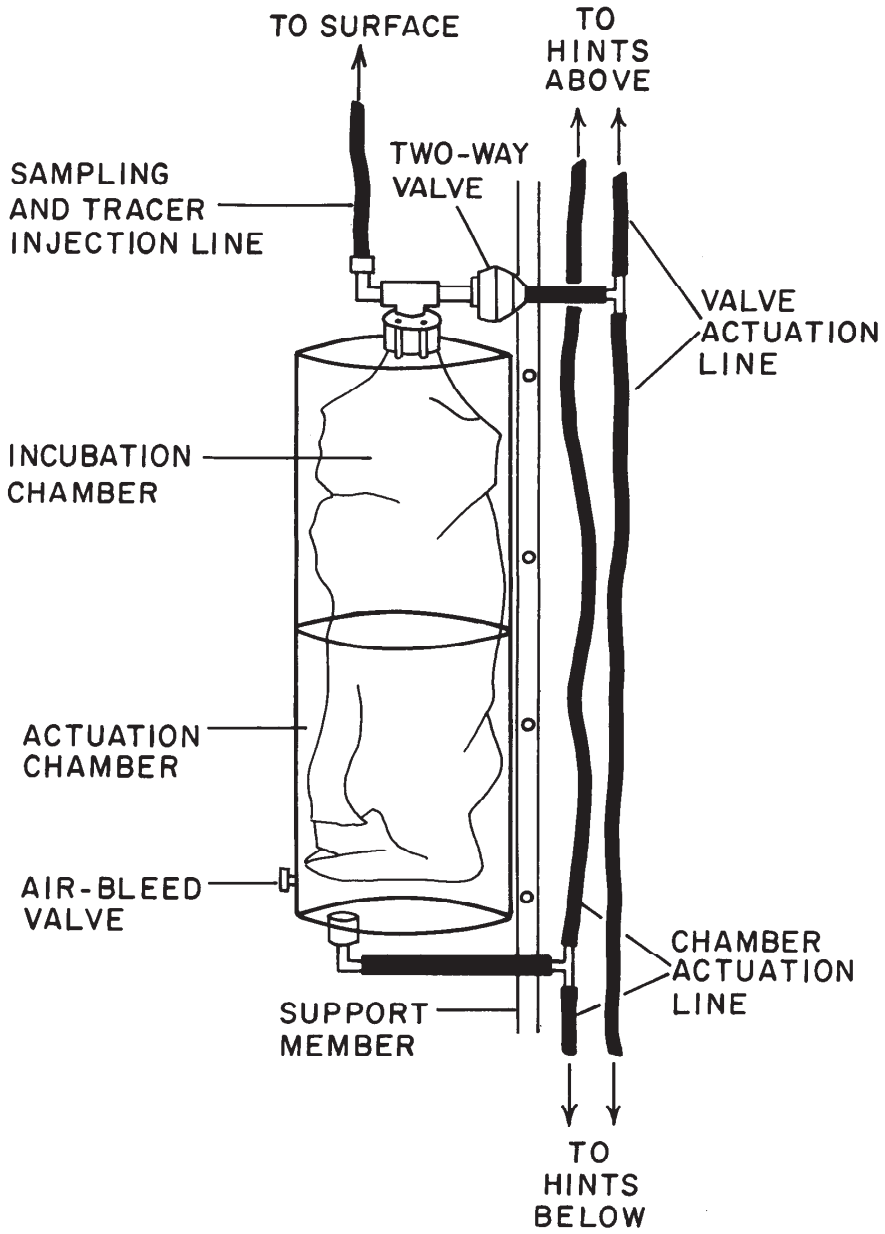

Fig. 1. Schematic diagram showing components of the hydraulic in situ time-series sampler (HINTS). The two-way valve and chamber actuation lines are connected in series for the number of chambers on the array. Each HINTS has a separate subsampling and tracer injection line that communicates directly with the surface. Tracer isotope solution is added to empty HINTS. The two-way valve is closed to ambient water by applying suction to the valve actuation line. Water is then pumped out of the actuation chamber via the chamber actuation line. This process draws water from the surface down the sampling and tracer injection line into the incubation chamber. Meanwhile, isotope is injected into the sampling and tracer injection line through a septum with a syringe. A volume of chaser equal to the dead volume of the sample line (dependent on depth of the HINTS and equal to 18 ml m^{-1}) is drawn into the incubation chamber to ensure that all the tracer has been introduced. Filling the HINTS with ambient water is accomplished by pressurizing the valve actuation line, which opens the two-way valve to the ambient water. Water is pumped out of the actuation chamber via the chamber actuation line causing ambient seawater to be drawn into the incubation chamber. At a rate of 1.5 liters min^{-1} it takes about 11 min to fill the 16-liter incubation chamber. Several incubators can be filled at once. The HINTS is sampled by closing the two-way valve and pumping water into the actuation chamber, forcing the incubator contents up the tracer injection sample line and into the waiting sample bottle. The dead volume of seawater in the sample line must be discarded to ensure that water held in the sample line is cleared before a sample is collected.

intensive and has three drawbacks. First, with so much handling there is the possibility of sample contamination. Second, it is difficult to incubate a large number of bottles under strictly uniform conditions. Third, on-deck incubations have difficulty simulating depth-dependent variability in temperature and light quality. No sampler accurately simulates the variable light field experienced by phytoplankton due to turbulent motion in the water column.

