MAMALA BAY STUDY

MICROBIAL ASPECTS OF POINT AND NON-POINT SOURCE POLLUTION IN MAMALA BAY: BATHER AND LIGHT EFFECTS ON SEWAGE-INDICATOR BACTERIA

PROJECT MB-7

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5 JULY, 1995

1 EXECUTIVE SUMMARY

The present study contributed to the effort to generate an integrated coastal management plan for Mamala Bay by examining the influences of bathers, sunlight and protistan predators on three indicator bacteria, *Escherichia coli,* enterococci and *Clostridium perfringens*, in Mamala Bay seawater. The effects of bathers on nearshore densities of indicator bacteria were investigated in three diel studies involving three sampling sites (Waikiki Beach, Ala Moana Beach and the mouth of the Ala Wai Canal), in peak use sampling at Waikiki Beach over a 6 day holiday weekend, and in two morning-afternoon sampling surveys at 21 sites spanning the Waikiki shoreline from Ala Moana to Sans Souci Beach. The effects of sunlight and other natural mortality factors on decay rates of indicator bacteria were investigated in 2 day incubations of sewage-spiked seawater under 5 simulated *in situ* light conditions, in 8 short-term incubations during peak hours of winter sunlight, and in 8 experiments involving the disappearance rates of fluorescently labeled bacteria.

In preliminary studies at Waikiki Beach, there was a strong correlation between the concentrations of indicator bacteria (fecal coliforms and enterococci) and the numbers of bathers. The expanded diel studies yielded less consistent results, possibly due to location- and indicator-specific differences. Nonetheless, the following generalities about beaches, bathers, and bacteria emerged: There was generally a distinct coupling between time of day (a surrogate measure of bather density) and bacterial concentrations; tides had no demonstrable effect. An "afternoon effect", in which bacteria were more prevalent in the broad beach survey at 1600 h than at 0600 h, was clear. The beach surveys also showed that the Ala Wai Canal was a significant source of coastal pollution and identified other potential "hot spots" of elevated concentration of indicator bacteria, particularly on the eastern end of the survey where *E. coli* was high in a semi-enclosed swimming area, where *C. perfringens* was high in the vicinity of the Waikiki Aquarium, and where enterococci were generally high in the

afternoon. Nearshore transects generally indicated decreased bacterial concentrations with increasing distance from shore.

Of the 3 indicator bacteria tested, *C. perfringens* was not deactivated by sunlight. Thus, it provides a relatively conservative tracer for the movement and mixing of water masses contaminated with mammalian feces. *C. perfringens* decays in seawater at low rates consistent with the grazing activities of naturally occurring protistan consumers (0.01 to 0.04 h^{-1}). *E. coli* and enterococci do show strong deactivation by visible wavelengths of sunlight, and both are affected to about the same extent. Rates of photodeactivation are highest for light conditions at shallower depths and diminish for conditions in deeper waters. Light at 3% of sea surface intensity shows a negligible deactivation effect. The photodeactivation of indicator bacteria appears to be a complex process, potentially confounded by the physiological state of the organism, adaptation to the organism's previous light environment, and/or differences among the different bacteria in mixed assemblages. Photodeactivation rates are substantially lower for a given light dose when measured over a full photoperiod rather than peak sunlight hours.

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2 INTRODUCTION

The general objective of the Mamala Bay Study is to develop an integrated coastal management plan to maintain environmental quality and minimize human health risks on the south shore of Oahu (Mamala Bay Study Plan). Although the original impetus for the study was to understand the fate and impact of deep-water sewage discharge into the bay from the Sand Island Sewage Treatment Plant, the project required assessments of alternate point- and non-point sources of pollution -- including bathers, boaters and land runoff -- which confound the interpretation of sewage discharge effects. A major multidisciplinary effort was also needed to resolve the pathways and probabilities of health risks from pathogenic microbes which enter a complex and time-variable physical environment from numerous sources and are subject to death or deactivation from both biotic and abiotic processes. The present study contributes to this effort by investigating the effects of bathers on near-shore abundances of pollution indicator bacteria and the processes effecting decay rates of indicator bacteria in Mamala Bay seawater.

Since it is generally not practical to assay directly for the microorganisms associated with human health risks in marine and freshwaters, common enteric bacteria, like fecal coliforms, enterococci and *Escherichia coli*, are used routinely as indicators of water quality. Ideally, indicators are as or more resistant to environmental stress than associated pathogens, so that their presence serves as a conservative indication of potential health risk (Sloat and Ziel 1991). In this regard, the bacterium, *Clostridium perfringens,* is perhaps most useful as an indicator in Hawaiian waters because its spores are associated with mammalian feces and quite resistant to environmental degradation in seawater (Fujioka and Shizumura 1985). Though considered less stable in seawater, the enterococcus subgroup of fecal streptococci is also used routinely in water quality sampling in Hawaiian waters (Fujioka et al. 1990). In contrast, fecal coliform bacteria, including *E. coli*, are less useful in pointing to specific sources of anthropogenic pollution because they thrive in tropical terrestrial environments and undergo rapid deactivation in

seawater (Fujioka 1981, Fujioka and Shizumura 1985). Nonetheless, their presence can indicate nonspecific and fairly recent contamination.

Since water quality is routinely determined relative to established standards for indicator microbes, it is important in the context of an integrated coastal management plan for Mamala Bay to understand the various process by which these organisms are added to and lost from the marine environment. The role of bathers as a potentially significant non-point source of bacteria to the nearshore environment is relevant in this regard. Cheung et al. (1991) has shown, for example, that bathers exerted a profound influence on densities of indicator bacteria at Hong Kong beaches. We studied this potential effect at Waikiki beaches to assess the magnitude of daily fluctuations in pollution indicators and to provide a basis for distinguishing locally generated bather effects from pollution due to other point and non-point sources. The results of our sampling investigations at Waikiki beaches and the Ala Wai Canal complement R. Fujioka's routine monitoring program for indicator bacteria at selected sites, C. Gerba's assessments of human health risks from pathogenic microbes at Waikiki swimming beaches, and more intensive studies of microbial populations and physical circulation in the Ala Wai Canal.

The second aspect of our research was to investigate decay rates for the common indicator bacteria in Mamala Bay seawater, emphasizing the deactivation effect of sunlight. The sunlight effect is well documented for enteric bacteria in seawater (Gameson and Saxon 1967, Gameson and Gould 1974, Chamberlain and Mitchell 1978, Fujioka et al. 1981, Davies and Evison 1990). Working in Hawaiian waters, for example, Fujioka et al. (1981) found a rate of loss of 90% of fecal coliforms in 0.5 to 1.5 h, and a somewhat slower, but also rapid, decline in fecal streptococci (90% loss in 1 to 3 h). This decline may represent real death or simply a physiological shift to a nonculturable dormant state (Barcina et al. 1990, Davies and Evison 1991, Gonzáles et al. 1992), from which the still viable cell can recover under appropriate circumstances (e.g., Xu et al.

1982, Colwell et al. 1985). Fujioka et al. (1981) and Curtis et al. (1992) attributed most of the deactivation potential of sunlight to the visible wavelengths, and the effect appears to be at least accelerated by, if not ultimately due to, hydrogen peroxide or singlet oxygen generated by photochemical reactions (Arana et al. 1992, Curtis et al. 1992, Sikorshi and Zika 1993). Our particular interest in the sunlight effect was understanding how it decreases with the depth-dependent extinction of light. This is relevant to the tracking and potential "aging" of the sewage plume from the Sand Island diffuser, which generally resides in water 30 to 50 m deep. Enhanced activity of longer-lived pathogens at depth could also present more of a health problem for scuba divers than might be inferred from the rates of bacterial decay in waters near to the surface. Our experiments on the effects of visible light on bacterial decay complement R. Fujioka's die-off studies with pathogenic microbes using a flow-through system and R. Hill's focus on viable but nonculturable bacteria. Our work also involved measurements of the rates of removal of bacteria from seawater by natural protistan predators, as this may be the most conservative estimate for the decay of dormant bacterial cells from the marine environment.

2.1 Scope of Work

This project examined the influences of bathers, sunlight and protistan predators on the abundances of three indicator bacteria, *Escherichia coli,* enterococci and *Clostridium perfringens*, in Mamala Bay seawater. The effects of bathers on nearshore densities of indicator bacteria were investigated in three diel studies involving three sampling sites (Waikiki Beach, Ala Moana Beach and the mouth of the Ala Wai Canal), in peak use sampling at Waikiki Beach over a 6 day holiday weekend, and in two morning-afternoon sampling surveys at 21 sites spanning the Waikiki shoreline from Ala Moana to Sans Souci Beach. The effects of sunlight and other natural mortality factors on decay rates of indicator bacteria were investigated in 2 day incubations of sewage-spiked seawater under 5 simulated *in situ* light conditions, in 8 short-term incubations

during peak hours of winter sunlight, and in 8 experiments involving the disappearance rates of fluorescently labeled bacteria.

2.2 Objectives

The general objectives of this project were:

1. To document the contribution of beach bathers to nearshore abundances of pollution-indicator bacteria, and
2. To determine the decay rates of pollution-indicator bacteria in Mamala Bay seawater.

2.3 Project Organization

The following individuals contributed to various aspects of the project research:

M. R. Landry - Professor, Dept. of Oceanography, Univ. of Hawaii

Principal Investigator with primary responsibility for design and implementation of bacterial decay experiments and for project synthesis.

F. C. Dobbs - Assistant Professor, Dept. of Oceanography, Old Dominion Univ.

Co-principal Investigator (Project P.I. before moving to ODU) with primary responsibility for design, implementation and interpretation of beach sampling.

L. Campbell - Assistant Researcher, Dept. of Oceanography, Univ. of Hawaii

Co-principal Investigator with primary responsibility for bacterial enumeration, including natural bacteria using flow cytometry.

L. Addessi - Graduate Student, Dept. of Oceanography, Univ. of Hawaii

Assisted in beach sampling experiments.

C. Allen - Research Assistant, Dept. of Oceanography, Univ. of Hawaii

Assisted in data analysis and prepared figures for final report.

J. Constantinou - Research Technician, Dept. of Oceanography, Univ. of Hawaii

Technical support in all aspects of beach sampling and decay experiments.

M. Deamud - Student, Florida Inst. Technology; REU student, Univ. of Hawaii

Assisted in all aspects of 1993 beach sampling and decay experiments.

M. Hristova - Student, Washington State Univ.; REU student, Univ. of Hawaii

Assisted in all aspects of 1993 beach sampling and decay experiments.

J. Kirshtein - Research Technician, Dept. of Oceanography, Univ. of Hawaii

Technical support in 1993 beach sampling and decay experiments.

H. Liu - Graduate Student, Dept. of Oceanography, Univ. of Hawaii

Assisted in beach sampling and decay experiments.

C. Moyer - Graduate Student, Dept. of Oceanography, Univ. of Hawaii

Assisted in beach sampling experiments.

H. Nolla - Research Technician, Dept. of Oceanography, Univ. of Hawaii

Technical support in 1993 beach sampling and decay experiments and flow cytometric analyses.

E. Parnell - Graduate Student, Dept. of Oceanography, Univ. of Hawaii

Assisted in beach sampling experiments.

K. Selph - Graduate Student, Dept. of Oceanography, Univ. of Hawaii

Assisted in beach sampling and decay experiments.

S. Vink - Graduate Student, Dept. of Oceanography, Univ. of Hawaii

Assisted in beach sampling experiments.

J. Wood - Student, Univ. of Florida; REU student, Univ. of Hawaii

Assisted in all aspects of 1993 beach sampling and decay experiments.

3 METHODS

3.1 Task Summary

The objectives of this project were addressed in the following research elements:

1. Preliminary sampling at Waikiki Beach to establish the magnitude of diel variations in abundances of indicator bacteria and their relationship, if any, to the density of bathers.

2. Mid-day sampling at Waikiki Beach over a 6-day holiday weekend to document day-to-day variations in the maximum concentrations of indicator bacteria and their relationship to beach use.

3. Replicated, intensive 24-h studies of diel variations in indicator bacteria at three sites, with early-morning and late-afternoon synoptic sampling at 21 locations and near-shore transect sampling to document broader temporal and spatial relationships.

4. An intensive 2-day summer experiment to estimate the decay rates of sewage-derived indicator bacteria under simulated *in situ* conditions and to distinguish the relative roles of light deactivation versus other natural mortality influences.

5. Follow-up *in situ* experiments to assess the magnitude of bacterial mortality due to the grazing activities of natural marine protists.

6. Follow-up experiments to assess deactivation rates of indicator bacteria during peak sunlight hours.

3.2 Task Methodology

3.2.1 Beach Sampling

Table 3.1 gives an overview of the beach sampling experiments and their relationships to the tasks defined in Section 3.1. Site abbreviations refer to Waikiki Beach (WB), Ala Moana Beach (AMB), and the Ala Wai Canal (AWC). All sampling site locations are noted in Fig. 3.1. Counts of bacterial populations include flow cytometric (FCM) analyses of total bacteria and specific plating media and procedures for fecal coliforms (mFC), *Escherichia coli* (mTec), enterococci (mEnt, mE), and *Clostridium perfringens* (mCp). Ancillary data included the number of people counted on the beach and in the water for a 35-m length of the shoreline ("bathers"), air and water temperatures (T), and salinity (S). DIEL 2 and 3 included additional long-shore and off-shore sampling as described below.

Water samples were collected in autoclaved, clear polycarbonate bottles of varying volumes (100 to 1,000 ml). At all stations except the Ala Wai Canal, the standard sampling depth was in water about 1-m deep. Sampling bottles were fastened near the end of a 1-m stick with a loop of silicone rubber tubing, opened underwater (10 to 20 cm deep), and allowed to fill completely with subsurface water while being held at arm's length away from the sampler. The filled bottles were capped underwater and placed in a black plastic bag in an insulated cooler to protect the samples from sunlight and temperature shock before processing. Samples from the Ala Wai Canal were collected by lowering a bucket from the seaward side of the Ala Moana Blvd. bridge, but these samples were otherwise handled similarly to those collected elsewhere.

Table 3.1 Summary of dates, sites, and variables measured for beach sampling tasks.

Exper.	Task	Dates	Sites	Bacteria	Other
DIEL 1	1	6/21-22/93	WB	FC, Ent	bathers
HOLIDAY	2	7/1-6/93	WB	FC, Ent	bathers
DIEL 2	3a	8/1-2/93	WB, AMB, AWC	Tec, E, Cp, FCM	bathers, T, S
DIEL 3	3b	8/29-30/93	WB, AMB, AWC	Tec, E, Ent, Cp, FCM	bathers, T, S

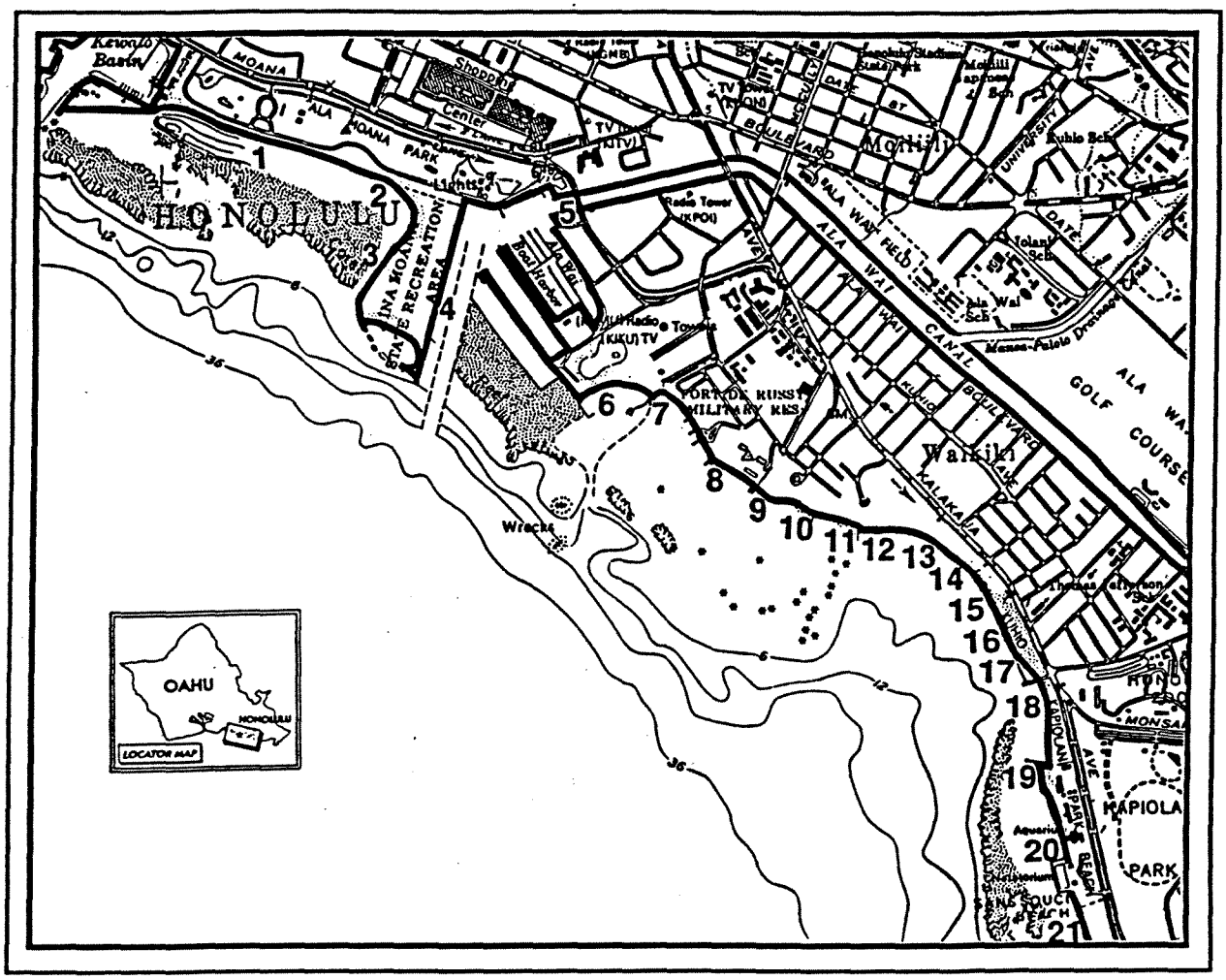

Fig 3.1 Map of the Honolulu waterfront from Kewalo Basin boat harbor to Sans Souci Beach. Numbers refer to sampling stations for DIEL 2 and 3 beach surveys. Routine sampling stations are, respectively, Ala Moana Beach (AMB = site #2), the Ala Wai Canal (AWC = site #5), and Waikiki Beach (WB = site #13). Adapted from U.S. Geological Survey, National Mapping Program.

The frequency and timing of sampling differed among the four experiments. For the preliminary experiment (DIEL 1), replicated water samples were taken at 4-h intervals for 24 h beginning at 1600 h on 21 June. Samples for the HOLIDAY experiment were collected only once per day (1500 h), during the period of maximum bather and bacterial abundances observed in DIEL 1. Sampling intensity was expanded for DIEL 2 and 3. For both experiments, replicated water samples were collected every 2 h for 24 h (13 time points), beginning at 0600 h. In addition, afternoon (1600 h) and morning (0600h) samples were taken from an additional 18 stations spanning the Honolulu waterfront from Ala Moana to Sans Souci Beach (Fig. 3.1). These samples were taken more-or-less synchronously using five teams of samplers each with specific site responsibilities. Also at 1600 and 0600 h, samples were collected from two transects extending 60-m offshore from the Waikiki Beach (WB) sampling site. Transect samples were taken at 10-m intervals using a measured line held by one investigator on the shoreward end while the second investigator took the sample from a surfboard.

Beach samples were generally made by two person teams, one of whom collected the water samples while the other recorded notes and ancillary measurements. Air temperatures were taken in the shade with a dry, hand-held mercury thermometer. Water temperatures were taken with the same thermometer in the water where bacterial samples were collected. Salinity measurements were made on separate water samples analyzed in the laboratory with a refractometer. The number of bathers in the water at the time of sample collection were enumerated for approximately 35-m sections of WB and AMB between preselected landmarks (e.g., palm trees).

3.2.2 Bacterial Decay Experiments

Table 3.2 gives a summary of experiments designed to assess the rates of decline of culturable indicator bacteria in Mamala Bay seawater and the relative contributions of sunlight deactivation and protistan predation to these losses. LIGHT 1 was the major

experiment for this component of the research and consequently yielded the bulk of the results. The additional experiments were conducted to provide supporting evidence from a different time of the year (LIGHT 2 and 3) and with a different approach (FLB 1 and 2).

Table 3.2 Summary of dates, conditions and variables measured for bacterial decay experiments.
Abbreviations for bacterial counts as in Table 3.1. I_o is incident solar irradiation, and $\%I_o$ is relative irradiation under incubation conditions.

Exper.	Task	Dates	Conditions	Bacteria	Other
LIGHT 1	4	7/26-28/93	Simulated *in situ*	Tec, E, Cp, FCM	T°C, I_o, $\%I_o$
FLB 1	5a	12/1-2/94	*In situ*	FCM	T°C
FLB 2	5b	12/2-3/94	Simulated *in situ*	FCM	T°C
LIGHT 2	6a	12/8/94	Simulated *in situ*	Tec, E	T°C, I_o, $\%I_o$
LIGHT 3	6b	12/14/94	Simulated *in situ*	Tec, E	T°C, I_o, $\%I_o$

3.2.2.1 LIGHT Experiments

Natural water samples for the LIGHT 1 experimental incubations were collected from Mamala Bay on the afternoon of 26 July 1993 at a station seaward and upwind from the Sand Island sewage diffuser. Acid (1 N HCl) washed 30-L Niskin bottles with internal silicone rubber springs were lowered open to 10, 20, 35, 50 and 60 m on a hydrowire and tripped at depth. Upon return to the surface, temperature was measured to the nearest 0.1°C on a stream of water from the bottles using a hand-held mercury thermometer (isothermal 26°C from the surface to 50 m, 24°C at 60 m). Water samples were gently drained into darkened 10-L, acid-cleaned polycarbonate carboys through a length of silicone tubing. The carboys were placed in insulated coolers for return to the laboratory.

A fresh sewage sample was collected on the day of the experiment from the Sand Island Sewage Treatment plant. To prepare the sample for the experiment, it was first strained through a 10-μm screen to break-up and/or remove larger particles. The particulates, including microbes, were concentrated and rinsed free of associated dissolved organic and inorganic enrichment by sequential centifugation (10,000 rpm) and resuspension in sterile phosphate buffered saline (PBS). The stock suspension of bacteria was prepared at about sewage density and added to incubation bottles at 1:1000 volume dilution.

As soon as the sewage bacteria were prepared, the experiment was started by dispensing water samples collected from each of the sampling depths into three 2.2-L acid washed, autoclaved polycarbonate bottles; two bottles from each depth were clear and one was completely wrapped with black electrical tape to serve as a "dark" control. Sewage bacteria were added to each bottle during the filling process to facilitate mixing (2 ml PBS suspension per bottle to achieve about 0.1% of microbial densities in raw sewage). The bottles were capped, rotated several times to complete mixing, and reopened for initial subsampling of bacterial populations.

The LIGHT 1 experiment was designed to start during the nighttime so that the rates of decline of indicator bacteria in light bottle "treatments" would track those in the dark "control" bottles until sunrise. Thereafter, the decay rates of bacteria in the light bottles were expected to diverge from the nighttime rates and the daytime rates in control bottles, giving, by difference, the decay rate directly attributable to light deactivation. The experimental bottles were maintained in temperature-regulated acrylic incubator boxes on the roof of the Marine Sciences Building, University of Hawaii at Manoa for exposure to the natural cycle of incident solar radiation (27 and 28 July 1993 were bright sunny days). The relative intensity and spectral quality of available light at the depths of water sample collection were simulated, within reasonable limits, using neutral density screening on bottles and blue acrylic panels on the boxes holding deeper samples. Fig.

3.2 shows the combined transmission characteristics of the acrylic incubator housing (6-mm clear Plexiglas), the tinted outer panels (3-mm Plexiglas 2069 light blue or 2424 dark blue), and the polycarbonate sample bottle, as measured relative to air transmission with a Beckman DU 640 Spectrophotometer. DNA-damaging UV-B wavelengths were effectively eliminated by the plastics in all incubators. The clear incubator (#1) transmitted about 80% of all visible (400-700 nm) wavelengths and sharply cut UV-A transmission below 380 nm. The light blue incubator (#2) reduced the availability of red and green visible light relative to the clear box, while the dark blue (#3) incubator transmitted only blue wavelengths between 410 and 510 nm. The additional treatments were achieved in incubators 2 and 3 using neutral density screening to reduce transmitted light by 70%. Relative light levels in the combined visible wavelengths were measured inside the bottles in the water-filled incubators using a Quantum Scalar Irradiance Meter (Biospherical QSL-100, San Diego). The 10-m sample experienced 70% of incident light (I_o) in incubator #1. The 20 and 35-m bottles experienced, respectively, 38% and 12% of incident light in incubator #2. The 50 and 60 m samples experienced 10 and 3% incident light, respectively, in incubator #3. The dark bottles gave no evidence of any light penetration. Incident radiation was not measured directly, but was provided as photosynthetically available radiation (PAR, μEin m^{-2} s^{-1}) from hourly observations at Kunia, Oahu (Hawaiian Sugar Planter's Association).