Our objectives were threefold: to conveniently collect and incubate a large volume of water in situ, to add isotopic tracer remotely, and to subsample from the surface without disturbing the incubator. We describe a large-volume incubator (HINTS: hydraulic in situ time-series sampler) that was designed to avoid possible artifacts resulting from containing small volumes (Venrick et al. 1977). We also wanted sufficient volume to provide a time series for a contemporaneous suite of chemical and biological analyses such as particulate C (PC), particulate N (PN), Chl *a*, O_2, ΣCO_2, NO_3^-, PO_4, SiO_4, and organism counts.

The HINTS is suspended beneath a surface float. The outer part of the device (Fig. 1) consists of two 9-liter Nalgene polycarbonate vacuum jars glued together. A bag made of a special trilaminate film obtained from the Packaging Center (Miami, Florida) and consisting of low-density polyethylene (LPE), nylon, and ethylene triacetate (ETA) inside the polycarbonate jars serves as the incubation chamber. The chamber actuation line connected to a pressure or suction source at the surface fills or empties the incubation chamber by pumping water into or out of the space between the incubation chamber and the rigid walls of the HINTS. The two-way valve at the top of each HINTS determines whether the incubation chamber is open to the ambient water or connected to the surface via the sampling and tracer injection line; it is operated by the valve actuation line connected to a source of pressure or suction at the surface. A typical sequence of spike, fill, and sample operations (Fig. 1) requires <20 min to collect and spike a 16-liter sample of water.

The adequacy of tracer mixing was evaluated by spiking the HINTS with rhodamine dye and then filling in the normal manner. The sample line was then connected to the flow cell of a Turner Designs fluorometer, and the dye concentration was then monitored continuously via strip-chart recorder as the sample was pumped out of the incubation chamber. The flatness of the recorder output showed that the dye was uniformly distributed throughout the volume of the incubation chamber by the simple action of filling. Also, after one flushing, little evidence of the rhodamine remained, and after two flushings there was no detectable dye.

The HINTS is made entirely of nontoxic plastics (polycarbonate, nylon, LPE) to minimize the risk of metal contamination during spiking and filling. The trilaminate film of the incubation chamber is very impermeable to dissolved O_2 (Fig. 2). The bag

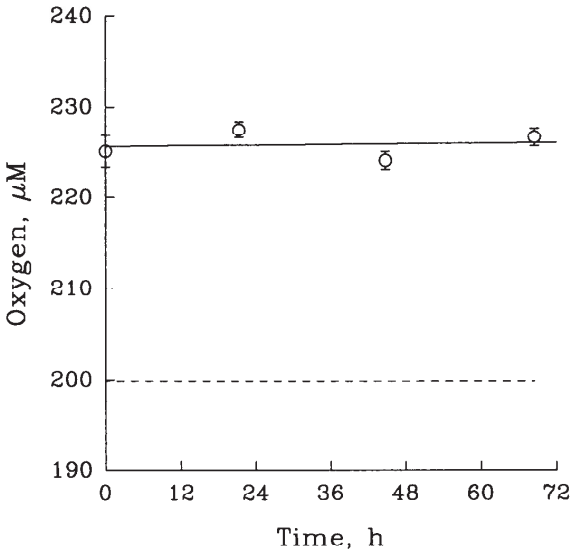

Fig. 2. Permeability to oxygen of the bags used to make the incubation chamber of the HINTS was tested by filling the HINTS with sterile, distilled water that had an initial O_2 concentration of 227 μM. About half of the incubation chamber volume was then discharged by pumping sterile, distilled water with an O_2 concentration of 200 μM (dashed line) into the actuation chamber. The result was an O_2 gradient across the bag of 27 μM, much larger than would normally be encountered in the field. Water from the incubation chamber was then sampled three times over 72 h, much longer than a normal 24-h incubation. Regression indicates that the rate of change [-0.01 ± 0.10 (95% C.I.) μM h^{-1}] was not significantly different from zero. For all practical purposes the incubation chamber is thus impermeable to O_2. Error bars are ±1 SE (n = 3).

Table 1. Concentrations (mean±SE) of O_2, ΣCO_2, and NO_3^- (from only one sampler) as a function of time for samples withdrawn from five replicate HINTS chambers. (No sample taken, —; not detectable, ND.) Only one incubator had sufficient water at the end of the experiment for ΣCO_2 analysis.

1990	Time (hours)	O_2	ΣCO_2	NO_3
		(mmol m^{-3})		
17 Jan	1330	—	1,960.8±0.8	1.21
17 Jan	1630	357.8±1.0	1,955.4±1.3	—
17 Jan	2100	354.8±0.2	1,959.7±1.0	—
18 Jan	0546	350.7±0.6	1,960.0±1.1	0.53
18 Jan	0746	349.9±0.3	1,957.1±2.2	—
18 Jan	1230	352.9±0.6	1,951.8±1.0	ND
18 Jan	1400	361.7±0.4	1,952.5	ND

and polycarbonate vessel are transparent to PAR but do absorb strongly in the UV. The tubing used for the sampling and tracer injection line is black nylon (Nylaflo), also impermeable to O_2 and opaque to prevent light shock during sampling and to discourage algal growth. The entire HINTS unit can be cleaned with a hot bleach solution (Clorox) or 10% HCl and flushed with freshwater.

Experiments to test the replication of results from multiple HINTS units were conducted from the end of the pier at the Graduate School of Oceanography (University of Rhode Island) on 17–18 January 1990. Five HINTS chambers were hung from the pier ~1 m below the surface. Net chemical changes in O_2, ΣCO_2, and NO_3^- were followed for 24 h.

Oxygen was determined by the Winkler method (Carritt and Carpenter 1966). To increase analytical precision, we detected the end-point amperometrically (Culberson and Huang 1987). ΣCO_2 determinations were made by coulometric back-titration of extracted CO_2 (Johnson et al. 1985, 1987). ΣCO_2 samples were collected in 100-ml glass-stoppered serum bottles and poisoned with $HgCl_2$. Samples for isotope enrichment and particulate C and N were filtered onto precombusted 47-mm GF/F glass-fiber filters. The particulate material was combusted with Pt-catalyzed copper oxide with Cu metal as reductant in evacuated and sealed Pyrex tubes at 550°C. Our laboratory comparison of this combustion method to quartz tubes at 900°C indicated that the 550°C combustion was complete for the surface-water particles analyzed. Combustion products were separated cryogenically on a vacuum preparatory system and quantified manometrically. N_2 gas was collected by Toepler pumping, and the isotopic composition of the gas sample was analyzed on a Nuclide 660 isotope-ratio mass spectrometer. Submarine, photosynthetically available, quantum scalar irradiance was measured with a Biospherical Instruments QSP-200 4π PAR sensor. Chl *a*, NO_2^-, and NO_3^- were measured with standard methods given by Parsons et al. (1984).