During the course of the 45.5-h experiment which ran through two full light periods, light bottles were subsampled for counts of total and indicator bacteria at time intervals ranging from 1.5 to 7.25 h (13 time points). The frequency between samples increased as the experiment progressed due to the need for greater subsample volumes to offset the anticipated decline in indicator populations. Subsampling operations during the daylight hours took place under a double thickness blue polyethylene tarp to minimize light shock to bottles incubated at low light conditions when the incubator lids were lifted. Dark bottles were subsampled at a reduced schedule (9 time points) to minimize their exposure to any light. Subsamples were protected from the light and taken

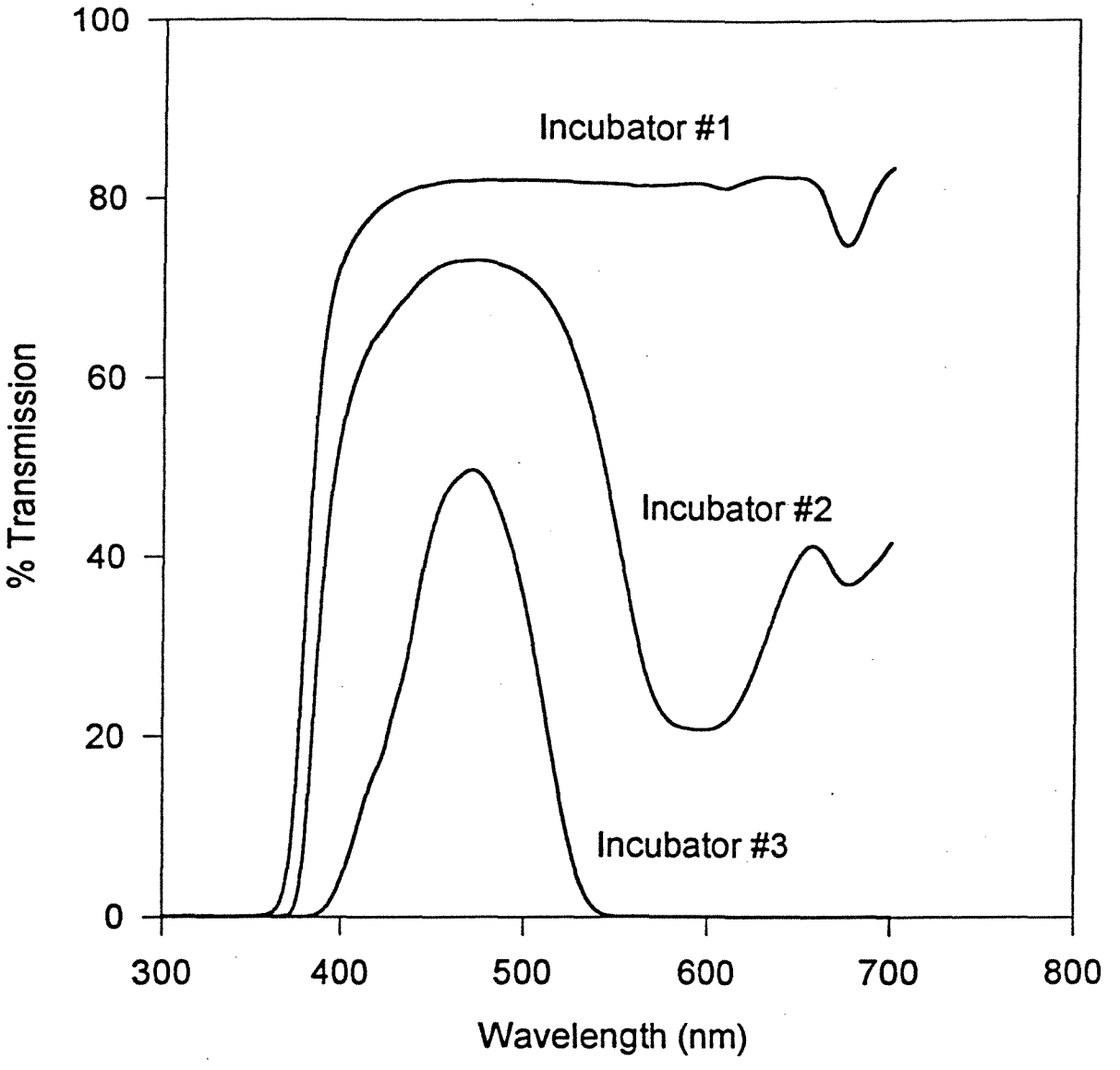

Fig. 3.2 Spectral quality of light transmission in incubation boxes. Spectra show the combined effects of all plastics (Plexiglass box, colored filters, and polycarbonate sample bottles) relative to light transmission in air (Beckman DU640 Spectrophotometer).

immediately to the laboratory (one floor below the incubators) for processing.

LIGHT 2 and 3 were short-term experiments conducted during sunny (12/8) and overcast (12/14) days in December 1994. All incubations involved near-surface seawater collected off Waikiki Beach (12/8) or Ala Moana Beach (12/14) in the early morning. The samples were spiked with seawater microbes from freshly collected samples from the Sand Island Treatment Plant at 0.1% of sewage densities. For LIGHT 2, the sewage was added directly to the seawater without additional preparation. For LIGHT 3, the sewage was prescreened, concentrated and washed (centrifugation), and resuspended before addition. Two light bottles and one dark control was prepared for each of four light treatments -- 76, 30, 11 and 6% of incident visible light. The bottles were incubated on the roof of the Marine Science Building during peak afternoon lighting (1230 to 1605 h - LIGHT 2; 1235 to 1535 h - LIGHT 3), during which they were subsampled for indicator bacteria at approximately hourly intervals.

3.2.2.2 FLB Experiments

FLB experiments involved the use of heat-killed, fluorescently labeled bacteria (FLB) as tracers to estimate the rate of decline of bacteria due to the grazing activities of naturally occurring protistan predators. FLB were prepared from cultures of *Vibrio damsella* (mean cell diameter of 1.1 μm) according to the fluorescein-staining protocol of Sherr and Sherr (1987). For use, they were introduced into replicated natural water samples and a 0.2-μm filtered-water "control" at a concentration of 5×10^3 cells ml^{-1}. Subsamples for flow cytometric enumeration were taken initially and after a 24-h incubation. The predator effect was estimated from the difference between disappearance rates determined for the natural water (with predators) and for the control water (without predators).

For FLB 1, individual tracer experiments were conducted from water collected from 5, 20, 40 and 60 m in the vicinity of the discharge diffuser for Sand Island Sewage

Treatment Plant. After inoculation with FLB and initial subsampling (about 1030 h local time), the bottles were attached at the depth of collection to a taut line between a subsurface float and bottom anchor. Final FCM subsamples were taken after 24-h *in situ* incubations.

For FLB 2, individual tracer experiments were performed on water samples collected at 5 m at the Sand Island diffuser and on near-shore, subsurface samples collected at the sites of previous beach sampling -- Ala Moana Beach (AMB), the Ala Wai Canal (AWC) and Waikiki Beach (WB). These samples were brought back to the laboratory where the FLB experiments were run under simulated *in situ* conditions in water-cooled incubators on the roof of the Marine Science Building.

3.2.3 Bacterial Counts

3.2.3.1 Total Heterotrophic Bacteria and FLB

Combined populations of heterotrophic bacteria and fluorescently labeled bacteria (FLB) were enumerated using the flow cytometric techniques of Monger and Landry (1992, 1993). Duplicate 1 to 3-ml samples were added to sterile cryovials, fixed with paraformaldehyde (0.2% final concentration) for 10 min, frozen in liquid N_2 and stored at -85°C until analysis. The samples were subsequently thawed, stained with Hoescht 33342 (1 μg ml^{-1}) for 1 h, and analyzed with a Coulter Model 753 flow cytometer equipped with dual 5W Argon lasers tuned to UV and blue wavelengths (Campbell et al. 1994). Hoescht 33342 stains the DNA of all microbial cells, giving them a blue fluorescence when excited by the UV laser. Heterotrophic bacteria were distinguished from larger algae and from co-occurring photosynthetic bacteria (*Prochlorococcus* and *Synechococcus* spp.) by their small light scatter signal and the absence of red autofluorescence due to chlorophyll (680 ± 40 nm). Fluorescein-stained FLB were distinguished by blue-laser stimulated green fluorescence.

3.2.3.2 Indicator Bacteria

Indicator bacteria were enumerated using media and procedures for specific species or groups. All indicators were concentrated on Gelman Metricel GN6 filters (47 mm diameter, nominal pore size 0.45 μm) membrane filters. For beach sampling at the WB and AMB sites, standard filtration volumes were 100 to 200 ml for fecal coliforms, *E. coli* and enterococci and 500 ml for *C. perfringens.* For AWC samples, standard filtration volumes were 10 to 50 ml for *E. coli* and enterococci and 25 ml for *C. perfringens.* Varying volumes were concentrated for the different bacteria investigated in decay experiments, and at least two and generally 3 to 4 volumetric dilutions were prepared for each target group to produce at least one plate on which a statistically reliable count could be read after the standard incubation period. The volumes of the subsamples increased progressively during the experiment to account for the anticipated decline in the populations of culturable bacteria. Colonies of indicator bacteria were counted using a fluorescent light and a magnifying lens.

Fecal coliform bacteria were enumerated on Difco mFC agar following incubation at 44.5°C (Difco Manual, 1984, pp. 351ff). To make our results equivalent to those from R. Fujioka's laboratory (Water Resources, University of Hawaii), rosolic acid was not used.

Escherichia coli were enumerated using the standard procedures of the American Public Health Association for natural bathing beaches (APHA, 1992, section 9213D). Filtered seawater samples were grown on mTec agar (Difco) for 2 h at 35°C followed by 22 h at 44.5°C. Presumptive *E. coli* colonies were counted after exposure to urea substrate.

Enterococci were enumerated by two methods. For the DIEL 1 and HOLIDAY experiments, we used Difco mEnterococcus agar (Difco Manual, 1984, pp 346ff). For the DIEL 2 and 3 and LIGHT experiments, we used Difco mE agar, nalidixic acid (Sigma)

and 2,3,5-triphenyl tetrazolium chloride (Sigma), without exposure to EIA substrate (APHA, 1992, section 9230 C). For both methods, the colonies were allowed to grow at 35°C for 48 h before enumeration. Comparison of the two methods on water samples from the 1600 and 0600 h shoreline surveys of DIEL 3 indicated that the mE agar generally gave comparable or higher colony counts than the mEnt agar.

Clostridium perfringens were enumerated on mCP media using 300 mg/L of indoxyl beta-D-glucoside, half the concentration suggested by Bisson and Cabelli (1979). Armon and Payment (1988) showed that concentrations as low as one-tenth those recommended originally do not affect the sensitivity of the medium, and the one-half concentration has been recommended for use in Hawaiian recreational waters (R. Fujioka, pers. comm.). Following membrane filtration, plates and spore-containing filters were placed into a glove bag with pyrogallol to scavenge oxygen. Three volumes of nitrogen were then exchanged, and while still in an anaerobic atmosphere, the plates were transferred to a canning jar and sealed tightly inside. The jar was removed from the glove bag and placed in an incubator at 45°C for 18 h. Red and dark pink colonies were counted as presumptive *C. perfringens* after the jar was opened and the colonies exposed to ammonium hydroxide.

4 RESULTS

4.1 Beach Sampling

4.1.1 DIEL 1 and HOLIDAY Experiments

DIEL 1 showed the expected pattern of beach use, with few people on the beach or in the water at night and an increasing number from early morning to mid-afternoon (Fig. 4.1). Maximum densities for the standard beach section were obtained between 1200 and 1600 h. A strong, positive correlation was found between the number of people in the water and the number of people on the beach ($r = 0.97$, $n=14$); on average, somewhat less than half of the total people were in the water at any given time. Counts of indicator bacteria in seawater samples varied with the diel abundance of people at the beach, with bacterial counts declining steadily from evening through the early morning, then increasing sharply by a factor of 6 to 10 during the day (Fig. 4.1). Although the plating methods used were less discriminating than those used to establish public health standards for recreational water quality, observed counts did not exceed the EPA standards (200 FC/100ml; 126 *E. coli*/100 ml; 33 enterococci/100 ml) for any of the time points sampled. Neither fecal coliforms nor enterococci varied with the highs or lows of the tidal cycle.

The number of people at Waikiki Beach was highest on 4 July during six days of HOLIDAY sampling (Fig. 4.2a). Corresponding peaks occurred in fecal coliforms and enterococci on 4 July (67 and 45 CFU/100ml, respectively); however, the abundances for enterococci were not significantly different for 1, 4 and 6 July (Fig. 4.2b). Taken together, DIEL 1 and HOLIDAY results showed that densities of indicator bacteria should vary fairly closely with the number of bathers at WB. The abundances and temporal patterns of indicator bacteria observed in these preliminary experiments were used in designing more intensive sampling programs for DIEL 2 and 3.

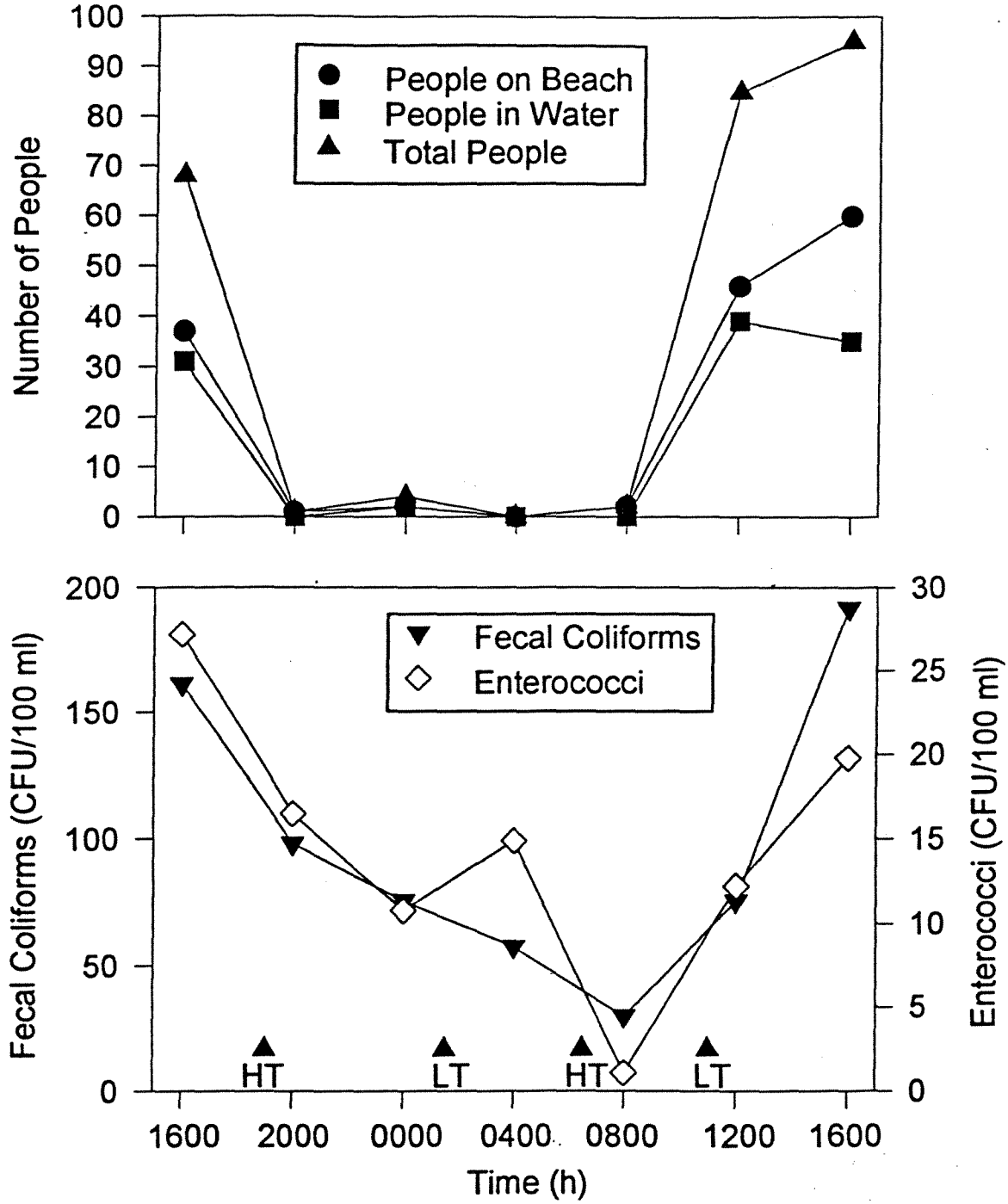

Fig. 4.1 Summary of results from DIEL 1 showing number of people on a 35-m length of coastline at Waikiki Beach on 21-22 June, 1993 and colony forming units (CFU/100 ml) of fecal coliforms and enterococci in seawater samples. Bacterial abundances are the means of two samples. The overall coefficients of variation for fecal coliforms and enterococci were, respectively, 7.5% (range 0.0 to 20.1%) and 14.9% (range 0.7 to 63.6%). HT=high tide, LT=low tide. Data in Appendix 8.1.

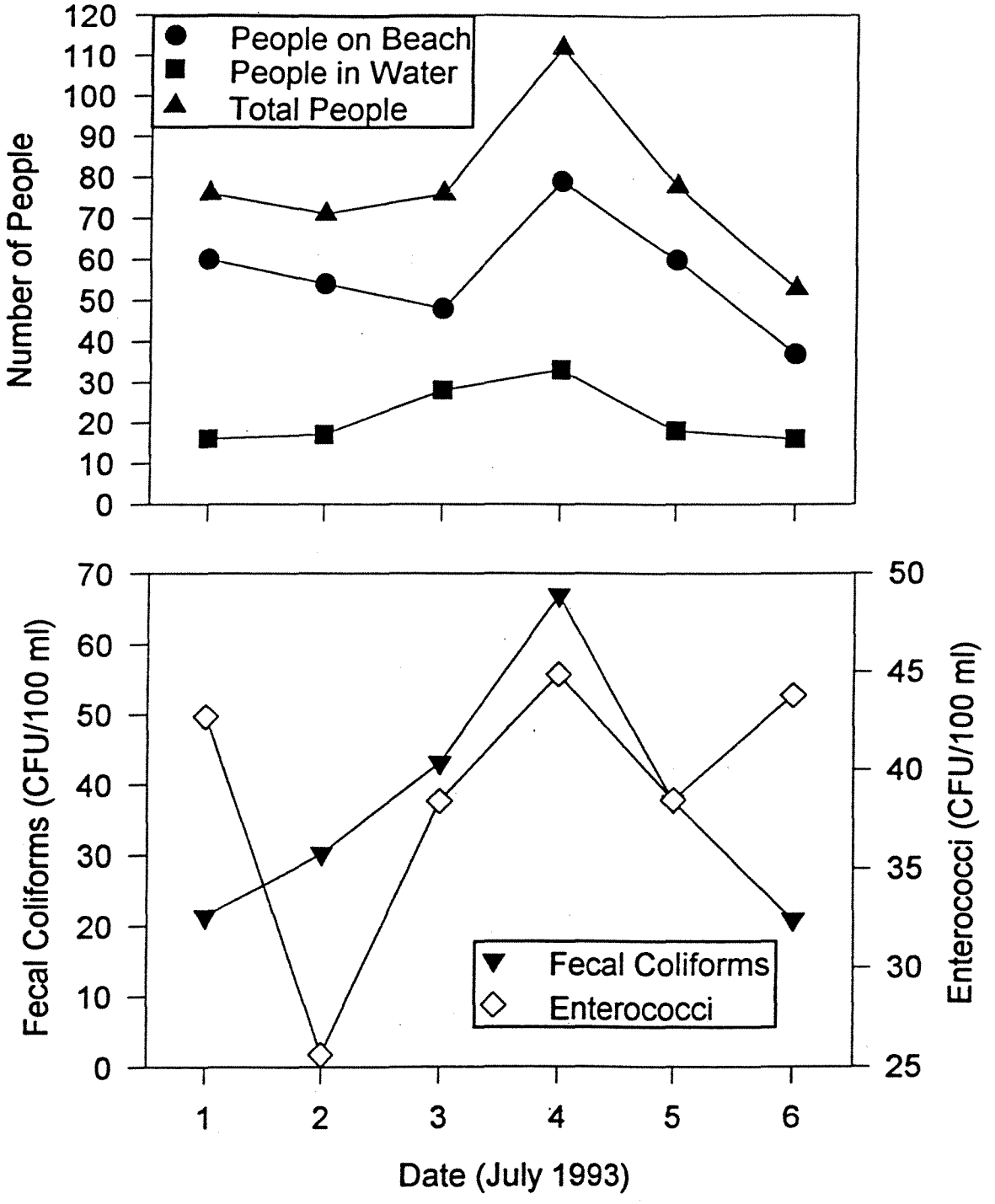

Fig. 4.2 Abundances of people and indicator bacteria on Waikiki Beach at 1500 h from 1-6 July 1993. Bacterial abundances represent the means of two samples. The overall coefficients of variation for replicated counts of fecal coliforms and enterococci were, respectively, 7.5% (range 1.0 to 42.8%) and 14.9% (range 3.4 to 58.8%). Data in Appendix 8.1.

4.1.2 DIEL 2 and 3 Experiments

4.1.2.1 Total Bacteria

High densities of bacteria are not necessarily indicative of polluted waters. In even the most pristine and oligotrophic open ocean ecosystems, natural marine bacterioplankton occur at densities well in excess of 10^5 cells ml^{-1}, surpassing by many orders of magnitude the concentrations of human enteric bacteria that would represent a public health hazard. Even the highest concentrations of total heterotrophic bacteria observed in Waikiki and Ala Moana Beach water ($\approx 10^6$ cells ml^{-1}) are more or less typical of clean coastal waters (Figs. 4.3 and 4.4). The much higher concentrations of bacteria in the Ala Wai Canal are, however, consistent with eutrophication.

During both DIEL 2 and 3, total bacteria showed a strong diel phasing at all stations with minima in late morning or early afternoon and maxima in the evening (Figs. 4.3 and 4.4). The diel range was about a factor of two for all three sites and both experiments. Overall, the minima and maxima at the AWC appeared to occur several hours later than at WB and AMB. The temporal pattern is consistent with the expected natural growth cycle of marine bacterioplankton which grow at the expense of organic substrate produced during the daylight hours by photosynthetic organisms (e.g., Landry et al., In press b). Organic enrichment at the swimming beaches may also have increased during the daytime due to the higher density of people, but this effect, if any, is likely to be of secondary significance.

Given the diel abundance pattern defined above, the 0600 and 1600 h survey sampling was not ideal for capturing the full daily range of variability in heterotrophic bacteria. Nonetheless, most stations showed elevated population abundances at 1600 h. Replicated offshore transects at WB demonstrated, for instance, that the afternoon enhancement of heterotrophic bacterioplankton was remarkably uniform (Fig. 4.5), much more so than would be expected if it were due to anthropogenic influences or tidal

redistribution. On the broader beach surveys, the effect was more evident to the east of station 8 (Fig. 4.6), and more erratic in the vicinity of the Ala Wai Canal, which characteristically has relatively high bacterial concentrations in the early morning (Figs. 4.3 and 4.4).

4.1.2.2 Diel Cycles of Indicator Bacteria:

Mean counts of *C. perfringens* during DIEL 2 were greatest at the AWC, with peak values $\geq$ 10 CFU/100 ml, 5 to 30 times greater than those at WB and AMB (Fig. 4.7). At all three sites, but least so at WB, the between-sample variability was high, indicating a patchy distribution of the bacterial spores in the water. There was no indication of tidal pattern to *C. perfringens* counts. In fact, despite filtration of replicate 500-ml samples at WB and AMB, the abundances were so low as to make analysis of daily cycles meaningless. Consequently, sampling for *Clostridium* spores during DIEL 3 was limited to the morning and afternoon beach surveys.

Of the three areas sampled during DIEL 2, samples from the AWC consistently contained the highest concentrations of *E. coli*, as high as 3,000 CFU/100 ml but generally less than 500 CFU/100 ml (Fig. 4.8). Peak values occurred at 2200 h. At WB, the pattern was largely consistent with the fecal coliforms in DIEL 1; a peak value was recorded at noon, but concentrations otherwise increased until 1800 h before decreasing throughout the night. With the exception of one noon replicate, no WB samples contained more than 20 *E. coli* /100 ml. Concentrations were lowest at AMB (always <8 CFU/100 ml). Moreover, no pattern in abundance was apparent at AMB, except that the peak value coincided with that at AWC (2200 h).