On 17 January 1990 water temperature was 1.8°C, salinity was 32‰, and Chl *a* concentration was 3.4 mg m^{-3}. Microscopic examination indicated that the phytoplankton was dominated by *Skeletonema costatum*. The HINTS were filled at 1330 hours. The chambers were then sampled six times over 24 h to determine the net chemical changes in O_2, ΣCO_2, and NO_3^- (Table 1). The HINTS chambers received 2.30 Einst m^{-2} d^{-1} of PAR on 17 January and 3.36 on 18 January. Replication between HINTS averaged ±1.4 (SD) μmol O_2 $liter^{-1}$ and ±2.3 (SD) μmol CO_2 $liter^{-1}$. NO_3^- samples were taken from only one of the units.

Oxygen consumption at night was −8±2 (95% C.I.) μM (Fig. 3A). Production on 18 January between 0746 and 1400 hours was 11.8±1 (95% C.I.) μM. The data for ΣCO_2 show the expected decline during the day and increase at night but have interesting variations (Fig. 3B). CO_2 production at night occurs mostly during the first 5 h of darkness rather than at a roughly constant rate as does O_2 consumption. Also note that uptake of C begins sometime between 0524 and 0746 hours, while O_2 production begins only after 0746. This pattern does not necessarily imply dark fixation of C, because the first measurable increase in light intensity occurred between 0650 and 0705 hours. Nighttime production of ΣCO_2 was 4.6±2 (80% C.I.) μM. Consumption of ΣCO_2 between 0524 and 1400 hours on 18 January was −7.5±3 (80% C.I.) μM. Chl-specific net production rate was 0.35 μmol C (μg Chl *a*)$^{-1}$ h^{-1}. This rate is reasonable for 2°C. Sakshaug (1977) reported a net photosynthetic rate for *S. costatum* (SK6C) at 4°C of

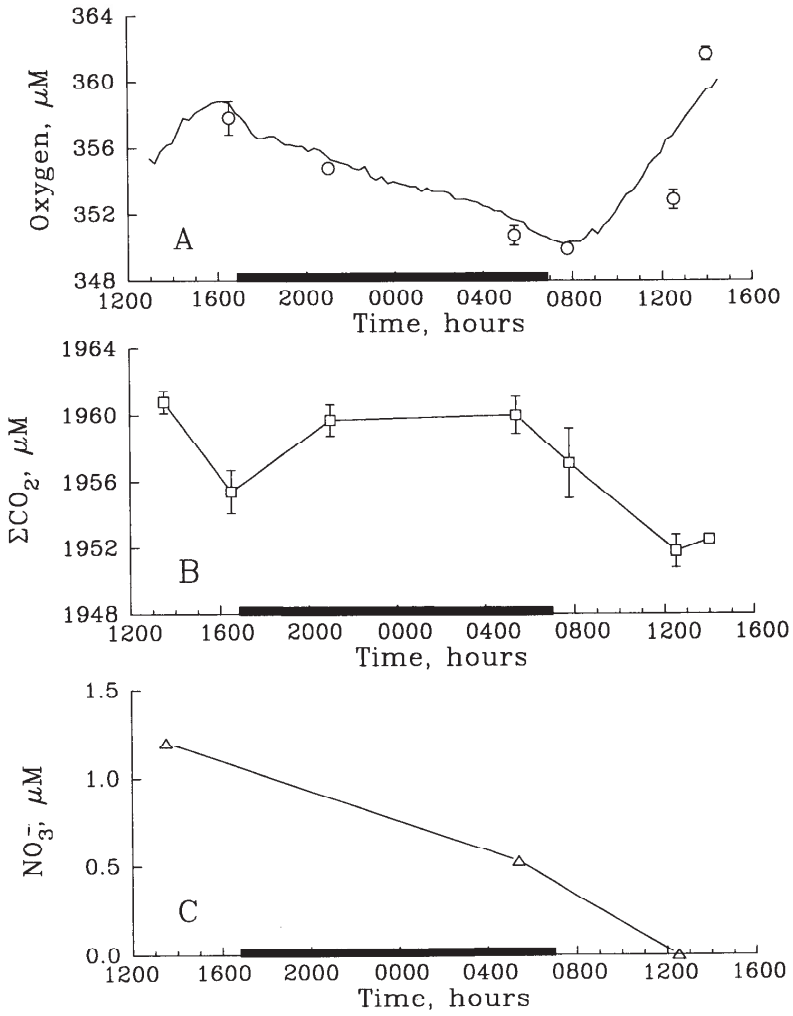

Fig. 3. Time series collected from five replicate HINTS in lower Narragansett Bay on 17–18 January 1990. A. Symbols with error bars (±1 SE) are the average O_2 from the five HINTS. Solid line is a continuous record from an O_2 sensor mounted in a transparent chamber similar to the HINTS and is shown for comparison. B. Symbols with error bars (±1 SE) are the average ΣCO_2 from the five HINTS. C. Symbols represent single analyses of NO_3^- from just one HINTS.

0.33 μmol C (μg Chl *a*)$^{-1}$ h^{-1}. Yoder (1979) reported rates for the same clone ranging from 0.20 at 0°C to 0.39 μmol C (μg Chl *a*)$^{-1}$ h^{-1} at 5°C.

Sampling frequency for NO_3^- (Fig. 3C) was not high enough to describe a diel pattern of NO_3^- uptake. A tracer uptake experiment was not attempted. Without an early morning sample it is impossible to say whether NO_3^- uptake can occur at very low light intensities as was found for CO_2, or if the light intensity must exceed 24 μEinst m^{-2} s^{-1} as appeared necessary for O_2 production. Sometime between 0546 and 1230 hours, NO_3^- available to the phytoplankton within the HINTS chambers was exhausted. Other forms of N such as NH_4^+ and urea were not measured. Uptake of NO_3^- between 0546 and 1230 hours was 0.53 μM. Uptake between 1330 on 17 January and 1230 hours on 18 January was 1.21 μM.

The utility of HINTS for tracer uptake experiments was tested on 14 November 1990 by running a comparison against a conventional 2-liter polycarbonate bottle. A second aspect of this experiment was to determine whether significant settling of particles occurred in the HINTS. A HINTS unit was suspended from the URI pier at a depth of 2 m and filled with bay water. Some

Table 2. Summary of Chl *a*, initial NO_3^- concentration, net community O_2 and ΣCO_2 production, and community PQ (mean±SE).

	Time (hours)	Chl *a* (mg m^{-3})	$[NO_3^-]$	ΔO_2	$\Delta\Sigma CO_2$	PQ
			(mmol m^{-3})			
17 Jan 90	0746–1400	3.4	1.2	11.8±0.5	−7.5±1.1	1.6±0.2
14 Nov 90	1315–1600	1.1	5.9	3.6±0.2	−2.2±0.5	1.6±0.4
17 Jan 91	0700–1010	3.1	8.5	3.0±1.0	−2.3±1.0	1.3±0.7
17 Jan 91	1317–1530	3.1	8.5	10.7±0.6	−7.9±0.5	1.4±0.1

of the 16 liters of bay water collected in the HINTS was pumped to the surface and used to fill a 2-liter polycarbonate bottle. $^{15}NO_3^-$ was injected into the tracer injection line and drawn into the HINTS along with 700 ml of bay water as a chaser. Ambient NO_3^- concentration was 5.9 μM, and 1.0 μM of $^{15}NO_3^-$ was added. Sufficient $^{15}NO_3^-$ was added to the 2-liter bottle to produce a similar 1.0-μM enrichment. The 2-liter bottle was then suspended at the same depth as the HINTS. Periodically the HINTS and the 2-liter bottle were agitated by tugging on the lines to simulate the motion they would normally experience at sea from wave action. After 5 h a 1-liter sample was withdrawn from the HINTS and filtered for analysis of particulate C and N (PCN) and isotopic enrichment. The 2-liter bottle was shaken vigorously to ensure that any particles that may have settled during incubation were resuspended before the contents were filtered.