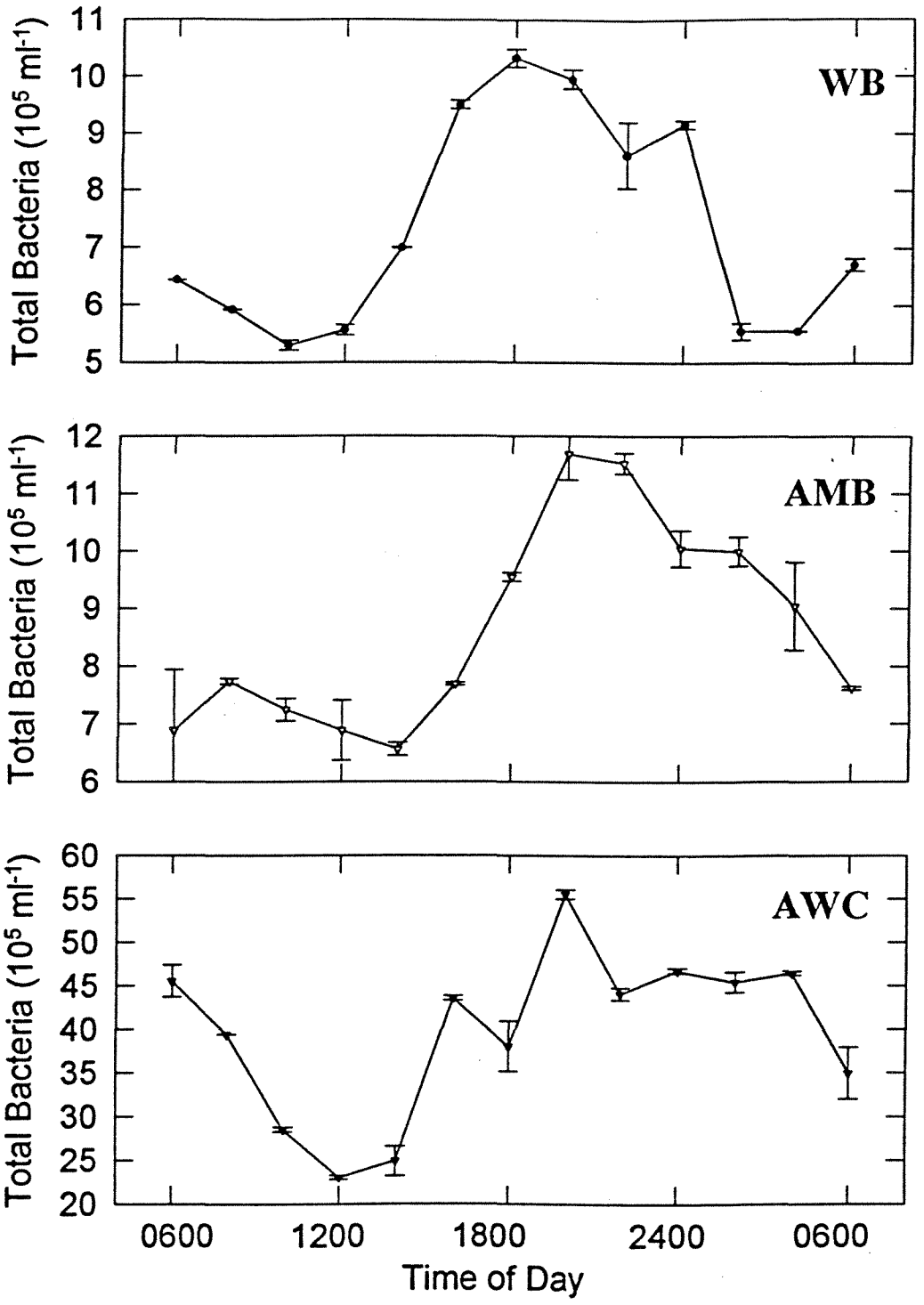

Fig. 4.3 Daily cycles of total bacterial abundances (10^5 cells ml^{-1}) at Waikiki Beach, Ala Moana Beach and the Ala Wai Canal during DIEL 2. Data in Appendix 8.2.

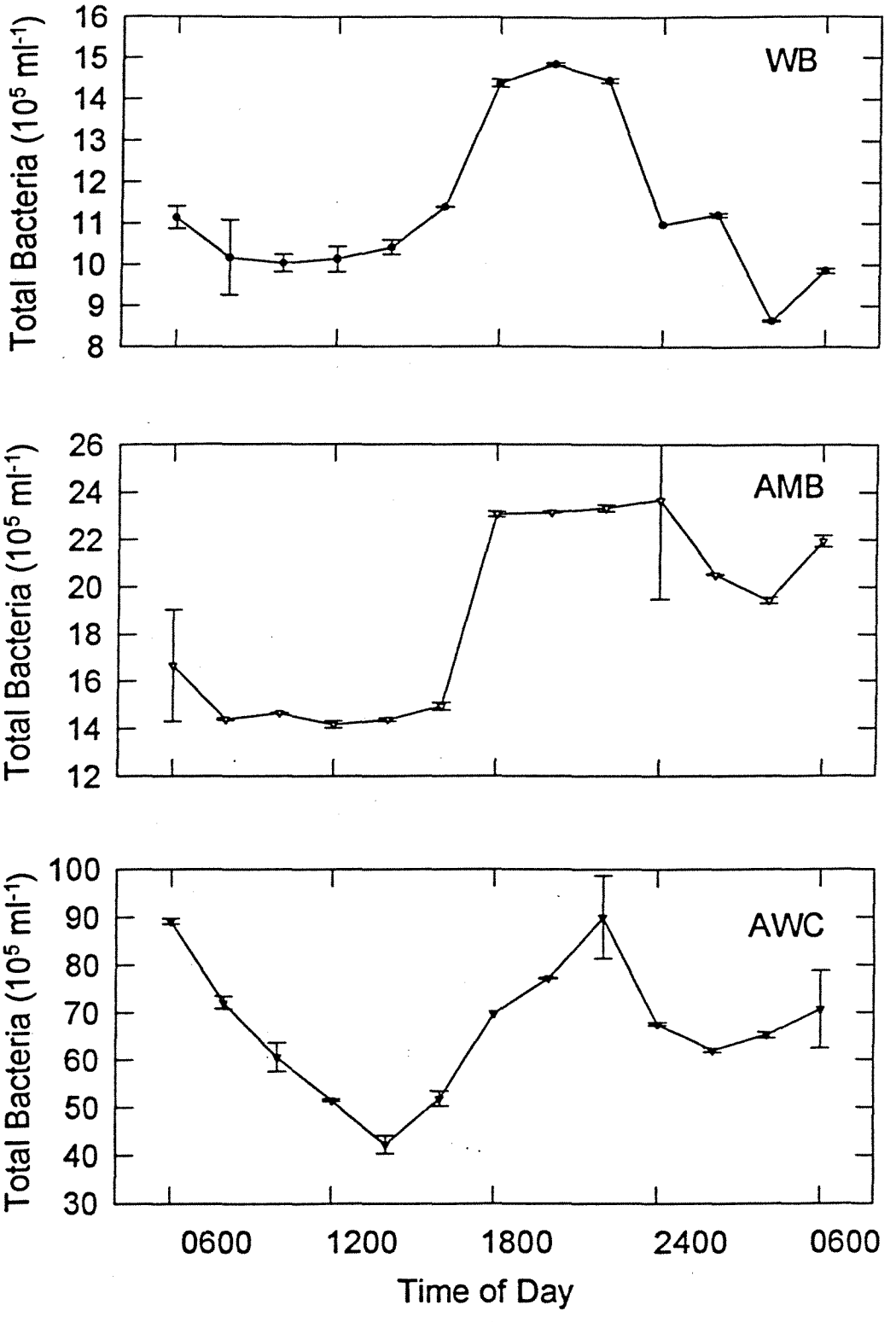

Fig. 4.4 Daily cycles of total bacterial abundances (10^5 cells ml^{-1}) at Waikiki Beach, Ala Moana Beach and the Ala Wai Canal during DIEL 3. Data in Appendix 8.3.

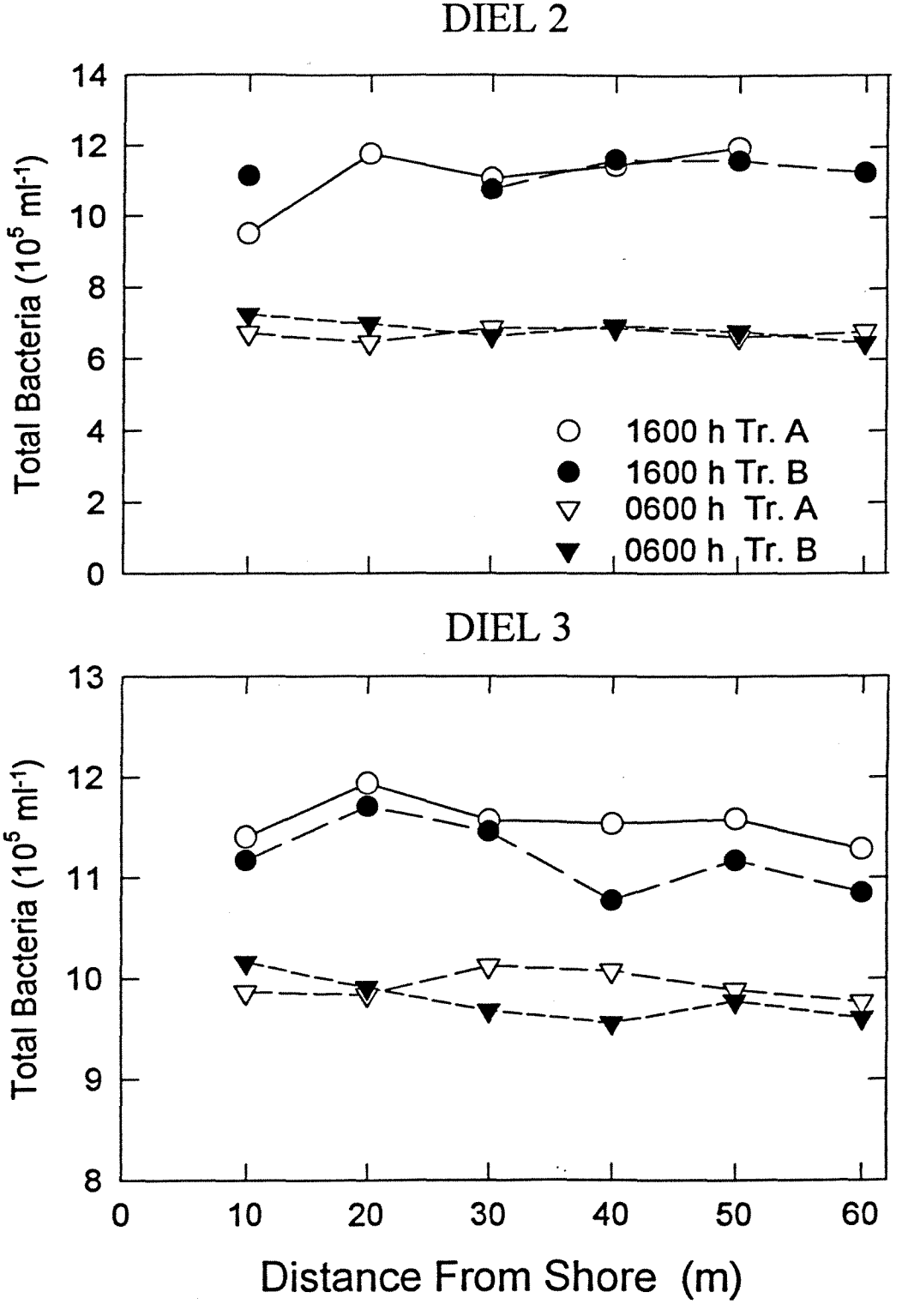

Fig. 4.5 Distributions of total heterotrophic bacteria (10^5 cells ml^{-1}) on 60-m transects perpendicular to Waikiki Beach during DIEL 2 and DIEL 3 experiments. Samples were taken on replicate transects 50 m apart at 1600 h and at 0600 h on the following day. Data in Appendix 8.4.

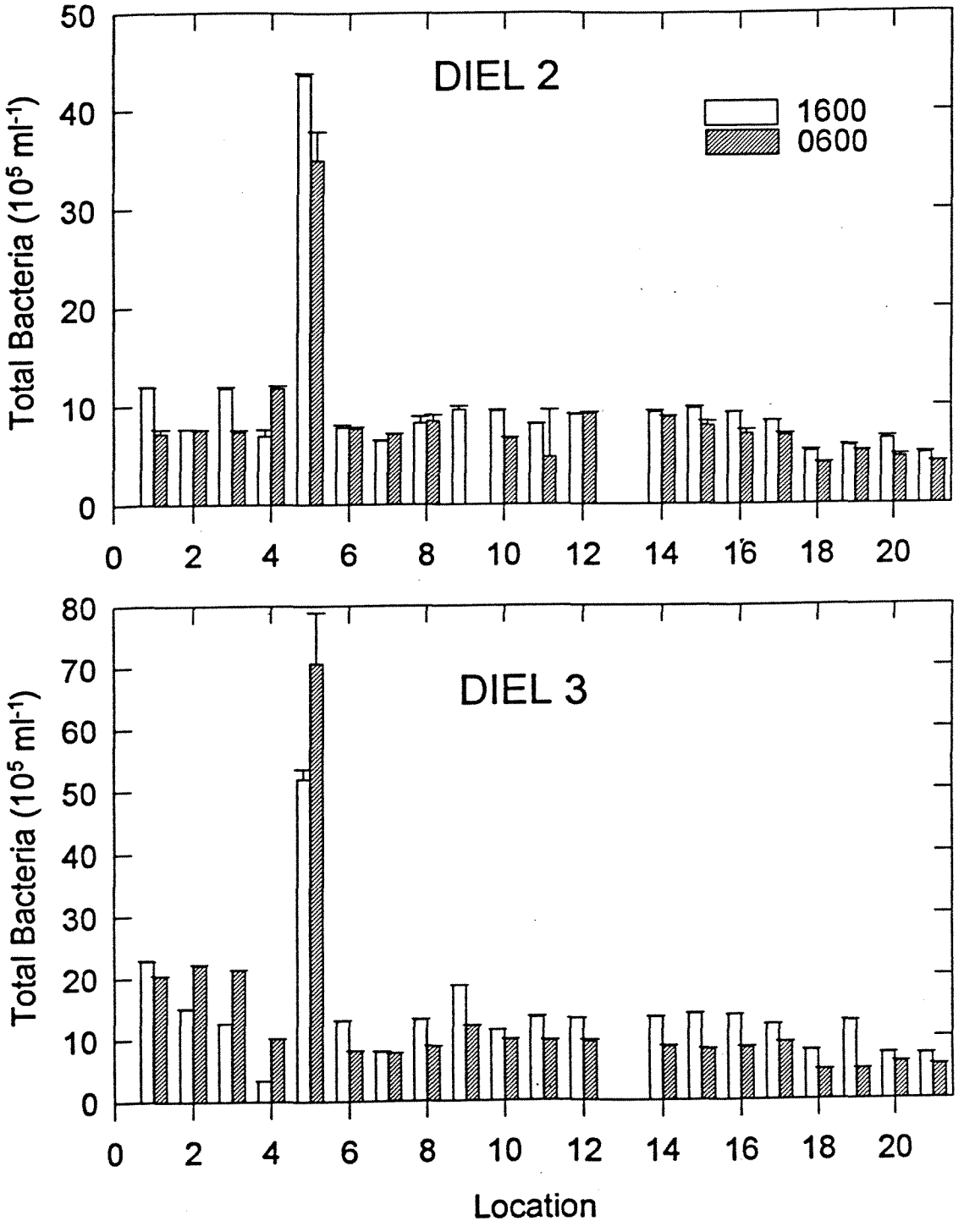

Fig. 4.6 Abundances of heterotrophic bacteria (10^5 cells ml^{-1}) from Ala Moana Beach (site 1) to Sans Souci Beach (site 21) for 1600 and 0600 h beach surveys during DIEL 2 and DIEL 3 experiments. Other sampling site designations as noted in Fig 3.1. Data in Appendix 8.5.

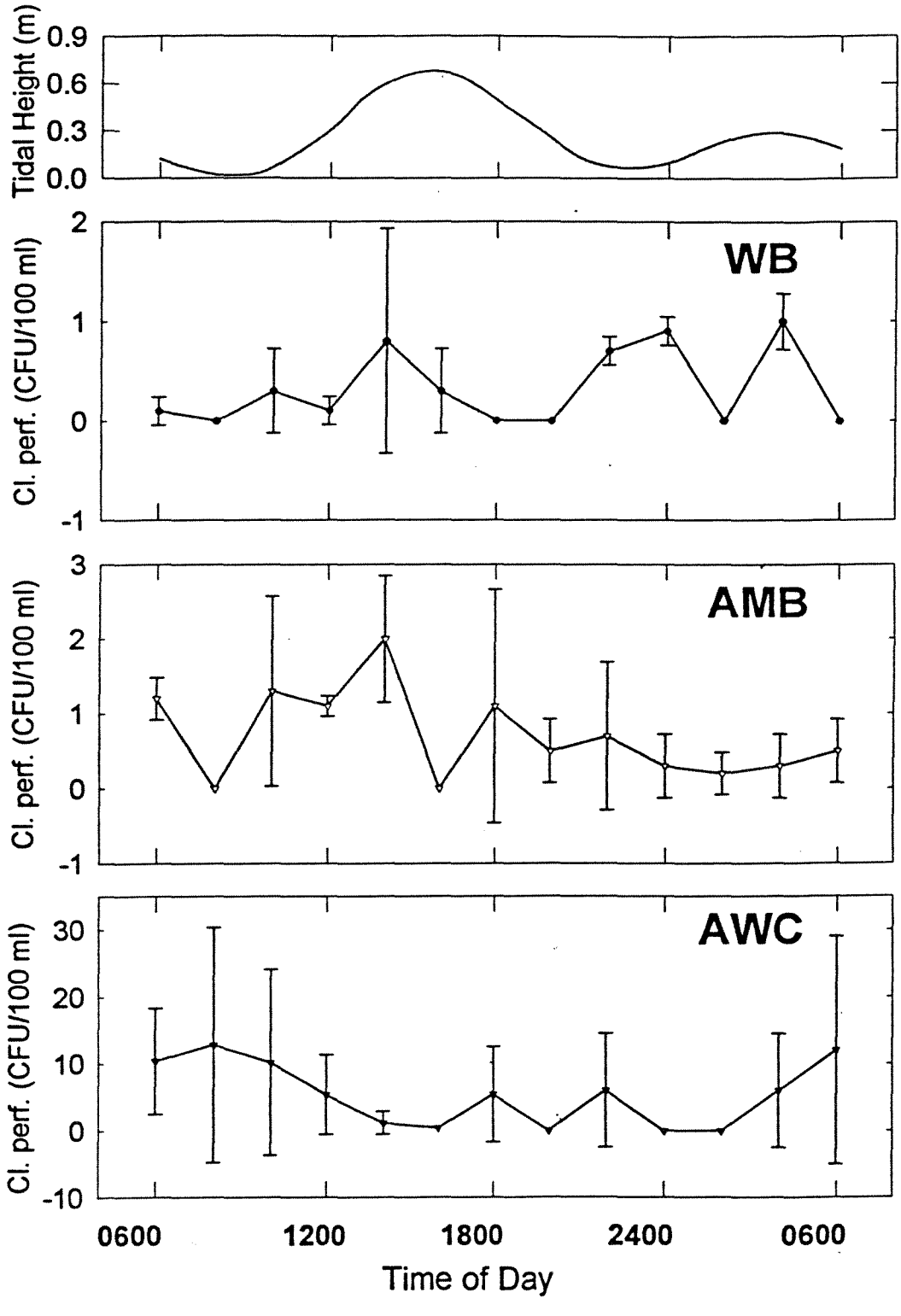

Fig. 4.7 Daily cycles of abundances for *Clostridium perfringens* (CFU/100 ml) at Waikiki Beach, Ala Moana Beach and the Ala Wai Canal during DIEL 2. Values represent the means of replicated 500-ml samples ± 1 standard deviation. Data in Appendix 8.2.

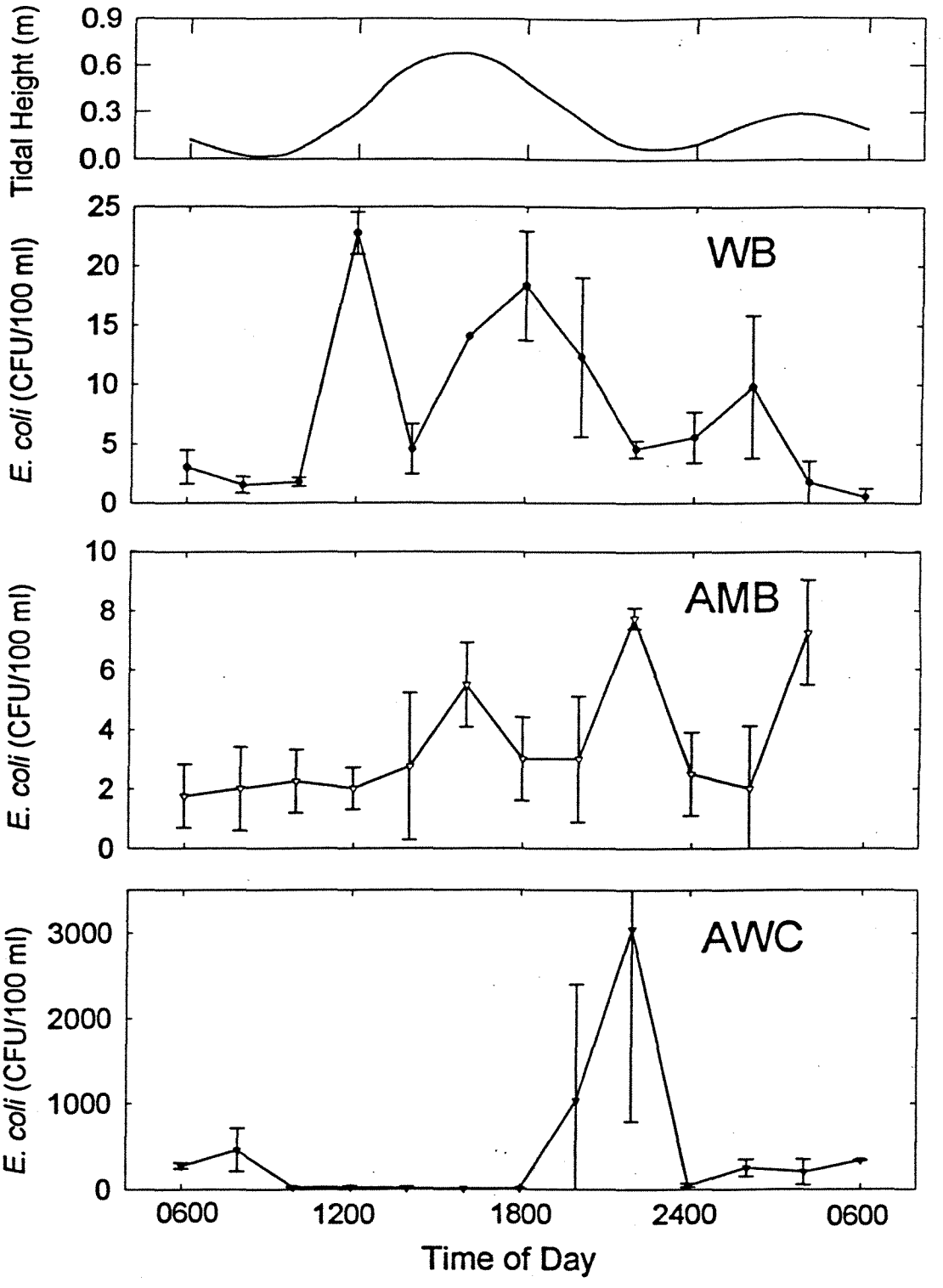

Fig. 4.8 Daily cycles of abundances for *Escherichia coli* (CFU/100 ml) at Waikiki Beach, Ala Moana Beach and the Ala Wai Canal during DIEL 2. Values represent the means of replicated 200-ml samples (100-ml samples at AWC) ± 1 standard deviation. Data in Appendix 8.2.