Atom% enrichment, PN, and PC agreed to within 9% in this single experiment. The results suggest that, at least over a 5-h period, settling of particles is not a problem in the HINTS. Presumably at sea or on a large lake wave action would restrict particle settling. Although this trial is unreplicated, the good agreement between the HINTS and the conventional bottle method suggests that the HINTS can be used to expedite tracer productivity studies.

Finally, we compared net chemical change and tracer uptake in the HINTS. This experiment was performed on 17 January 1991 at the same site as the earlier experiments. Chl *a* concentration was similar to that encountered in January 1990 (Table 2). Daytime net production was slightly higher than in 1990 (i.e. 15.4 vs. 11.8 mmol O_2 m^{-3}). The NO_3^- concentration in 1991 was 8.5 mmol m^{-3} and showed no measurable change over the incubation period (not shown). There was a small but measurable uptake of $^{15}NO_3^-$ between 0700 and 1330 hours, i.e. 0.019 mmol N m^{-3}. The sharp increase in production observed in both O_2 and ΣCO_2 in the afternoon was not reflected in the NO_3^- uptake rate (Fig. 4), and in fact there was a net decrease in the atom% enrichment of the particulate material at this time. These results indicate the unique diurnal information that can be provided by the time-series studies that the HINTS facilitates. For example, relative to the time-zero sample, there was the usual net increase in atom% over the day. The 1330 sample suggests, however, that N uptake was not uniform over time. Dilution of the ^{15}N label caused by rapid nitrification could account for the decrease in atom% enrichment (Ward et al. 1989) but would require very high rates of NO_3^- uptake and nitrification in the light. More likely, the decrease in atom% ^{15}N was due to dominance of uptake by regenerated N such as NH_4^+ or urea.

The weighted average of the four measurements of net community photosynthetic quotient($\Delta O_2/-\Delta\Sigma CO_2$, Table 2) is 1.4± 0.3 (95% C.I.). There is considerable interest in the measurement of community PQ because it should respond to the form of combined nitrogen utilized. Platt et al. (1989) have suggested that a relationship exists between the *f*-ratio and PQ. The theoretical PQ of a community utilizing NH_4^+ or urea as the dominant source of N is 1.1±0.1 (SD), and for NO_3^- it is 1.4±0.1 (SD) (Laws 1991). The range of values is small, so the precision of the PQ measurements must be high. If we want to resolve changes in PQ of 0.05, then ΔO_2 and $\Delta\Sigma CO_2$ must be measured with a standard error of