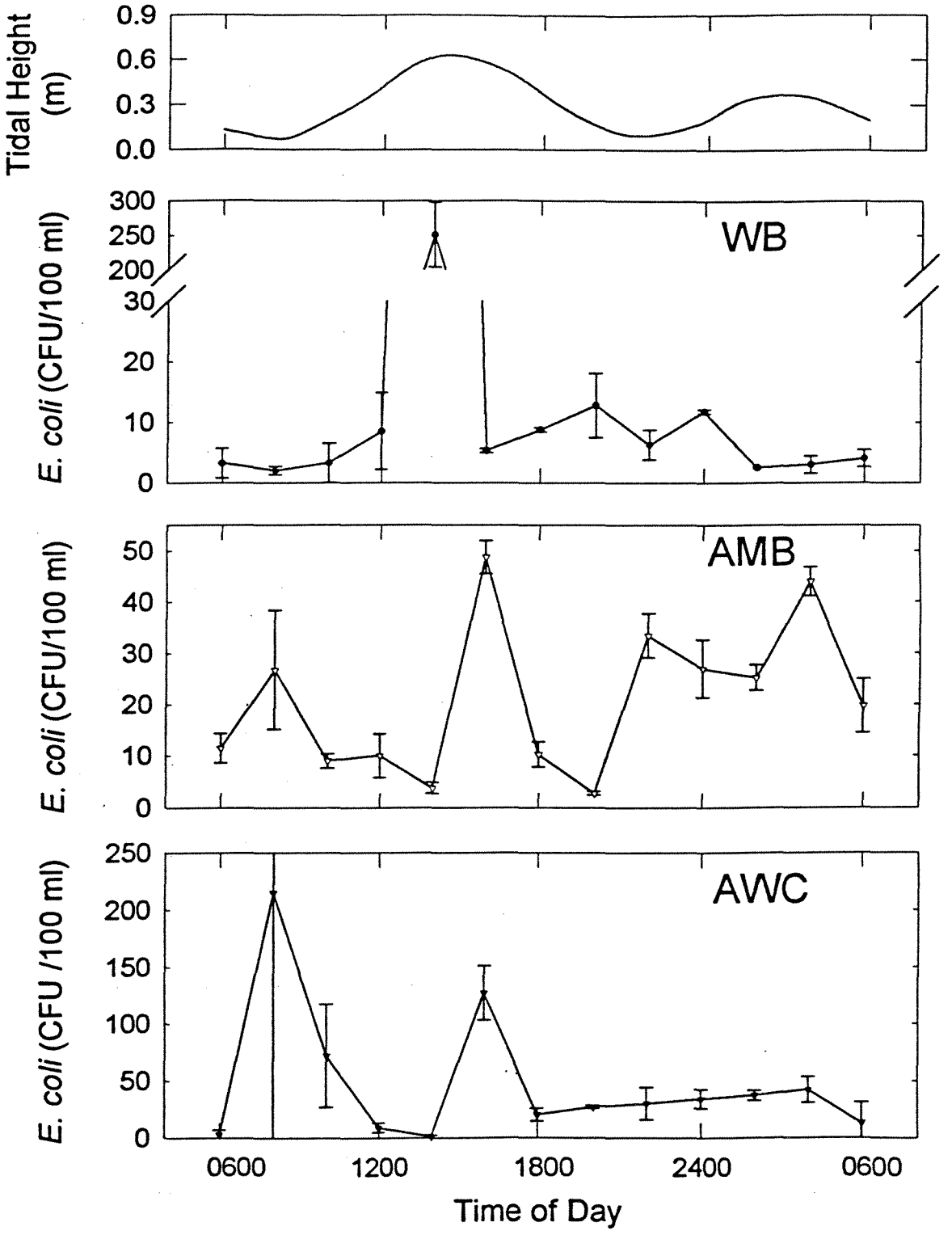

Fig. 4.9 Daily cycles of abundances for *Escherichia coli* (CFU/100 ml) at Waikiki Beach, Ala Moana Beach and the Ala Wai Canal during DIEL 3. Values represent the means of replicated 200-ml samples (50-ml samples at AWC) ± 1 standard deviation. Data in Appendix 8.3.

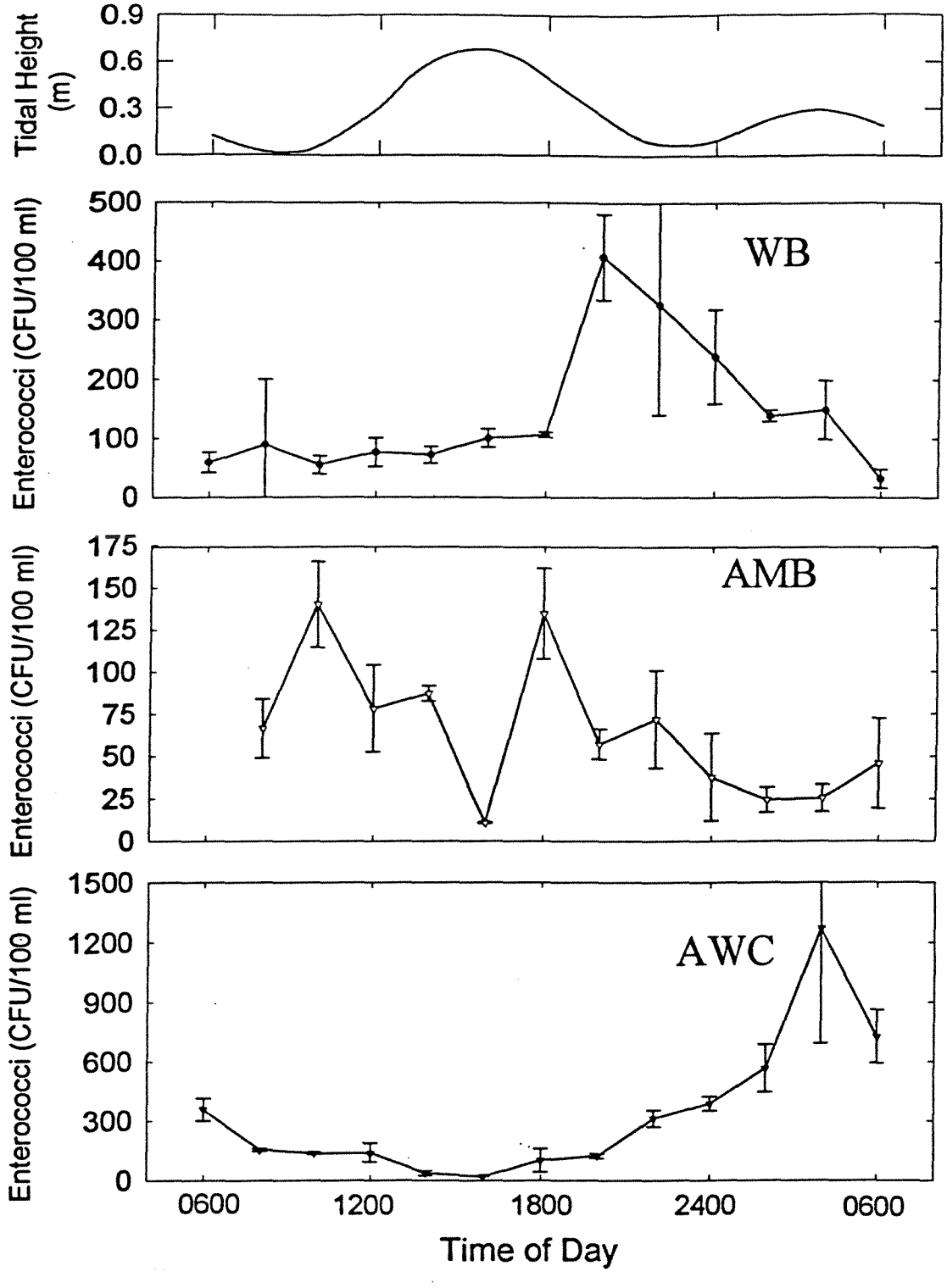

Fig. 4.10 Daily cycles of abundances for enterococci (CFU/100 ml) at Waikiki Beach, Ala Moana Beach and the Ala Wai Canal during DIEL 2. Values represent the means of replicated 200-ml samples (100-ml samples at AWC) ± 1 standard deviation. Data in Appendix 8.2.

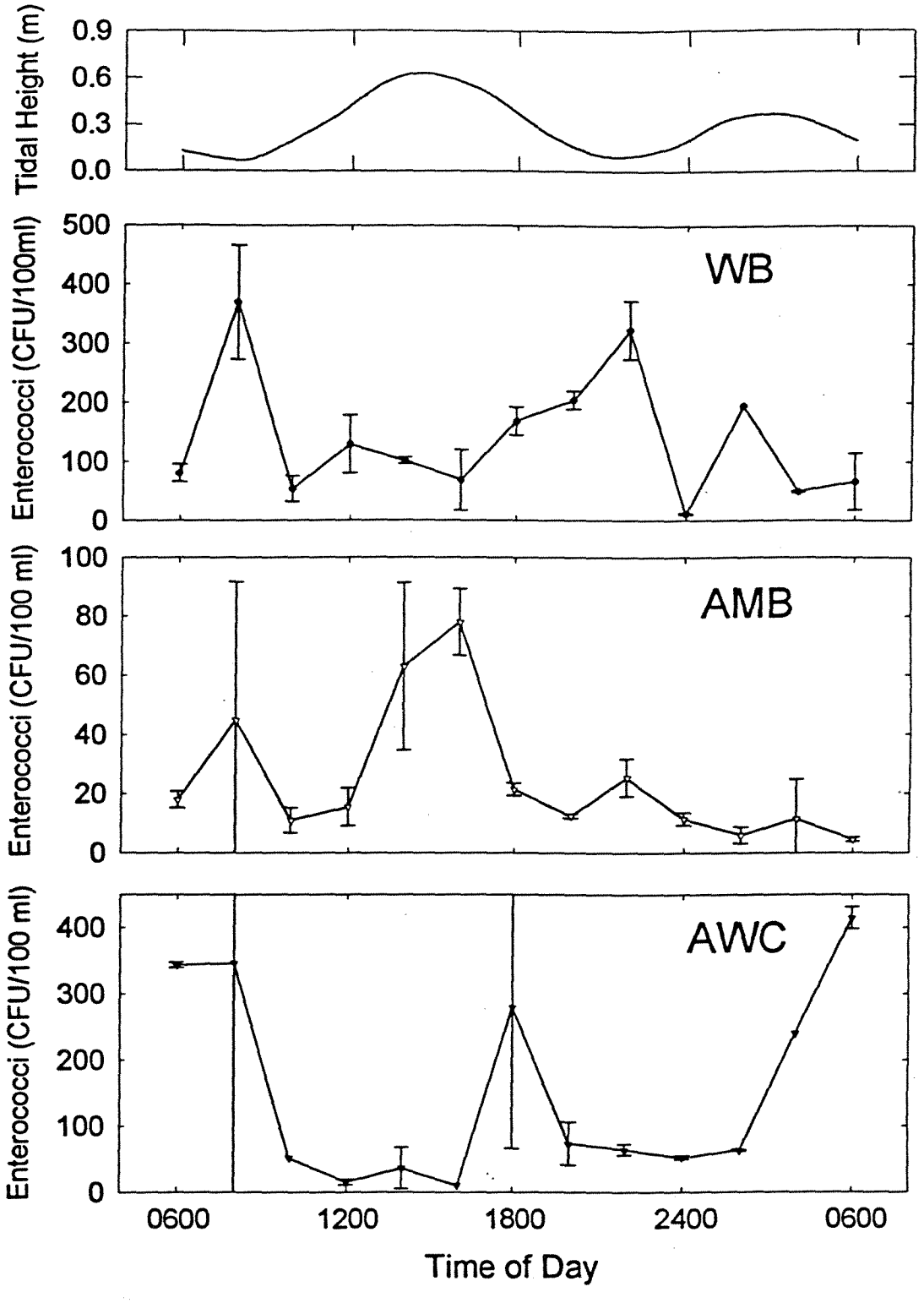

Fig. 4.11 Daily cycles of abundances for enterococci (CFU/100 ml) at Waikiki Beach, Ala Moana Beach and the Ala Wai Canal during DIEL 3. Values represent the means of replicated 200-ml samples (50-ml samples at AWC) $\pm$ 1 standard deviation. Data in Appendix 8.3.

The highest mean value of *E. coli* during DIEL 3 was obtained at WB (1400 h) and coincided with a flood tide (Fig. 4.9). This point was exceptional, however, as WB counts otherwise were <12 CFU/100 ml and concentrations generally rose during the day and decreased at night. *E. coli* counts showed a dynamic range of about 5 at AMB and AWC and were generally higher at AWC. There was no apparent tidal or day/night pattern at AMB. The two highest values from AWC were associated with an ebbing tide.

The AWC samples collected from 0200 to 0600 h gave the highest concentrations of enterococci during DIEL 2 -- 500 to 1,200 CFU/100 ml (Fig. 4.10). In more than half the samples from WB, however, values exceeded 100 CFU/100 ml, occasionally greater than corresponding values from AWC. The pattern in abundance at WB was similar to that for *E. coli*, except that the peak value and decline of the enterococci was shifted 8 h in time. As with other indicator bacteria sampled at AMB, enterococci were relatively low (but still generally >50 CFU/100 ml) and there was no apparent tidal signal in their abundance. Over the course of sampling, however, counts were higher during the day and decreased at night.

During DIEL 3, counts exceeding 300 enterococci/100 ml were obtained in samples from both AWC and WB while values at AMB did not exceed 80 CFU/100 ml (Fig. 4.11). Both peaks at WB occurred during a flood tide, and high counts occurred during the ebbing tide at AWC. Peak bacterial values at AMB were recorded at both low and high water; overall, concentrations rose during the daytime and decreased at night.

Abundances of *E. coli* and enterococci were not appreciably different between the two diel studies at Waikiki Beach (Figs. 4.8 to 4.11) with one notable outlier, the 1400 h samples from DIEL 3. *E. coli* counts were higher during DIEL 3 at AMB, and the reverse was true at the AWC. Counts of enterococci were greater during DIEL 2 than DIEL 3 at both AMB and the AWC (Figs. 4.10 and 4.11).

On the whole, there was no obvious link between the tidal cycle and counts of *E. coli* and enterococci at either WB or AMB (Figs. 4.12 and 4.13). At AWC, *E. coli* counts were higher on the ebbing tide than on the flooding tide during DIEL 2 (Fig. 4.12). No tidal effect was evident for counts of enterococci during DIEL 2, but counts were 3-4 times greater for ebbing versus flooding tides during DIEL 3 (Fig. 4.13).

The presence of a tidal signal at AWC, but not at WB or AMB, is consistent with the salinity data for these three sites (Fig. 4.14). While the salinity at the two beaches was essentially constant at 33 and 34 ppt, it fluctuated at AWC, but not always in a way that could be explained simply by the influx of offshore water. The AWC effluent, lower in salinity than Mamala Bay, moves back and forth across the mouth of the AWC with the tides and mixes in a complex manner with seawater. Other factors that potentially confound the interpretation of abundance pattern for indicator bacteria in the AWC include the inadvertent leakage or intentional nighttime dumping of shipboard sewage from the large numbers of boats docked at the Canal's mouth and the strong vertical stratification that develops in the AWC after heavy rains.

4.1.2.3 Beach Surveys of Indicator Bacteria

Two sites exhibited high numbers of *C. perfringens* in DIEL 2 shoreline surveys (Fig. 4.15). Site 5 was at the mouth of the Ala Wai Canal and site 20 was near the Waikiki Aquarium (Fig. 3.1), which houses marine mammals. Sites 4 and 5 also showed elevated levels of *E. coli* and enterococci at the mouth of the AWC, where the displacement of morning (stn. 5) and afternoon (stn. 4) peaks is probably attributable to tidal transport. Early morning levels of *E. coli* were relatively low for all of the Waikiki swimming beaches, except site 17, which is largely enclosed by a breakwater that limits flushing. The substantially higher levels of *E. coli* during afternoon sampling at most of the popular Waikiki swimming beaches are consistent with bather influences. There was not a close correspondence between *E. coli* and enterococci abundances for most of the

shoreline survey. *E. coli* were abundant during the afternoon from stations 9 to 17 and lower from stations 18 to 21, where enterococci were significantly elevated.

Results for the beach surveys during DIEL 3 were qualitatively similar in most respects to those for DIEL 2. Peaks of *C. perfringens* occurred only in the vicinity of AWC and the Waikiki Aquarium (Fig. 4.16). Sites 3 to 5 (Magic Island and Ala Wai Canal) and the site 17 breakwater were again areas of elevated *E. coli* counts. In addition, a third area, site 21 (Sans Souci Beach) had concentrations >200 *E. coli* /100 ml at 1600h. Enterococci counts were highest near the AWC (>400 CFU/100 ml) and showed the enhanced afternoon concentrations on the eastern portion of the survey (sites 16 to 21), as previously noted for DIEL 2.

The shoreline surveys were designed not only to address broad-scale distributions of indicator bacteria, but to investigate their time dependence as well. When afternoon and morning counts of *E. coli* and enterococci are compared, it is clear that abundances are generally higher at 1600 than at 0600 h (Fig. 4.17). This phenomenon is referred to as the "afternoon effect". The pattern holds for samples from both DIEL 2 and 3 and is expressed more strongly by the enterococci (77% of points below the 1:1 line) than by *E. coli* (56% of points below the 1:1 line). No pattern was detected in the scatter plot for *Clostridium.*

4.1.2.4 Nearshore Abundance Patterns

Transect sampling at WB during DIEL 2 revealed no nearshore abundance pattern for *C. perfringens* (Fig. 4.18). Consequently, transect samples were not collected for this bacterial indicator during DIEL 3. *E. coli* and enterococci exhibited similar patterns during DIEL 2. Their counts were generally higher at 1600 h than at 0600 h, and afternoon abundances for both indicators decreased with increasing distance offshore. A large difference in *E. coli* counts was seen between Transect A and B at 1600 h, suggesting a patchy distribution of bacteria.

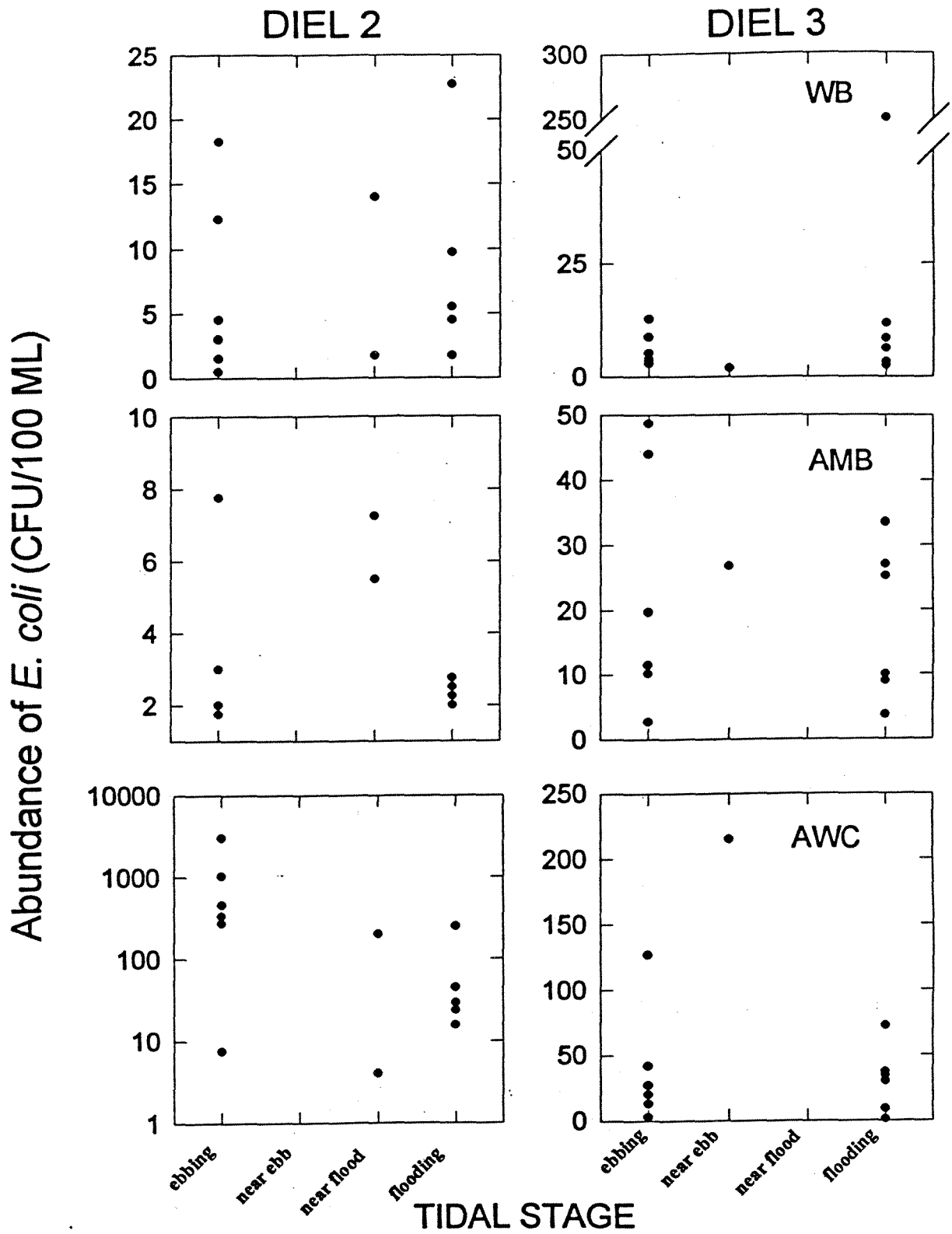

Fig. 4.12 Counts of *E. coli* as a function of tidal stage at Waikiki Beach (WB), Ala Moana Beach (AMB) and the Ala Wai Canal (AWC) during DIEL 2 and DIEL 3 experiments. "Near ebb" refers to a flooding tide within 10 min of low tide; "near flood" refers to an ebbing tide within 10 min of high tide.

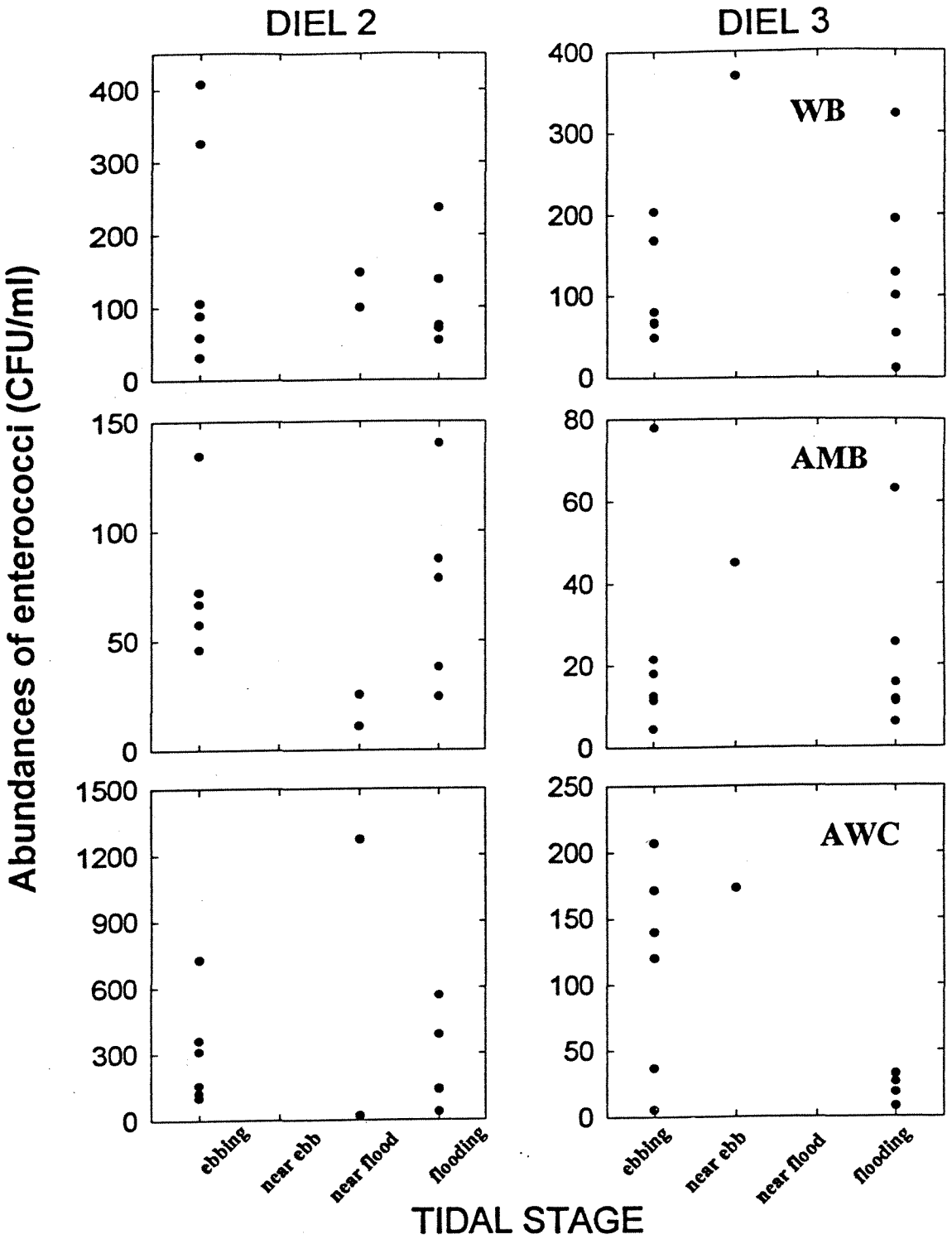

Fig. 4.13 Counts of enterococci as a function of tidal stage at Waikiki Beach (WB), Ala Moana Beach (AMB) and the Ala Wai Canal (AWC) during DIEL 2 and DIEL 3 experiments. "Near ebb" refers to a flooding tide within 10 min of low tide; "near flood" refers to an ebbing tide within 10 min of high tide.

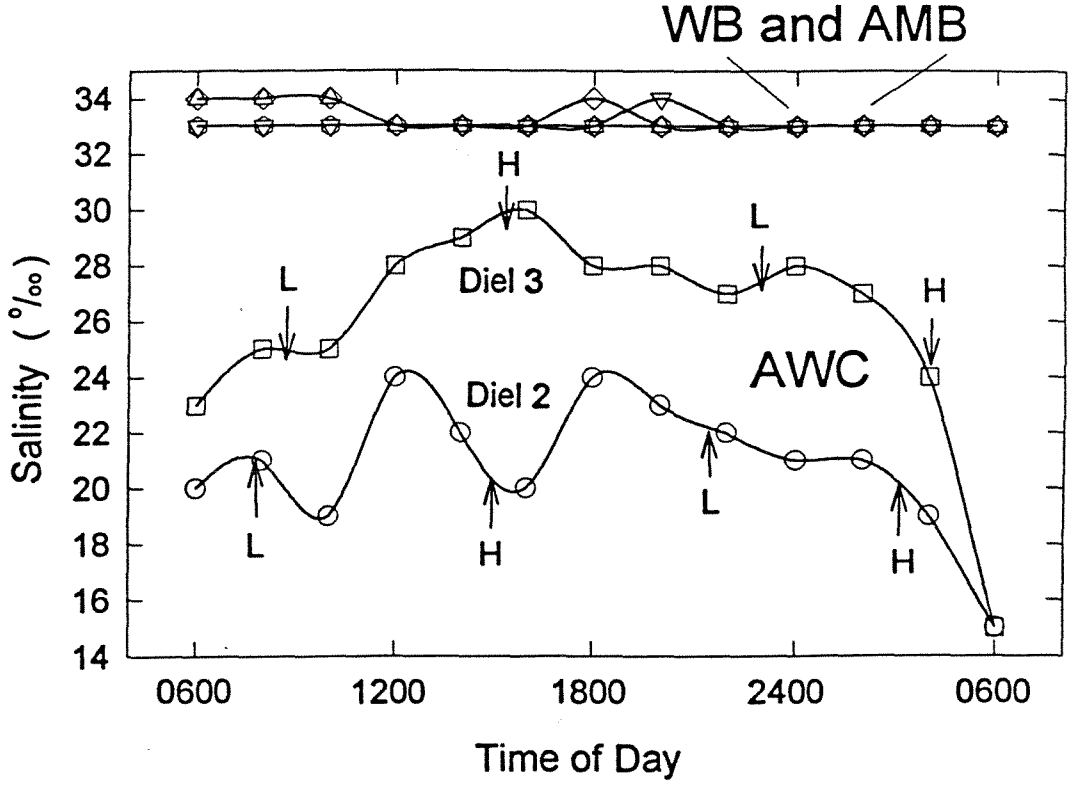

Fig. 4.14 Salinity (ppt) variations at Waikiki Beach (WB), Ala Moana Beach (AMB) and the Ala Wai Canal (AWC) during DIEL 2 and DIEL 3 experiments.

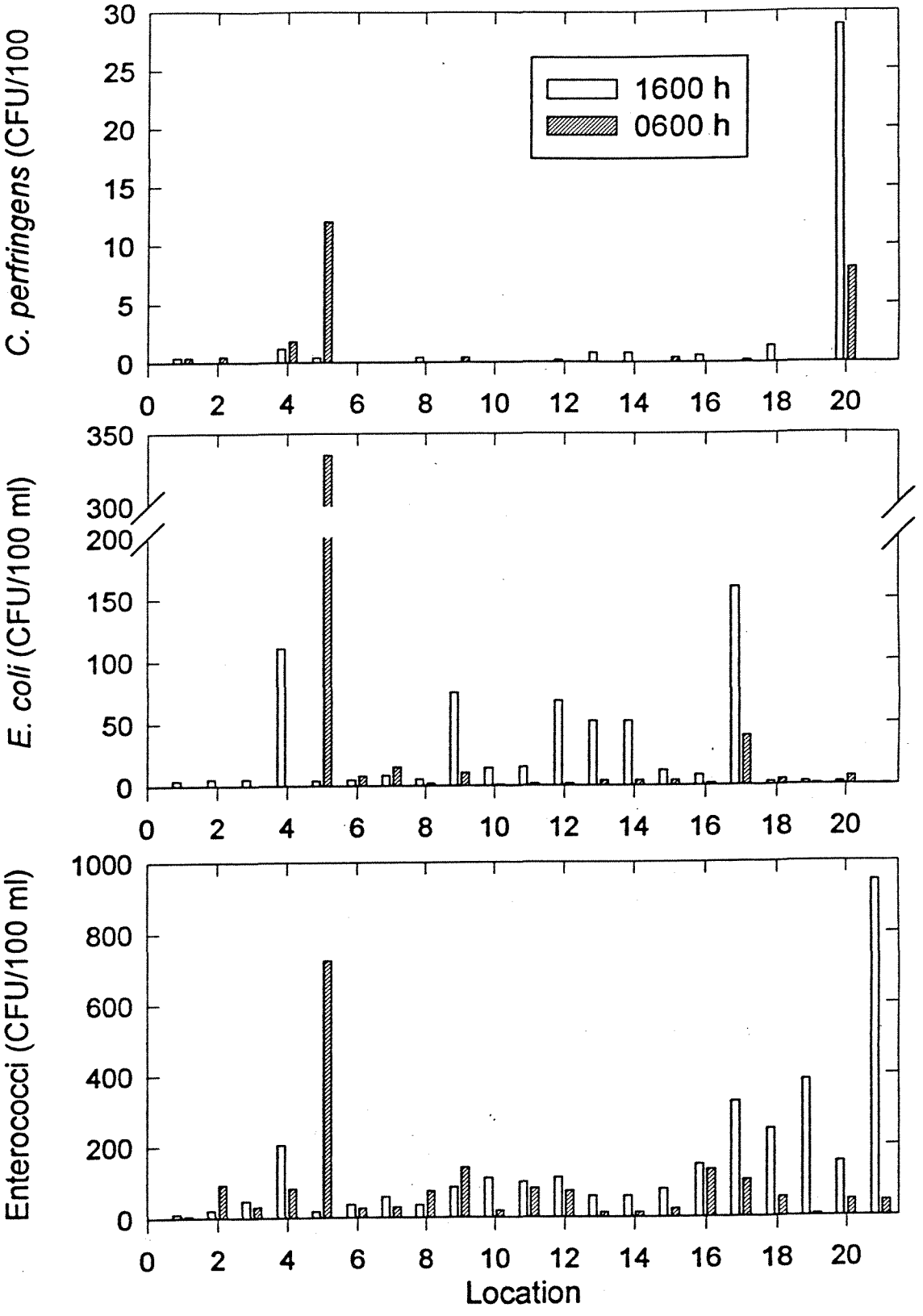

Fig. 4.15 Abundances of indicator bacteria for shoreline surveys at 1600 and 0600 h during DIEL 2. Site locations per Fig. 3.1. Sample volumes were 500 ml for *Clostridium perfringens* and 200 ml for *Escherichia coli* and enterococci. *E. coli* colonies from 0600 h samples at sites 1-5 were too numerous to be effectively enumerated. Data in Appendix 8.5.

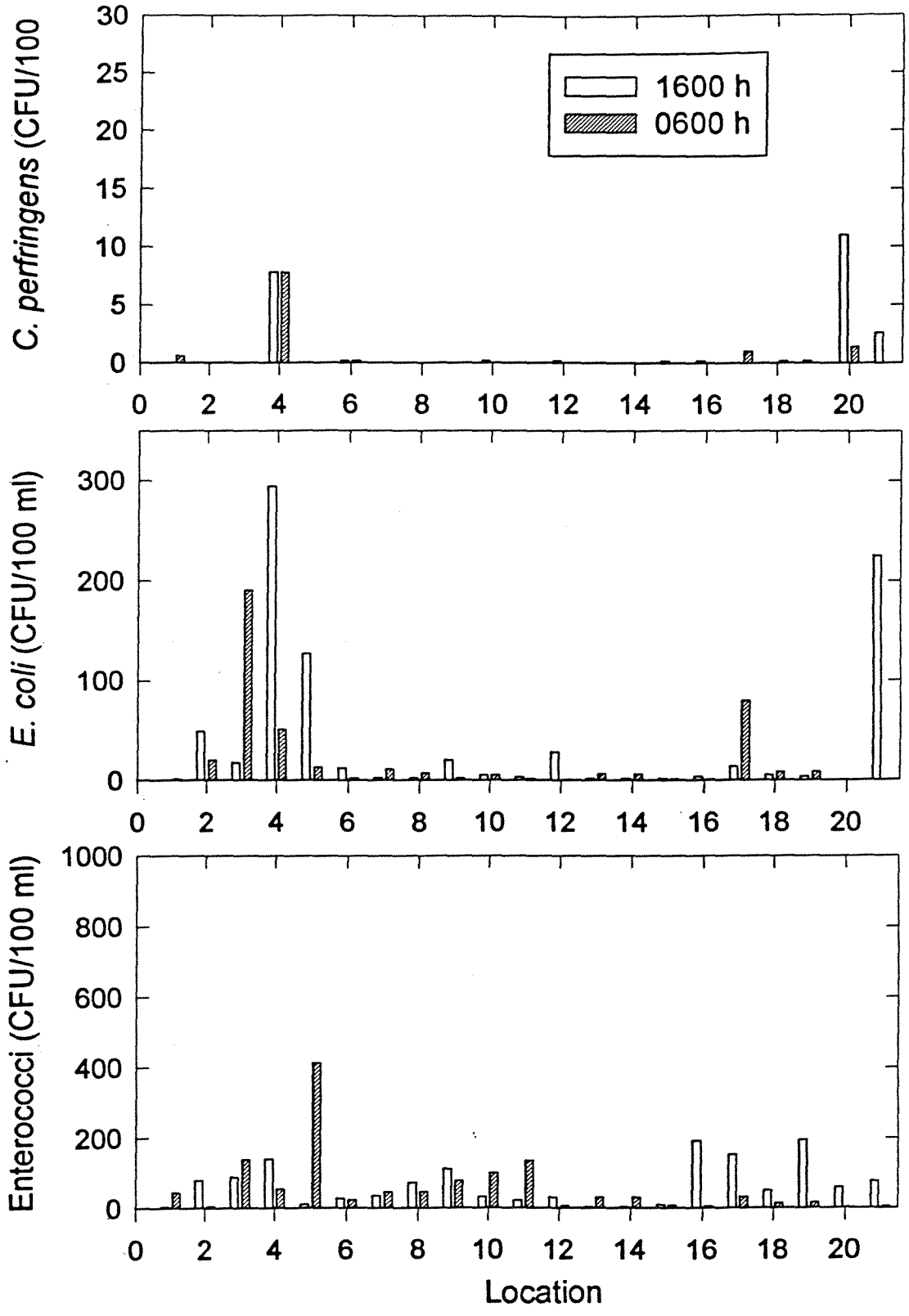

Fig. 4.16 Abundances of indicator bacteria for shoreline surveys at 1600 and 0600 h during DIEL 3. Site locations per Fig. 3.1. Sample volumes were 500 ml for *Clostridium perfringens*, 200 ml for *Escherichia coli* (50 at AWC) and 100 ml for enterococci (50 at AWC). Data in Appendix 8.5.

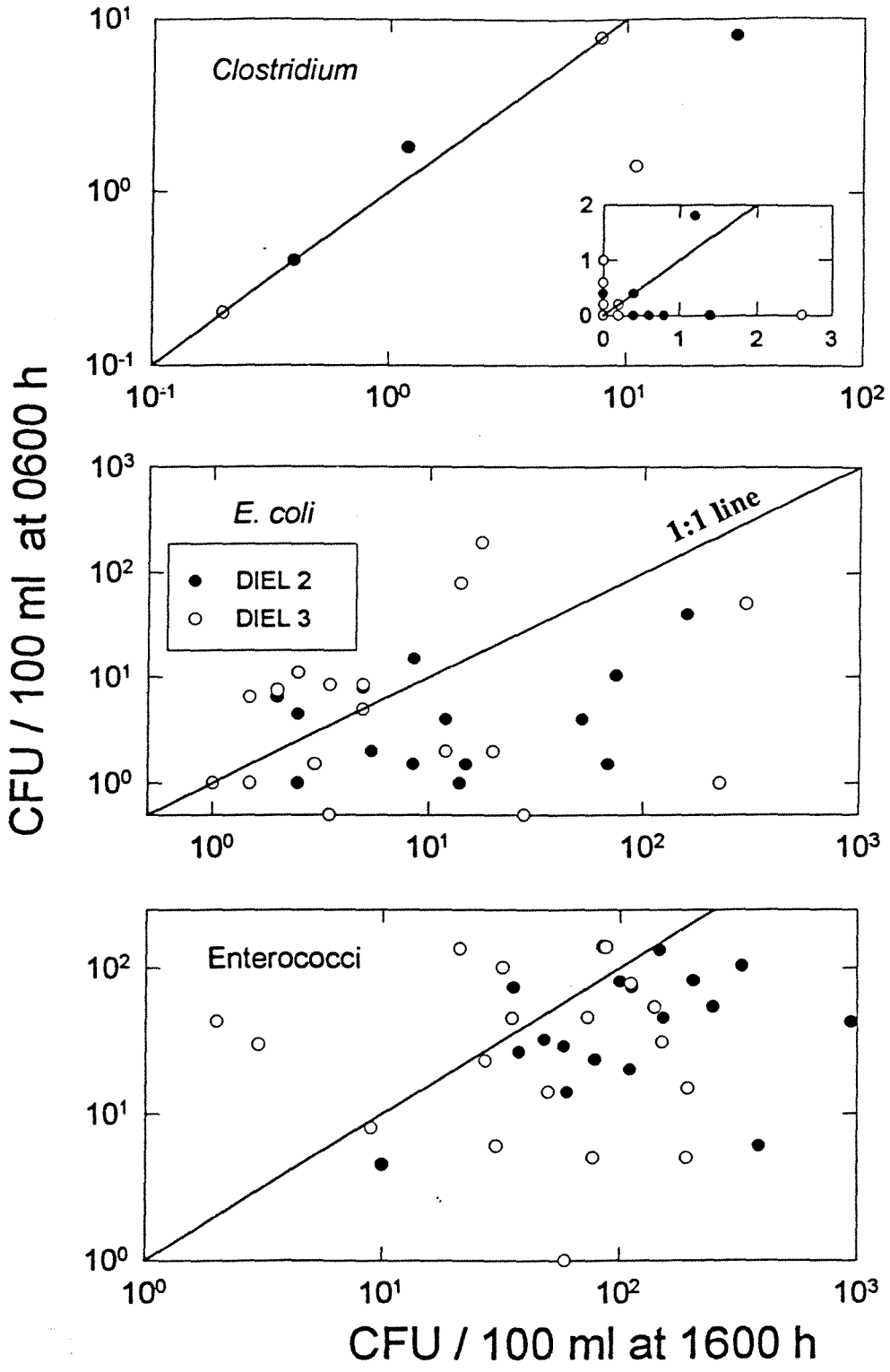

Fig. 4.17 The "Afternoon Effect". Scatterplots comparing the abundances of indicator bacteria in samples collected at 1600 and at 0600 h during DIEL 2 (open symbols) and DIEL 3 (closed symbols) experiments. Points below the 1:1 line indicate higher abundances in the afternoon. Distance from the 1:1 line represents an exponential difference between morning and afternoon samples.

The results from transect sampling during DIEL 3 were more complex than those from DIEL 2. Although counts tended to decrease with increasing distance from the beach, enterococci counts from Transect A at 0600 were highly variable (Fig. 4.18). Also, for both *E. coli* and enterococci, there was no clear difference between samples collected at 1600 h from those taken at 0600 h.

In nearly every daytime transect, concentrations of *E. coli* or enterococci decreased with increasing distance (out to 60 m) from the Waikiki shoreline (Fig. 4.18). This result is consistent with the idea that bathers are a significant source of indicator bacteria, since most were observed to be close to shore. Thus, the transect results may be interpreted as a dilution curve, in which indicator bacteria shed by bathers are mixed with offshore waters. Inactivation of bacteria by sunlight could also contribute to this phenomenon by decreasing the numbers of culturable bacteria transported seaward.

Bacterial concentrations were sometimes quite different in samples collected from parallel transects only 50 m apart. Localized channeling of water by the sills, depressions, channels and reefs along the nonhomogeneous shoreline may be one of the factors responsible. Indeed, with some knowledge of favored bathing sites, tidal stage and detailed water flow to and from the shoreline, an astute microbiologist could well take samples that minimize or maximize counts of indicator bacteria.

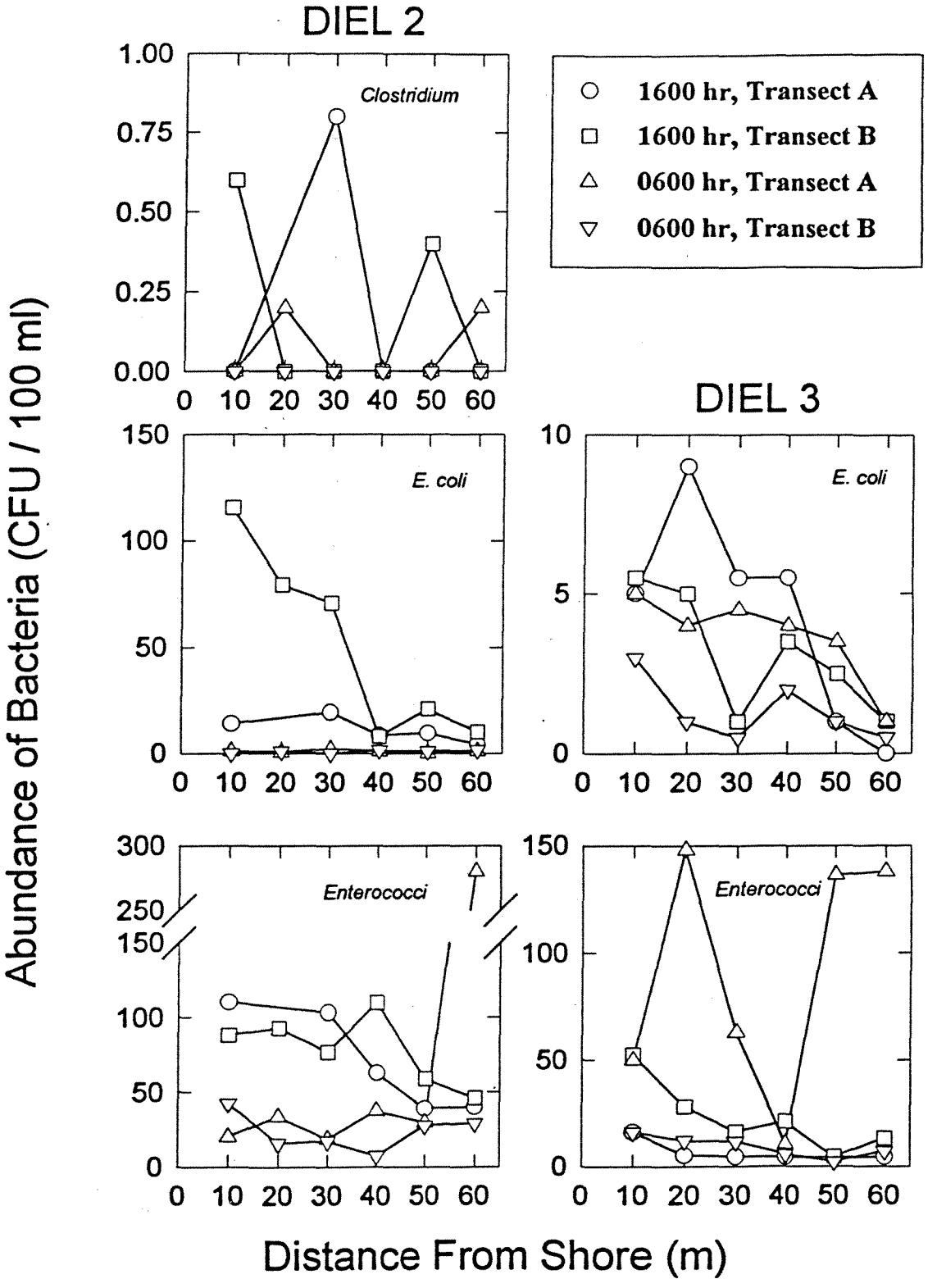

Fig. 4.18 Distributions of indicator bacteria on 60-m transects perpendicular to Waikiki Beach during DIEL 2 and DIEL 3 experiments. Samples were taken on replicate transects 50 m apart at 1600 h and at 0600 h on the following day. Data in Appendix 8.4.

4.2 Bacterial Decay Experiments

4.2.1 LIGHT 1

Figs. 4.19 to 4.21 show the declines in cell abundances of *E. coli*, enterococci and *C. perfringens* during the LIGHT 1 decay experiment. All indicator counts in the light treatments followed dark controls through the first 8 h of the experiment. Counts of *E. coli* and enterococci in the light treatments diverged significantly from mean dark controls at first light (Figs 4.19 and 4.20), but no light effect was noted for *C. perfringens* when either the highest two light levels or all light were compared to dark controls (Table 4.1). For *E. coli*, dark controls gave about a factor of 50 decline over two days, while the highest light treatments declined by more than 4 orders of magnitude and the lowest by about 2 orders of magnitude (Fig 4.19). For enterococci, the dark controls declined by about an order of magnitude, but the difference between decay rates in light treatments and controls were similar to those for *E. coli.*

Temporal patterns in decay rates of *E. coli* and enterococci are developed in detail for the various light treatments in Figs. 4.22 to 4.31. Mean rates of change in dark controls are represented in each of these figures by shaded histograms corresponding to the time intervals over which the rates apply. Controls for both indicators show a maximum decay shortly after sunrise on the first day of the experiment (about hour 10). This substantial decrease in bacterial culturability occurred independently in each of the controls without an apparent physical cause (light and temperature were controlled), and it is unlikely to be related to predators introduced with the sewage microbes since delicate protozoans would have been largely destroyed by prescreening and centrifugation and/or diluted to ineffective levels by the 1:1000 sewage addition. It is possible, on the other hand, that a disproportionate fraction of the bacteria passed the stress threshold resulting in loss of culturability at this time. Alternatively, they may have lysed due to viral infection -- either a regular cycle in the sewage holding tanks or synchronized by

increased encounters between bacteria and phages during the centrifugation steps used in preparing the sewage additions. Increased mortality rates in the controls were also noted prior to the start of the second light cycle (about hour 30). Whatever the cause of the variations in rates of decline in control bottles, these variations point clearly to the need for appropriate controls for interpreting treatment effects in bacterial decay experiments.

Table 4.1 Summary of mean exponential rates of decline (h^{-1}) observed in LIGHT 1 incubations.
Day 1= incubation times 0 to 22.25 h;
Day 2 = incubation times 22.25 to 45.5 h; Total = 0 to 45.5 h.

Condition	Day 1	Day 2	Total
E. coli			
Treatment 1	-0.167	-0.310	-0.236
Treatment 2	-0.162	-0.268	-0.216
Treatment 3	-0.157	0.008	-0.075
Treatment 4	-0.106	-0.058	-0.082
Treatment 5	-0.118	-0.066	-0.092
Dark Controls	-0.044	-0.083	-0.064
Enterococci			
Treatment 1	-0.146	-0.210	-0.178
Treatment 2	-0.093	-0.125	-0.109
Treatment 3	-0.076	-0.102	-0.089
Treatment 4	-0.077	-0.090	-0.084
Treatment 5	-0.112	-0.056	-0.084
Dark Controls	-0.034	-0.049	-0.041
C. perfringens			
Treatments 1 & 2	-0.013	-0.082	-0.048
All light treatments	-0.018	-0.061	-0.040
Dark controls	-0.013	-0.062	-0.038

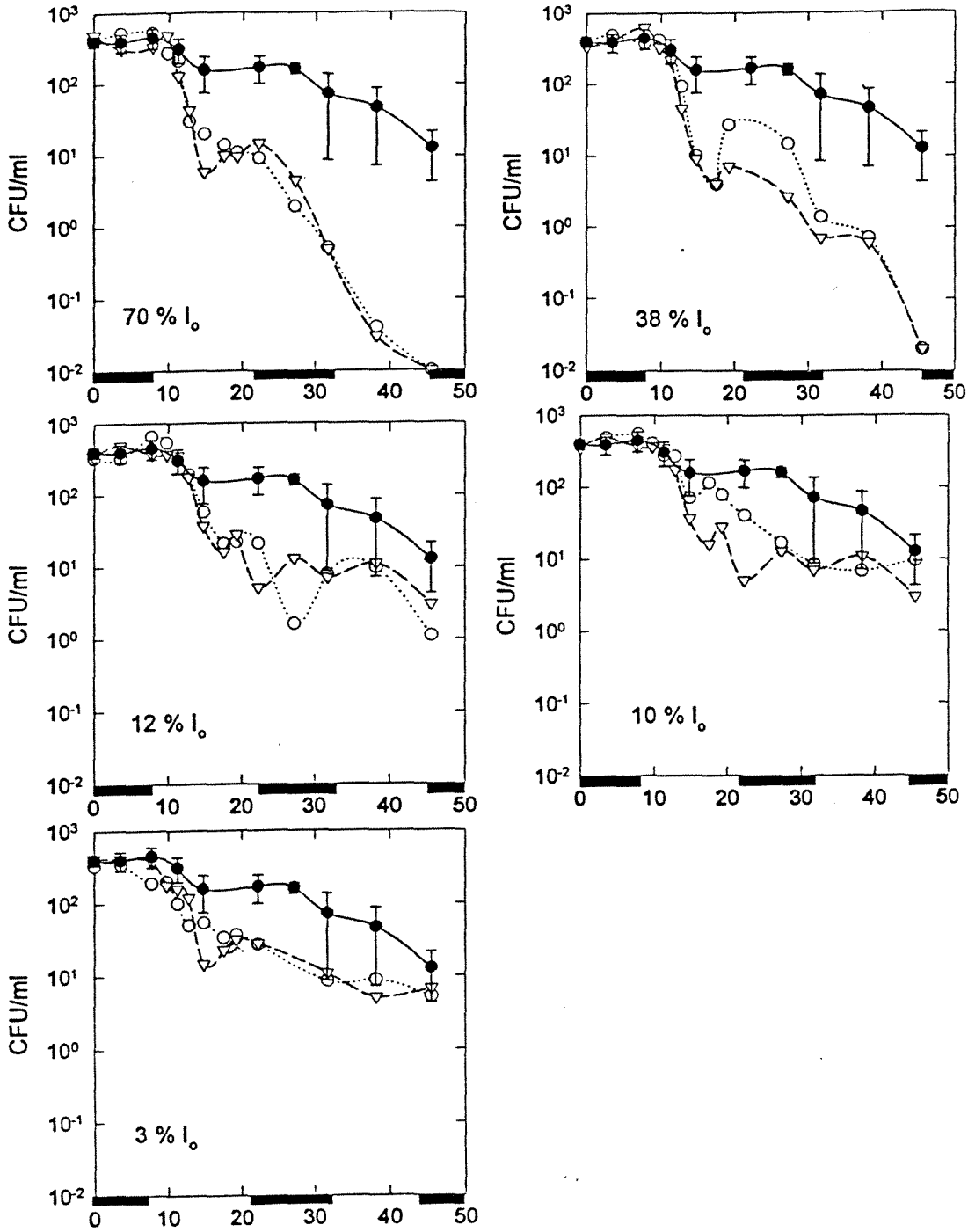

Fig. 4.19 Abundances of culturable *E. coli* (CFU/ml) during the LIGHT 1 experiment. Dark symbols show the mean counts of 5 dark controls; open symbols show individual counts in replicated light bottles. Light treatments are given as % I_o in the experimental incubators. Dark horizontal bars indicate nighttime. Data in Appendix 8.6.