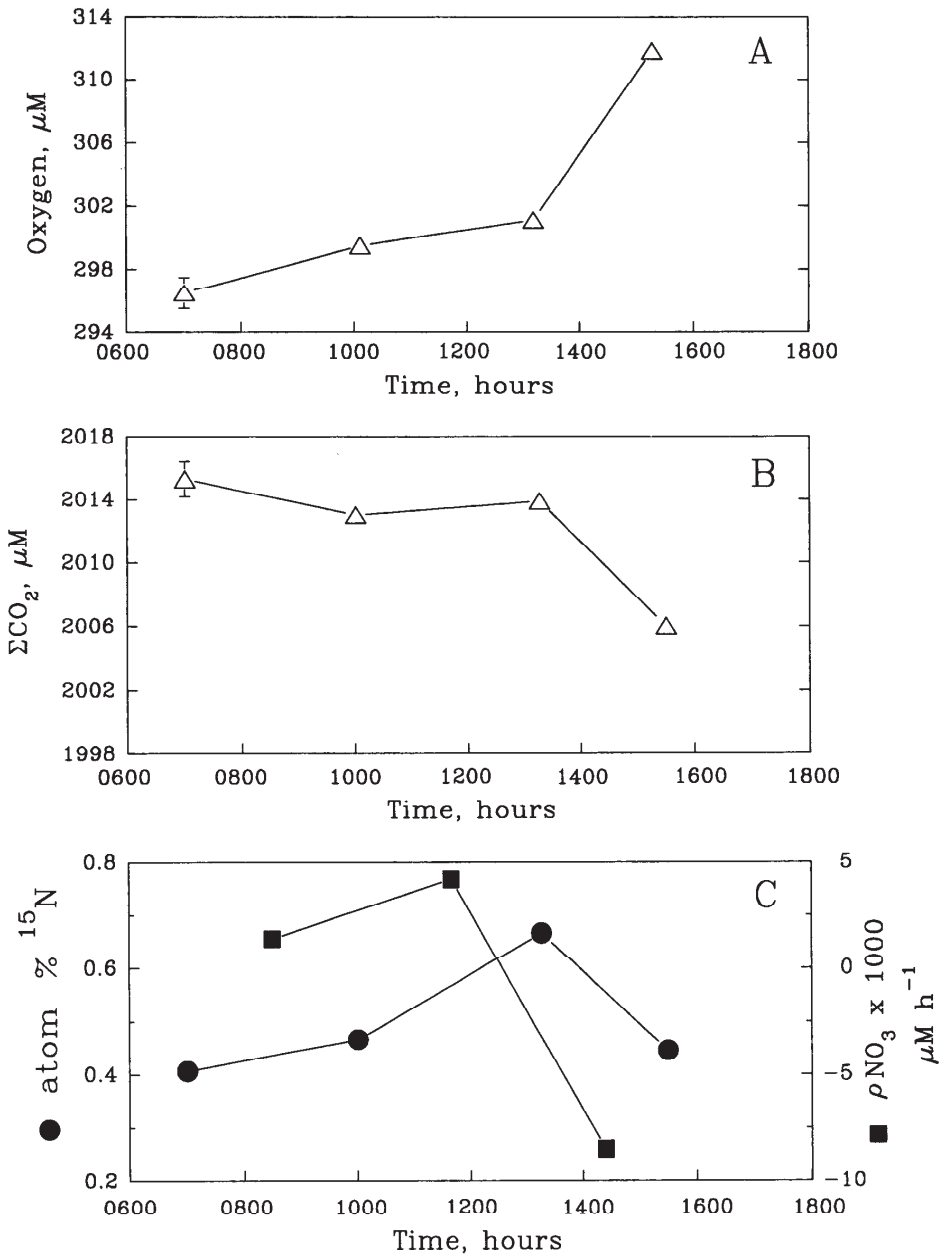

Fig. 4. Time-series observations made on 17 January 1991 in lower Narragansett Bay.

3%. If daily production is a relatively low 5 mmol C m^{-3} (production in Narragansett Bay ranges from 0.2 to 3.3 g C m^{-2} d^{-1} or 7–110 mmol C m^{-3} d^{-1} assuming a 3-m euphotic zone; Furnas et al. 1976) then, given an analytical precision of 0.3 μM for O_2, a standard error of 3% could be achieved by averaging four replicates. Given an analytical precision of 0.5 μM for the ΣCO_2 analysis, 10 replicates would be needed. Because of its large-volume incubator, the HINTS can easily provide the required number of replicates.

Experiments in which tracer measurements are compared to net chemical changes can answer many questions and clarify uncertainties regarding the relationship between net community production and ^{14}C- or ^{15}N-based measurements of total or new production. The HINTS was designed with such experiments in mind, and its use eliminates the variability introduced by per-

forming the tracer and net chemical change measurements in separate bottles.

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