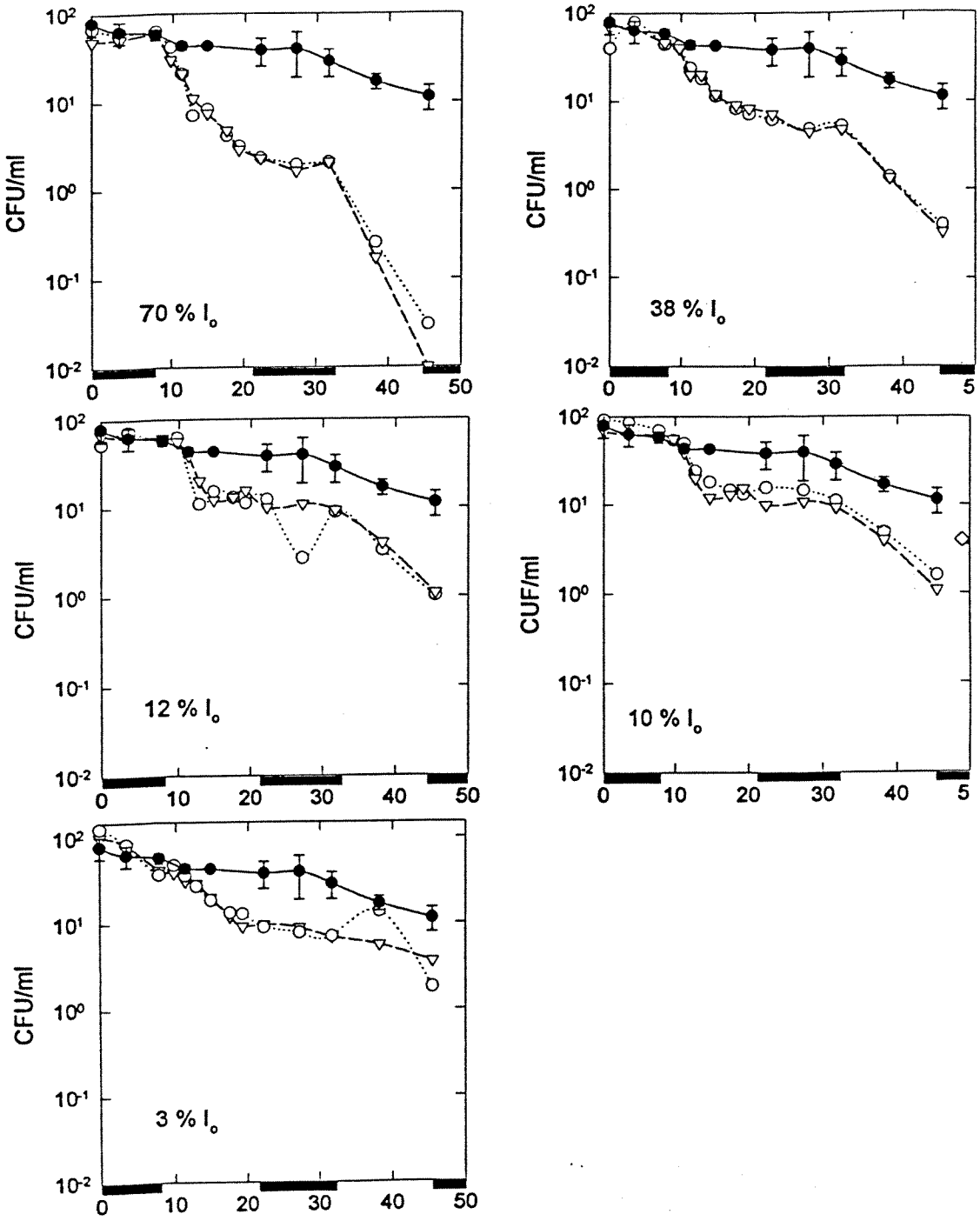

Fig. 4.20 Abundances of culturable enterococci (CFU/ml) during the LIGHT 1 experiment. Symbols as in Fig. 4.19.

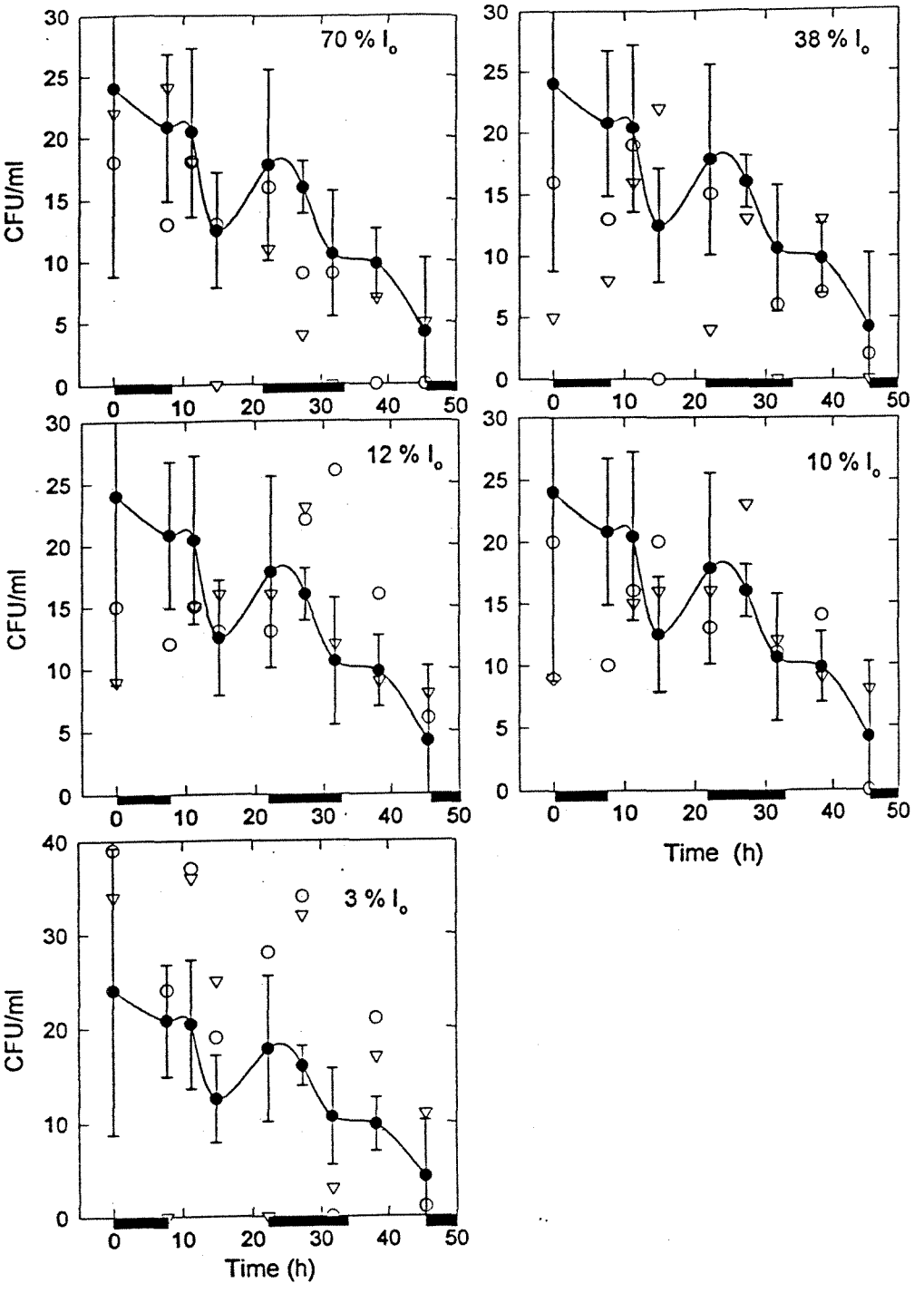

Fig. 4.21 Abundances of culturable *Clostridium perfringens* (CFU/ml) during the LIGHT 1 experiment. Symbols as in Fig. 4.19.

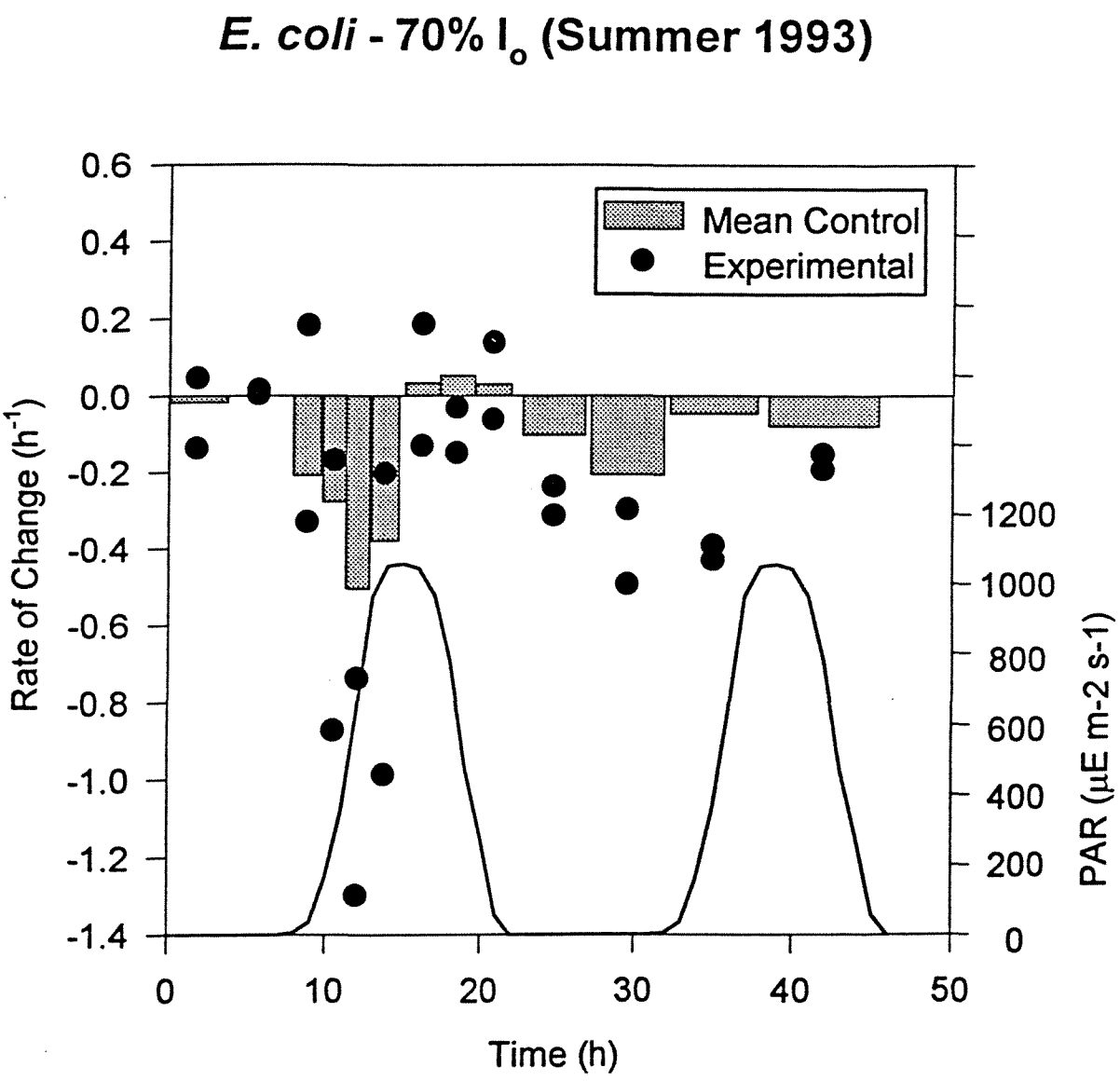

Fig. 4.22 Decay rate analysis for *E. coli* at 70% I_o during LIGHT 1. Shaded histogram shows the mean rate of change (h^{-1}) for 5 dark controls. Dark symbols show corresponding rates of change during the same time interval in replicate light bottles. Negative rate of change = population decline; positive rate = growth. Curves show light levels expressed as % I_o times measured PAR (photosynthetically available radiation) at Kunia Sugar Plantation, Oahu.

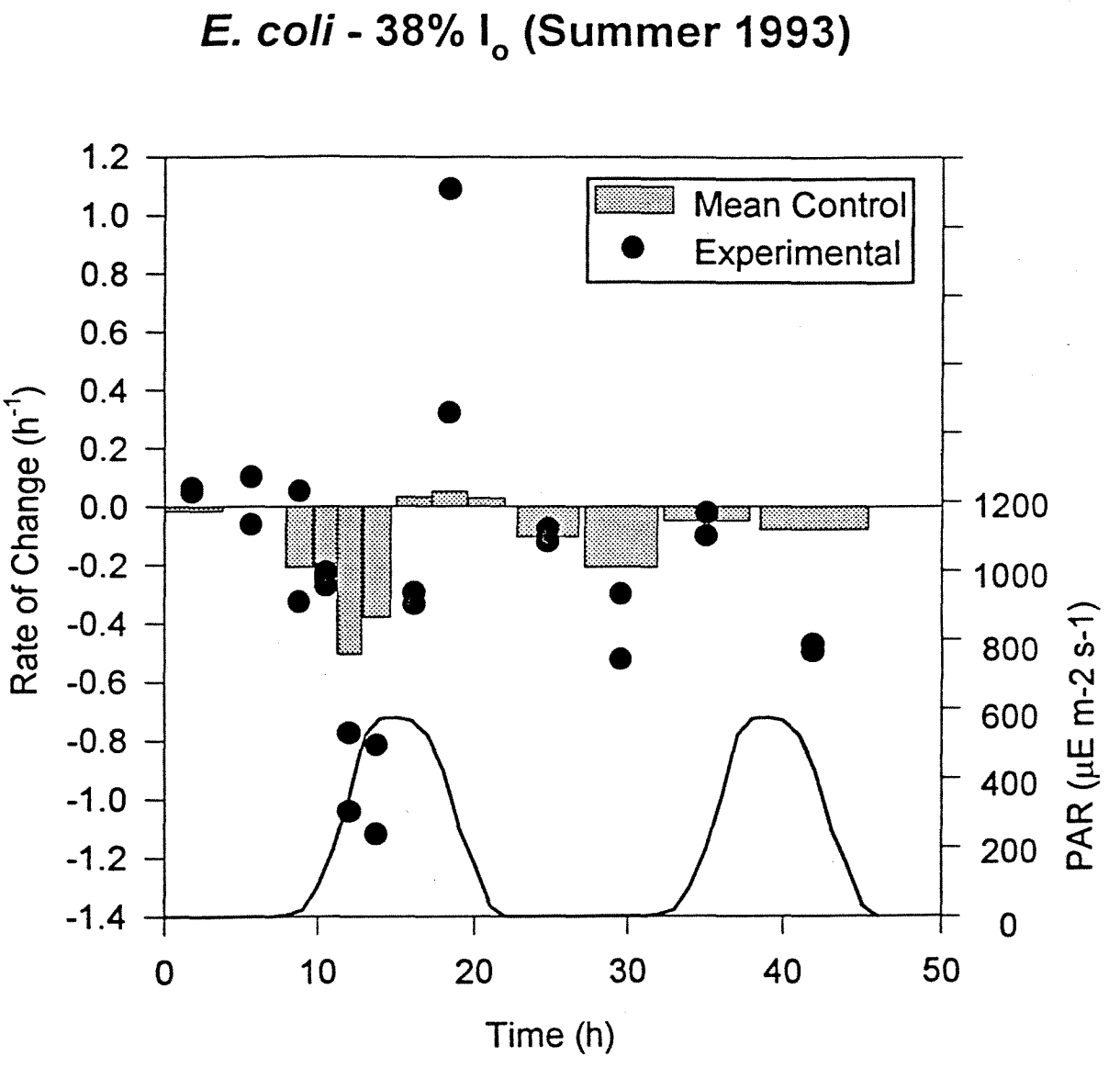

Fig. 4.23 Decay rate analysis for *E. coli* at 38% I_o during LIGHT 1. Symbols as in Fig. 4.22.

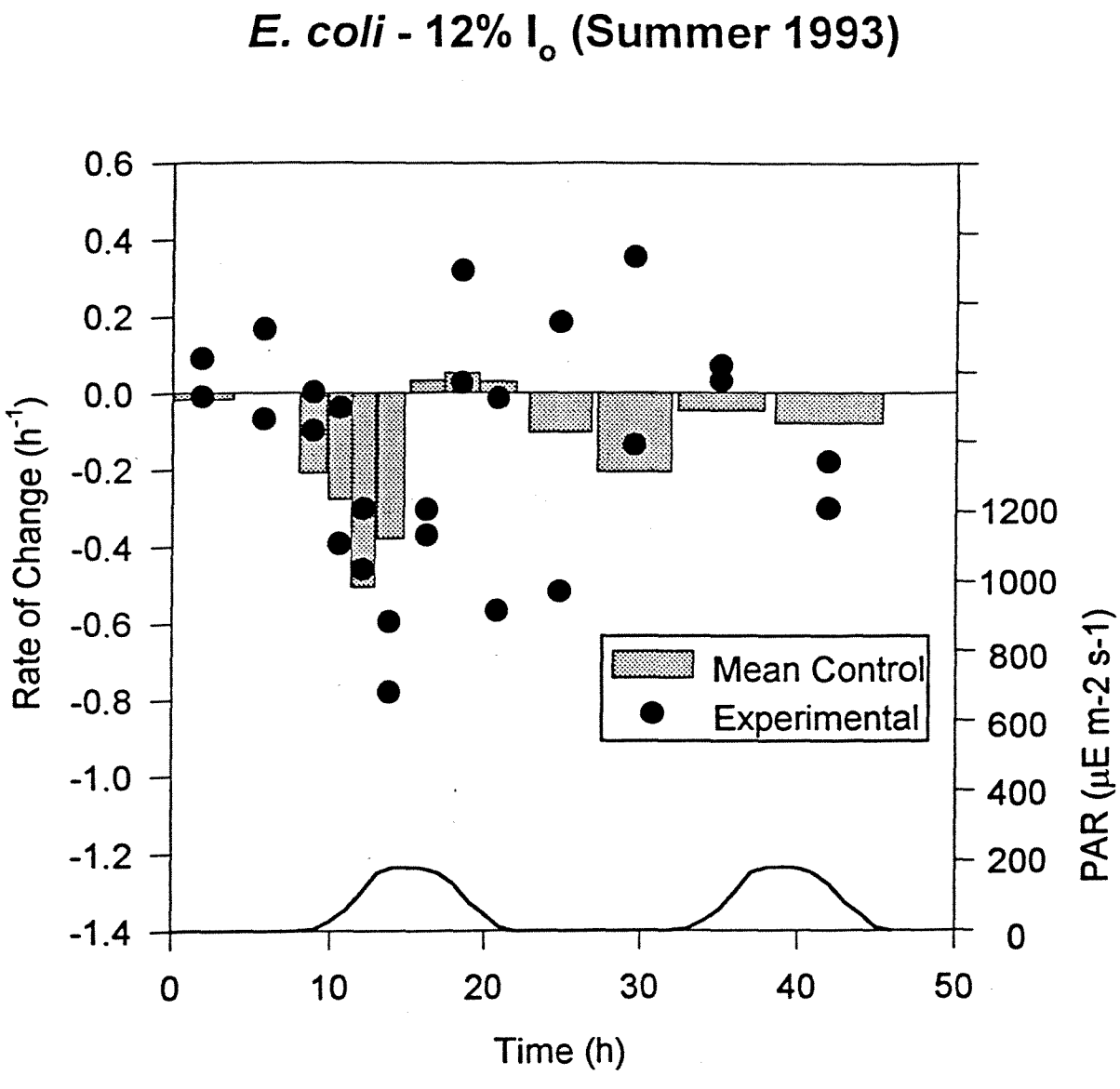

Fig. 4.24 Decay rate analysis for *E. coli* at 12% I_o during LIGHT 1. Symbols as in Fig. 4.22.

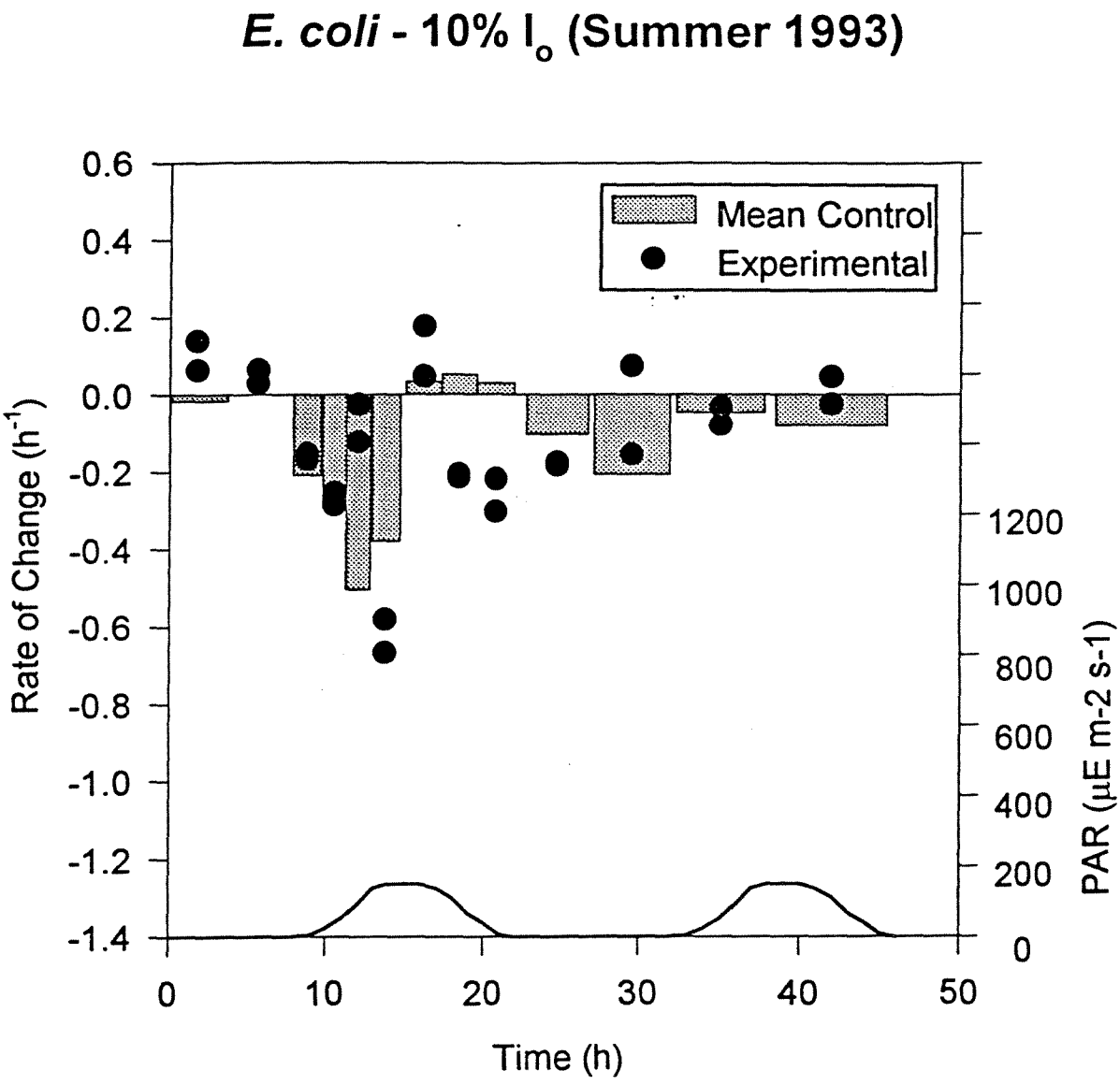

Fig. 4.25 Decay rate analysis for *E. coli* at 10% I_0 during LIGHT 1. Symbols as in Fig. 4.22.

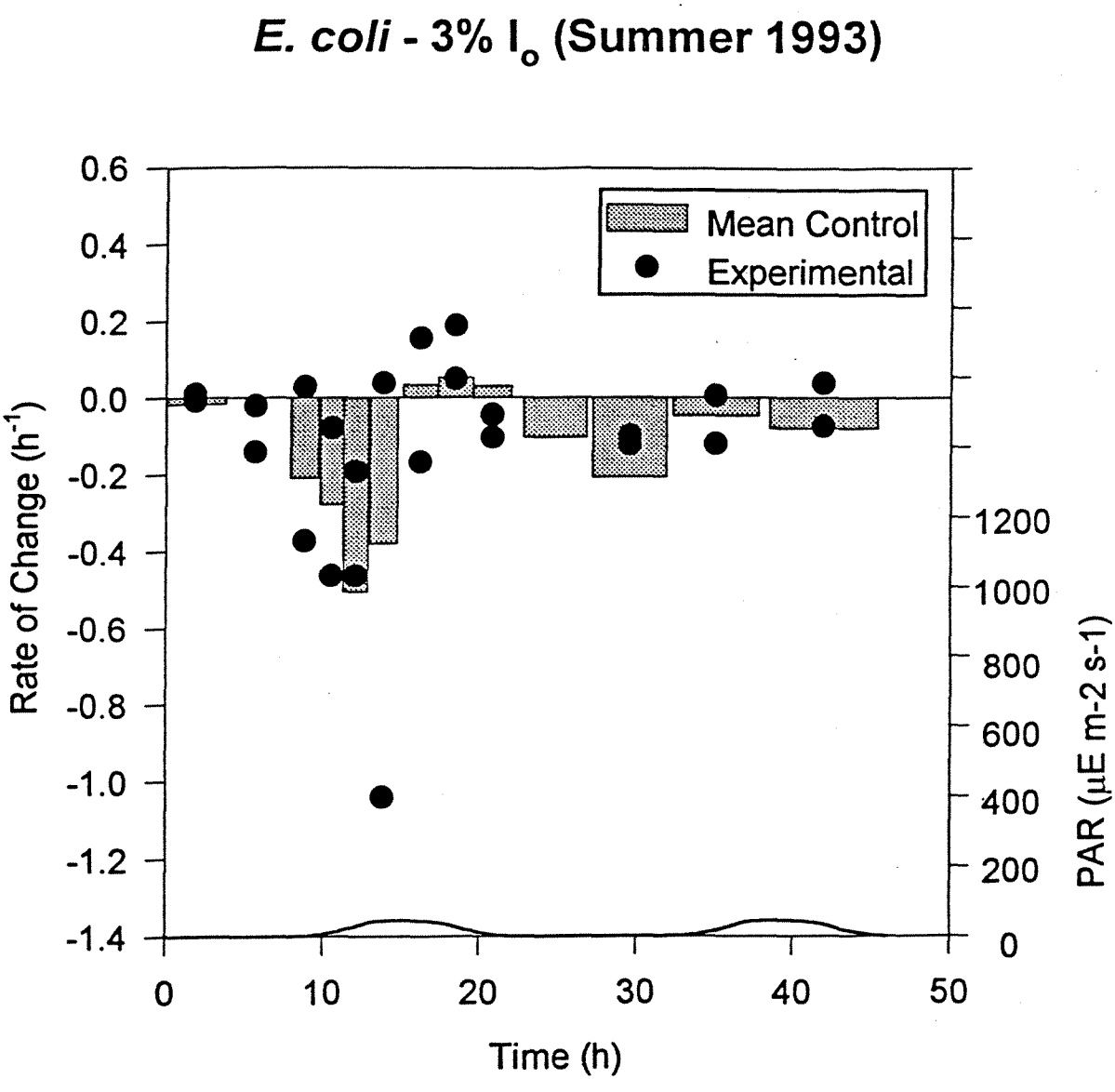

Fig. 4.26 Decay rate analysis for *E. coli* at 3% I_o during LIGHT 1. Symbols as in Fig. 4.22.

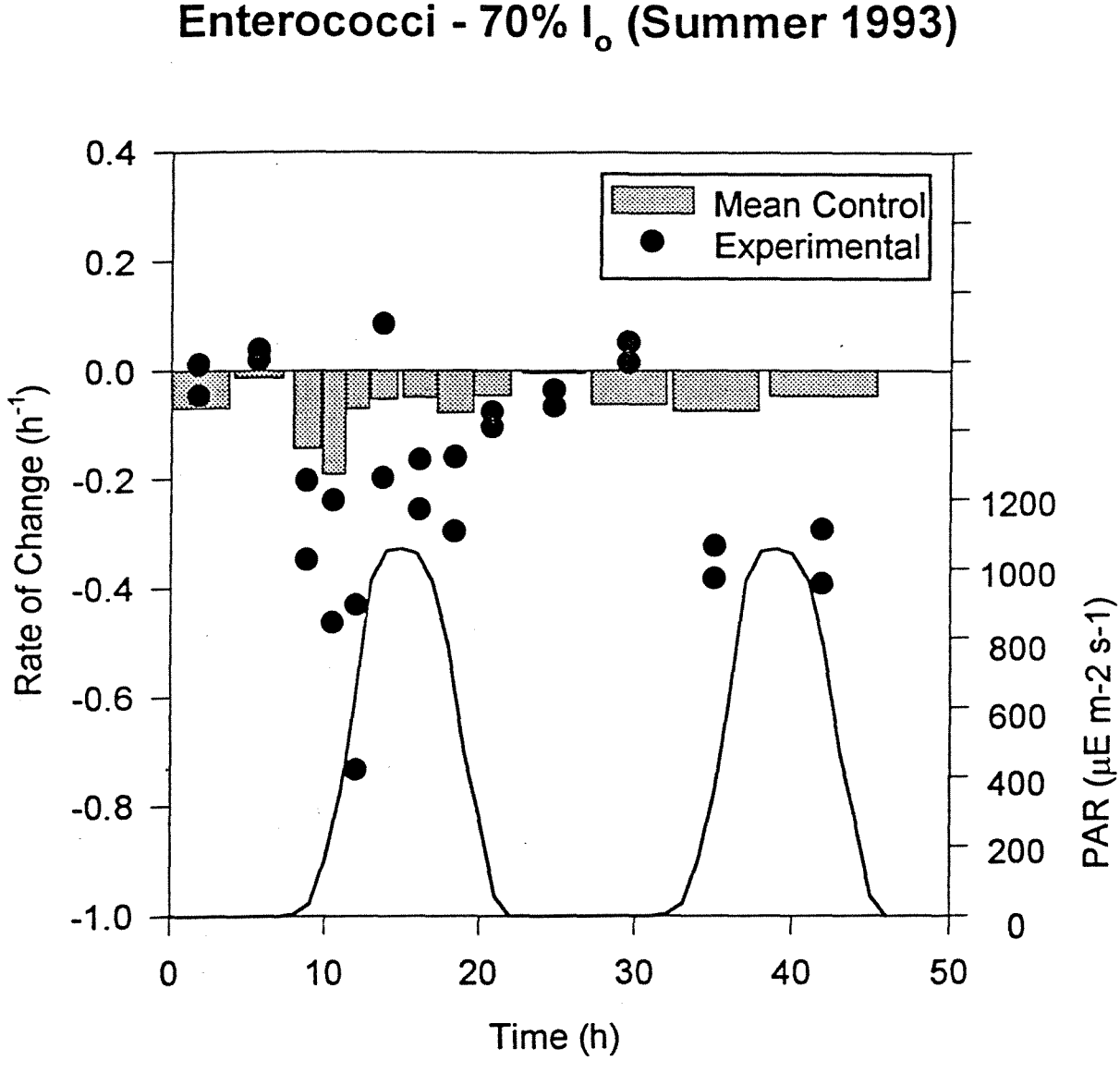

Fig. 4.27 Decay rate analysis for enterococci at 70% I_o during LIGHT 1. Symbols as in Fig. 4.22.

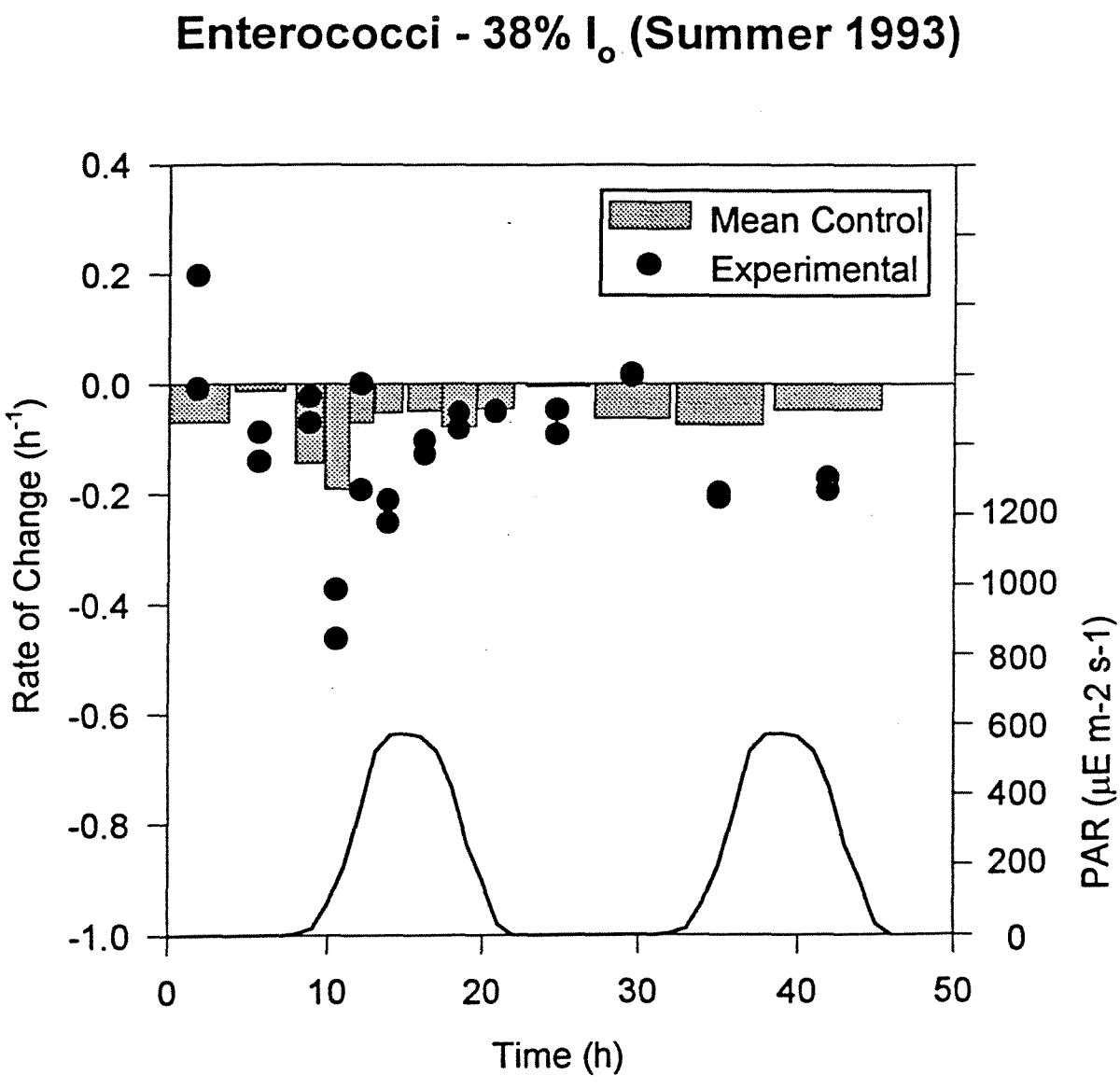

Fig. 4.28 Decay rate analysis for enterococci at 38% I_o during LIGHT 1. Symbols as in Fig. 4.22.

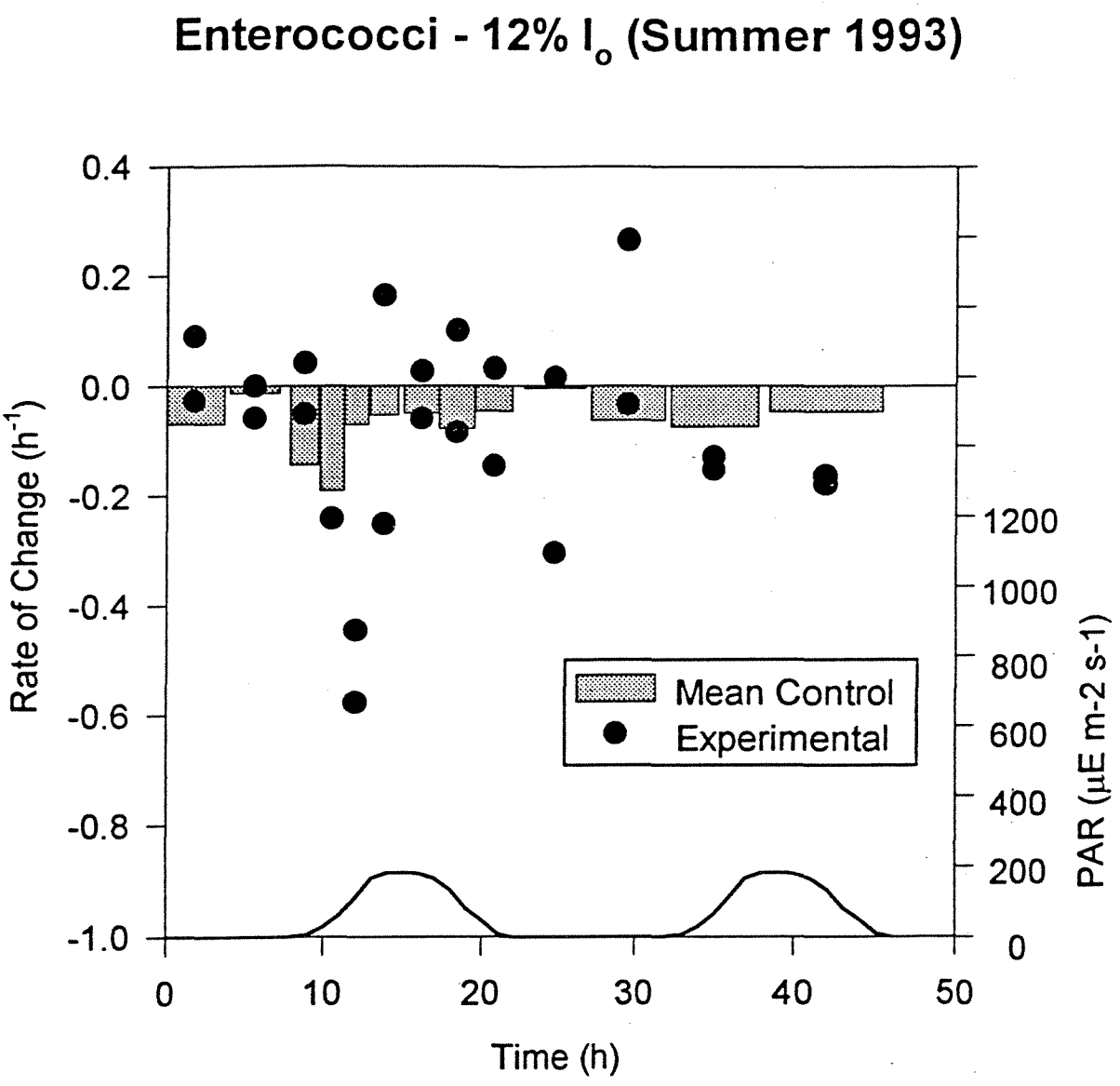

Fig. 4.29 Decay rate analysis for enterococci at 12% I_o during LIGHT 1. Symbols as in Fig. 4.22.

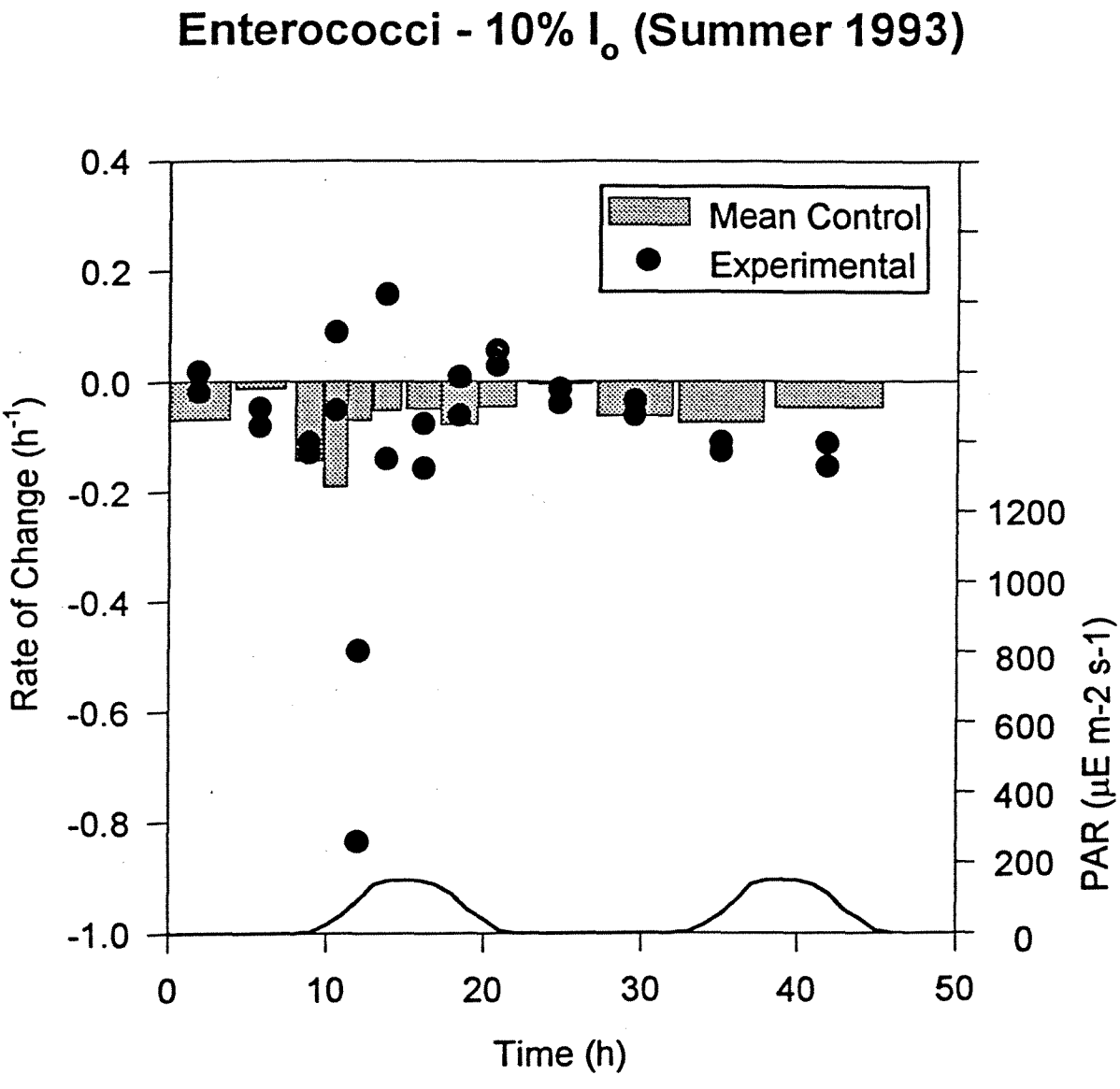

Fig. 4.30 Decay rate analysis for enterococci at 10% I_o during LIGHT 1. Symbols as in Fig. 4.22.

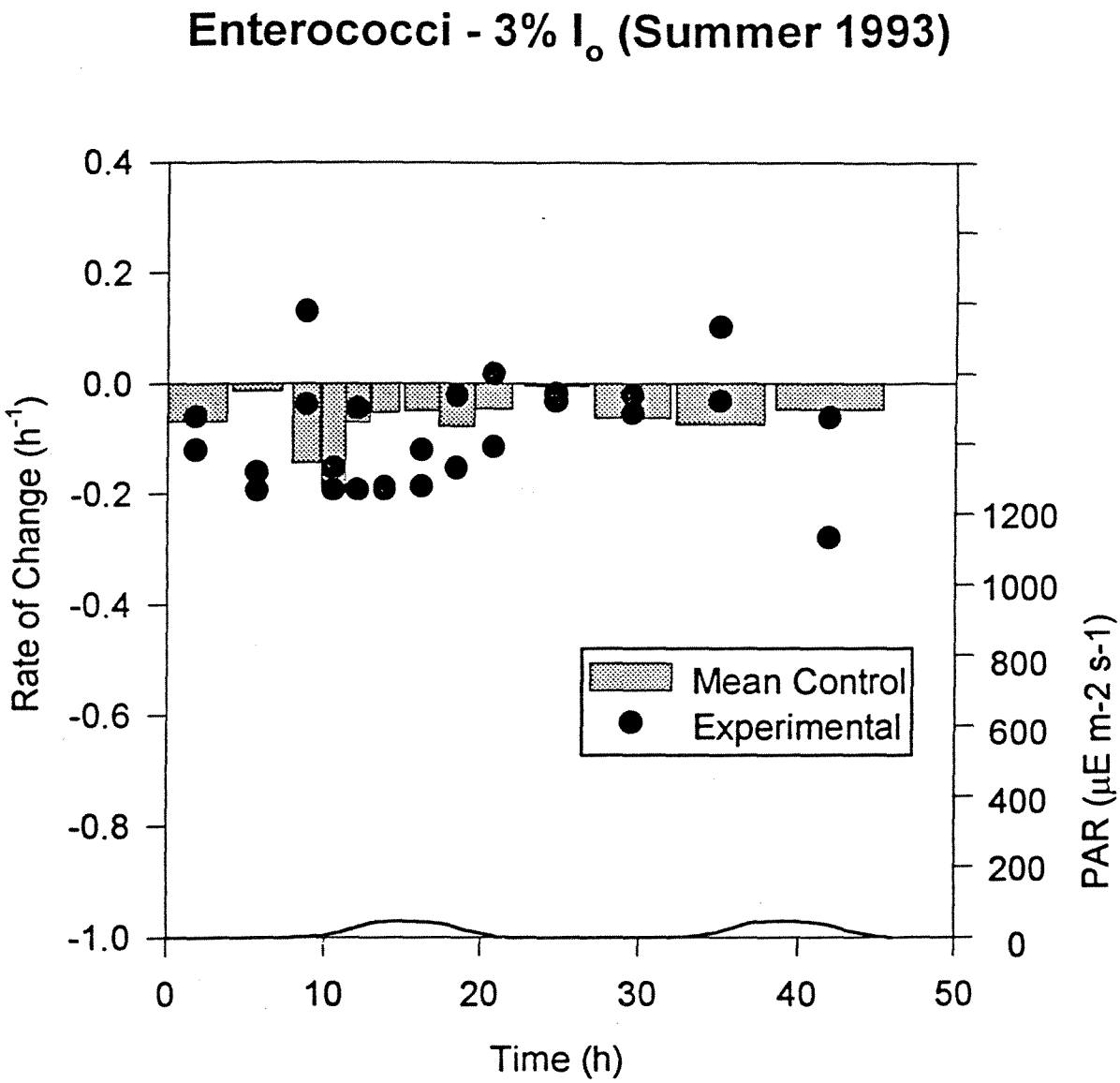

Fig. 4.31 Decay rate analysis for enterococci at 3% I_o during LIGHT 1. Symbols as in Fig. 4.22.

The effects of light on rates of bacterial inactivation are apparent in Figs 4.22 to 4.31 whenever the replicated rate estimates for the light treatments are more negative than the control rates. For most incubations, but particularly for those at the higher light levels, the deactivating effects of light are most significant early in the morning of the first day. After early morning exposure, rates of photodeactivation may even decline as incident light reaches its daytime maximum. There are many possible explanations for this observation -- the bacteria may either adapt to the light as it increases or the mixture of sewage microbes may contain individuals with very different physiological or genotypic susceptibilities to sunlight inactivation. Relatively low doses of sunlight were sufficient to deactivate the bulk of indicator bacteria in these incubations, while others were capable of passing through the period of highest light intensity while remaining active and culturable. For enterococci, the rates of bacterial deactivation in light treatments came back to low control levels during the nighttime following the first day of incubation. The cycle of sunlight deactivation was then repeated on the second day.

4.2.2 LIGHT 2 and 3

Figs. 4.32 to 4.35 show the changes in cell abundances of *E. coli* and enterococci during the LIGHT 2 and 3 decay experiments. Dark controls showed relatively low rates of decay or, in the case of enterococci in LIGHT 2 (in which se wage was added directly without washing out dissolved substrates), the cells even increased substantially during the incubation period. In comparison, counts in the light bottles dropped precipitously, in some cases to near zero in the first sampling interval (e.g., LIGHT 2 enterococci at 76% I_o; Fig. 4.33).

Time-averaged rates of decline were somewhat higher for *E. coli* during LIGHT 3 and slightly higher for enterococci during LIGHT 2 (Table 4.2). Consequently, neither indicator emerged as consistently more affected by photodeactivation. Enterococci may have been more susceptible to light deactivation in LIGHT 2 because they were in a state

of active growth, as seen in the rate difference between controls. Fujioka et al.'s (1981) results, also with sewage microbes from local treatment plants, led us to expect that fecal streptococci (approximately the same as our enterococci) would be significantly more resistant to light degradation than fecal coliforms (mostly *E. coli*). This may not be the case on a consistent basis if susceptibility is strongly influenced by physiological state.

Analyses of bacterial decay rates over the short subsampling intervals of LIGHT 2 and 3, revealed patterns similar to those observed for the LIGHT 1 experiment, except that the magnitudes of the decay rates were higher for comparable levels of light exposure (Figs. 4.36 to 4.43). At the highest light treatments, decay rates for both *E. coli* and enterococci began high ($\leq$ -1.2 h^{-1} in all cases) and declined to lower levels as the experiments continued (Figs 4.36 and 4.40). At progressively lower light levels, the highest decay rates were shifted to a later time interval, implying that the effect builds over time to some maximum. The dynamics of light deactivation are clearly complex, varying either with the physiological adaptations of individual organisms or reflecting different characteristics of populations in the mixed sewage community.

Table 4.2 Summary of mean exponential rates of decline (h^{-1}) observed in LIGHT 2 and LIGHT 3 experiments.

	LIGHT 2		LIGHT 3	
Treatment	***E. coli***	**Enterococci**	***E. coli***	**Enterococci**
Treatment 1 - 76% I_o	-1.024	-1.447 *	-1.501	-1.008
Treatment 2 - 30% I_o	-0.726	-0.828	-1.034	-0.424
Treatment 3 - 11% I_o	-0.392	-0.646	-0.521	-0.607
Treatment 4 - 6% I_o	-0.334	-0.473	-0.475	-0.413
Dark Controls	-0.196	0.179	-0.202	-0.005

All rates are for the full incubation period (* indicates rate for only the first sampling period).

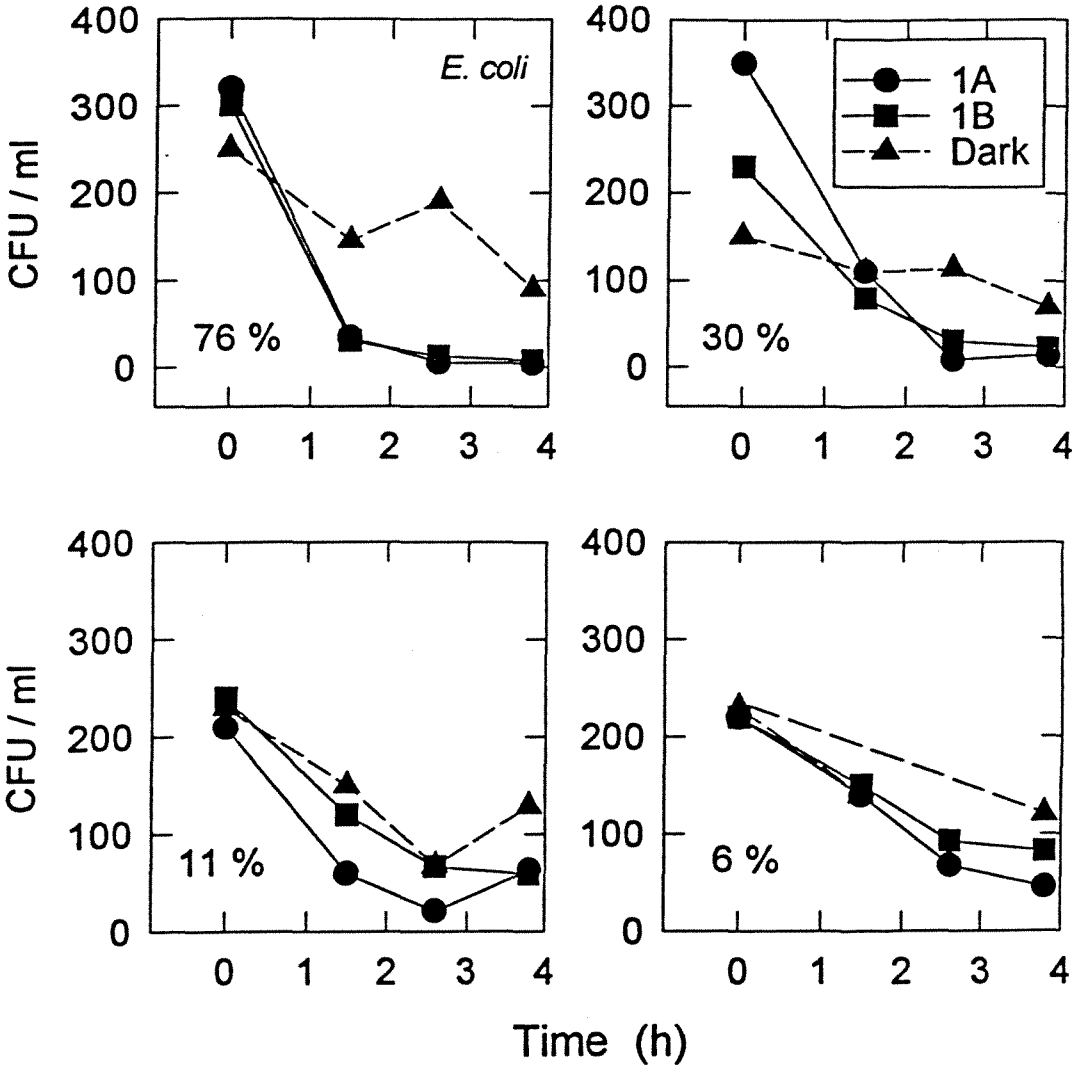

Fig. 4.32 Abundances of culturable *E. coli* (CFU/ml) during the LIGHT 2 experiment. Symbols show the mean counts of individual dark control and replicated light bottles. Light treatments are given as % I_o in the experimental incubators. Data in Appendix 8.7.

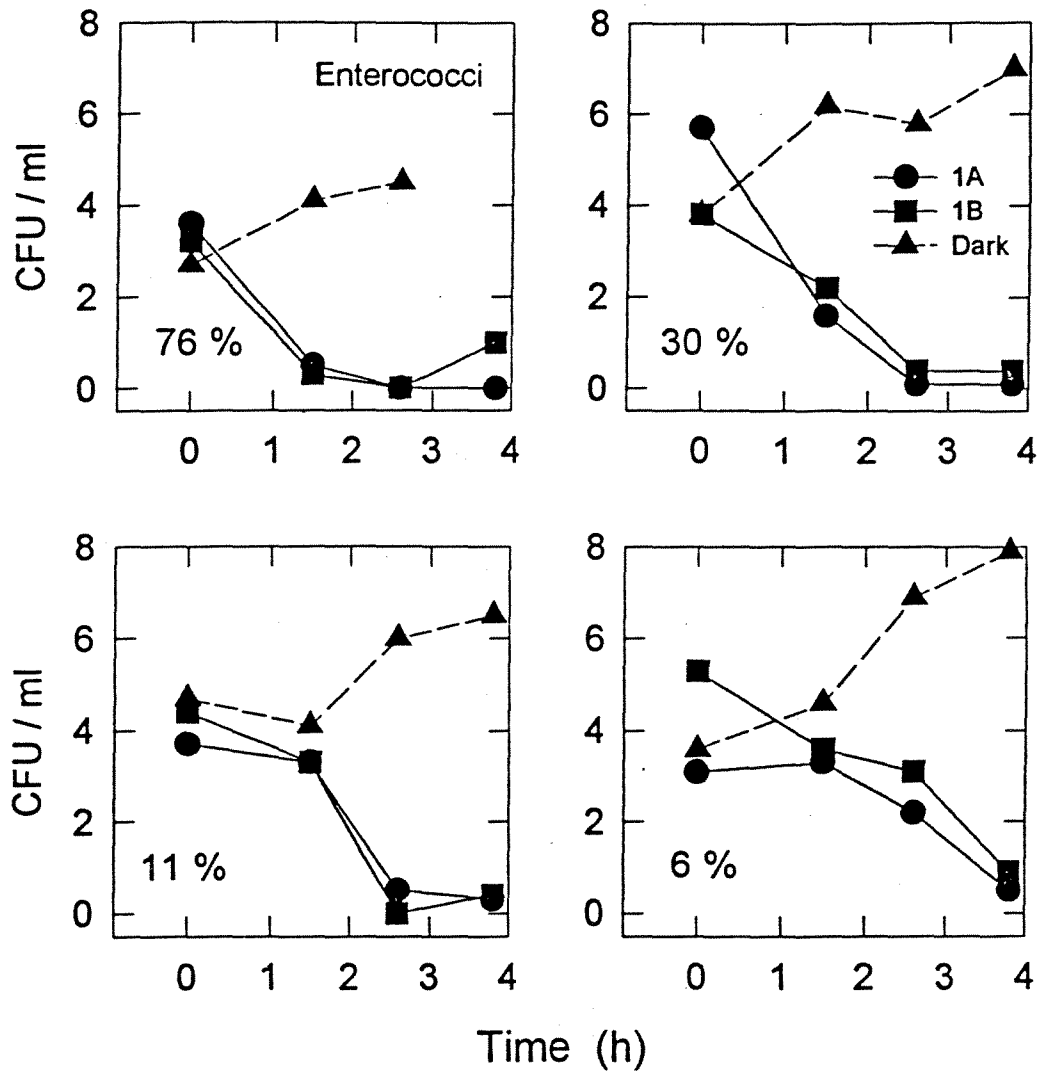

Fig. 4.33 Abundances of culturable enterococci (CFU/ml) during the LIGHT 2 experiment. Symbols as in Fig. 4.32.

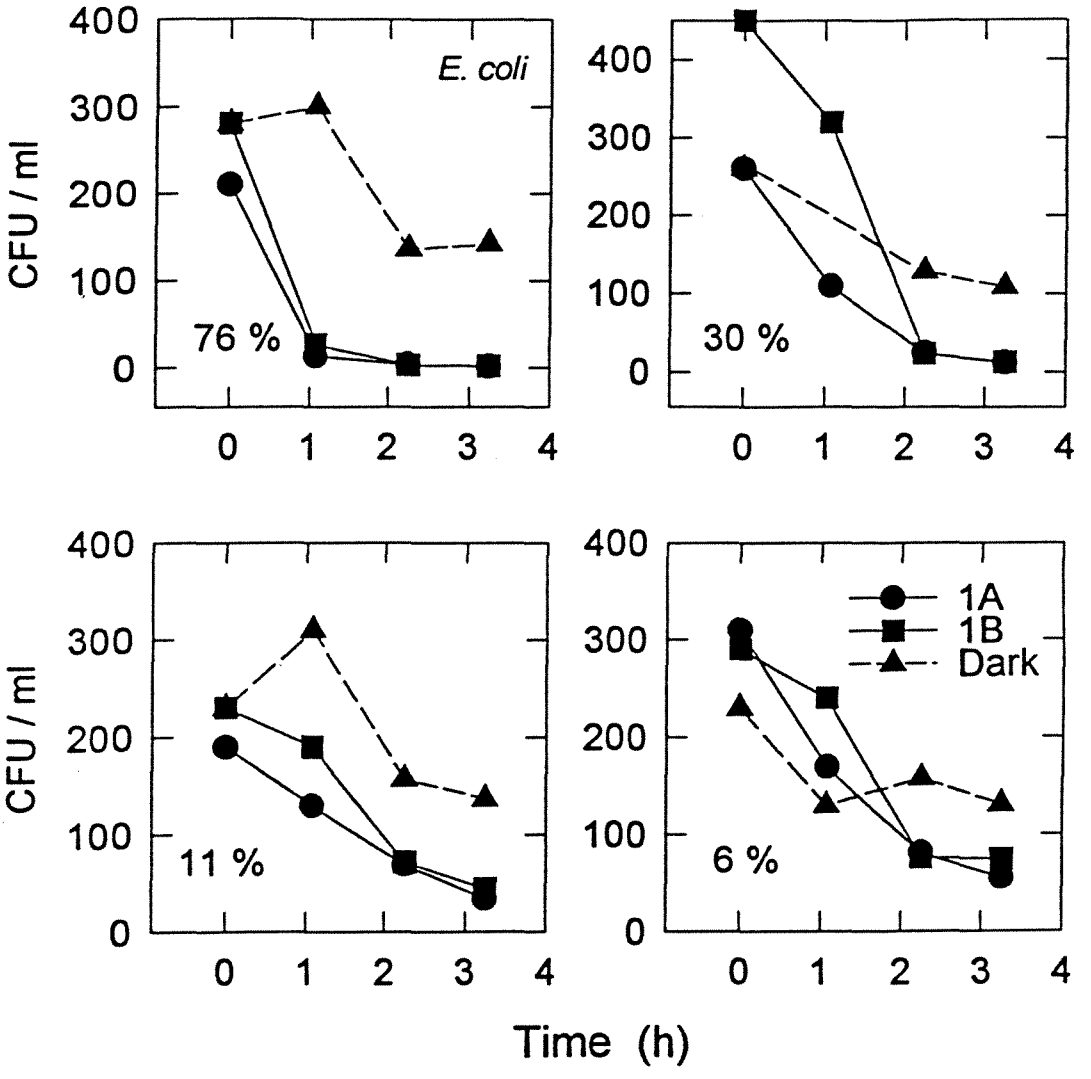

Fig. 4.34 Abundances of culturable *E. coli* (CFU/ml) during the LIGHT 3 experiment. Symbols as in Fig. 4.32.

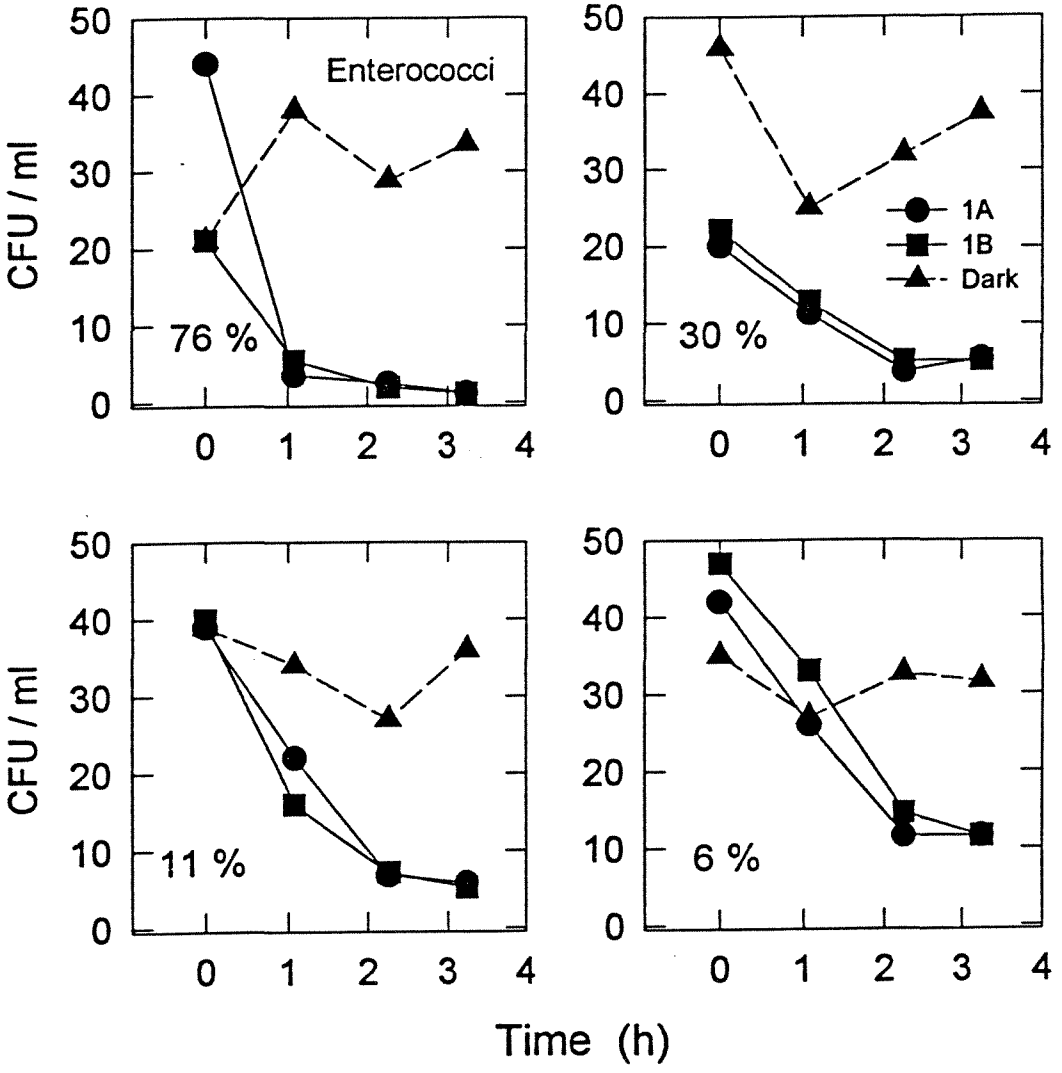

Fig. 4.35 Abundances of culturable enterococci (CFU/ml) during the LIGHT 3 experiment. Symbols as in Fig. 4.32

LIGHT 2 and 3 experiments differed from LIGHT 1 in exposing the bacteria to sunlight at peak intensity rather than gradually over the course of a natural daylight cycle. This latter approach was originally used to describe the potential of sunlight to deactivate indicator bacteria in Hawaiian waters (Fujioka et al. 1981). Given the response of bacteria to relatively low early morning light levels in LIGHT 1, the very dramatic effects at high noon are not surprising. Fujioka et al. (1981) noted exponential rates of decline for peak sunlight hours ranging from -1.5 to 4.6 h^{-1} for fecal coliforms and from -0.77 to -2.3 h^{-1} for fecal streptococci. These are many times the mean rates observed in LIGHT 1, but in the range of results for the LIGHT 2 and 3. Rates of decline in Fujioka et al.'s (1981) dark controls were comparable to those in LIGHT 1 (-0.048 to -0.11 h^{-1} and -0.027 to -0.064 h^{-1}, respectively, for the two indicators), implying that the main difference in these experiments was the manner in which light was presented.

The difference in incubation strategies for light decay experiments can have a profound effect on dose-rate predictions, as illustrated in Figs. 4.44 and 4.45. Decay rates due to photodeactivation are highlighted in these figures by subtracting from each light bottle the rate observed in dark controls (i.e., the zero line = the control rate). For experiments conducted over a full natural daylight cycle, the rate-dose effect is significant, but relatively modest. Experiments conducted over short incubation periods during peak sunlight hours clearly exaggerate the rate expectations when extrapolated to the full light dose experienced over the course of a day. Both of these results may have some applicability to decay rates in Mamala Bay. Where the question relates to the long-term residence and transport of indicators at given depths in the water column, the lower rates are likely to be more appropriate. If the issue is what will happen in the short-term to indicators introduced to seawater during the daytime (e.g., the bacteria associated with bathers), the higher rates are applicable. Regression statistics for these different rate-dose relationships are given in Table 4.3.

Table 4.3 Regression statistics for photodeactivation effects of sunlight as a function of light dose (Ein m^{-2} time $period^{-1}$). Rate (h^{-1}) = a x b (Light).

	E. coli			Enterococci		
Experiments	**a**	**b**	**r^2**	**a**	**b**	**r^2**
LIGHT 1 - Day 1	0.0785	0.0018	0.31	0.0464	0.0019	0.49
LIGHT 1 - Day 2	-0.0664	0.0114	0.80	0.0129	0.0051	0.89
LIGHT 1 - combined	0.0061	0.0066	0.48	0.0297	0.0035	0.67
LIGHT 2	0.122	0.0714	0.86	0.619	0.0969	0.87
LIGHT 3	0.225	0.155	0.94	0.385	0.0775	0.68
LIGHT 2 & 3 combined	0.238	0.0878	0.57	0.465	0.0992	0.72

4.2.3 FLB 1 and 2

FLB are commonly used in short-term studies of bacterivory by marine protists from epifluorescence microscopic observations of their rates of accumulation into the feeding vacuoles of individual organisms (Sherr et al. 1987, Gonzáles et al. 1990 a, b, Monger & Landry 1992, Landry 1994). In the present study, the decline of FLB in unfiltered seawater was attributed to the grazing activities of the entire protistan community, because the observed disappearance of FLB was negligible in filtered controls (mean rate = 0.0004 h^{-1}, n=7). Mortality rate estimates from these experiments ranged from 0.004 - 0.023 h^{-1}, corresponding to the removal of 10 to 43% of the standing stock of bacteria per day (Fig. 4.46). No significance is attached to the comparison of rate estimates for water from different sources, as such rates are likely to vary substantially from day-to-day as conditions change. The reduced grazing activity at deeper depths is, however, consistent with expectations (Landry et al. 1995, Liu et al. 1995, Landry et al., In press a).

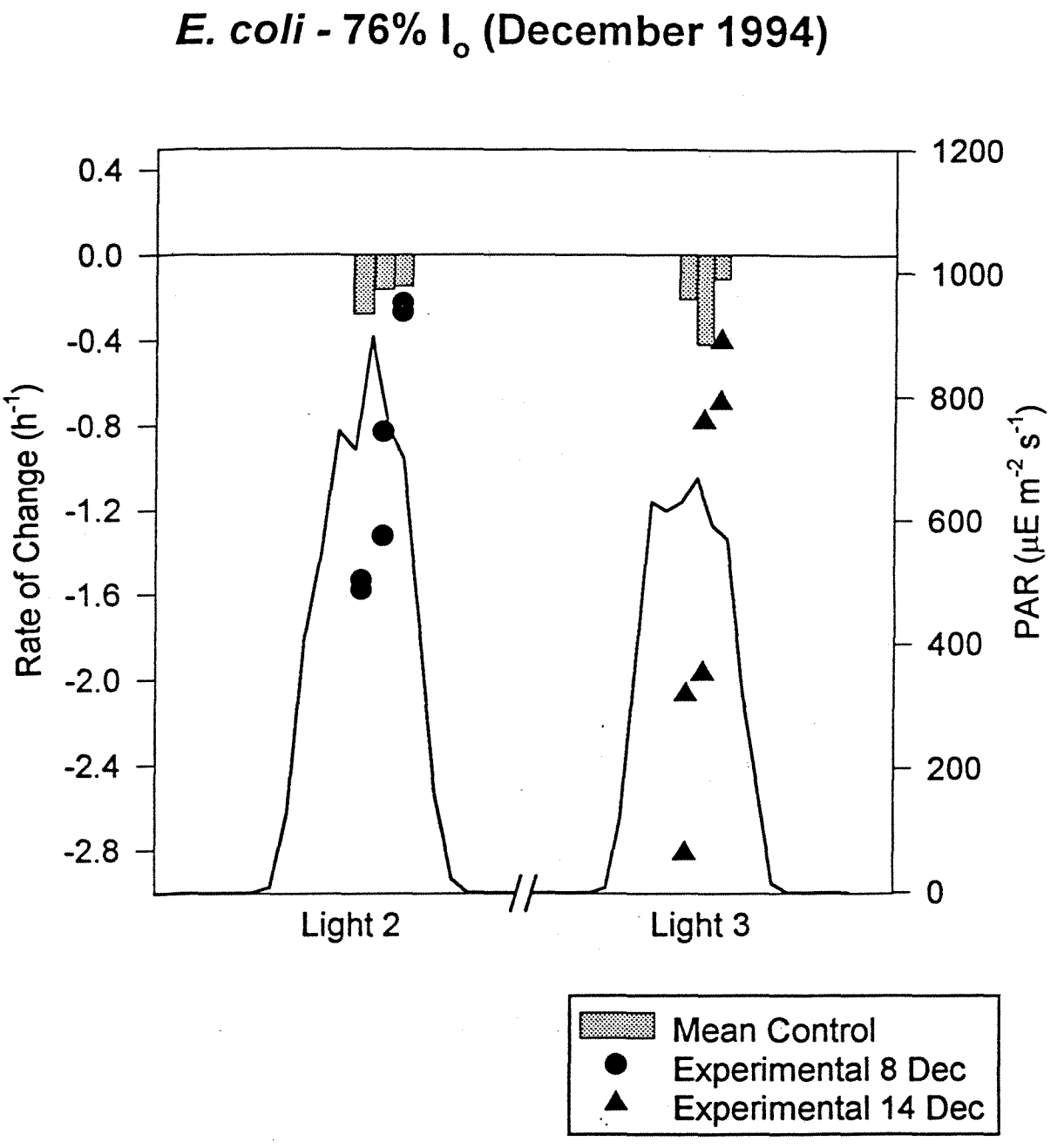

Fig. 4.36 Decay rate analysis for *E. coli* at 76% I_0 during LIGHT 2 and 3 experiments. Shaded histogram shows the mean rate of change (h^{-1}) for 4 dark controls. Dark symbols show corresponding rates of change during the same time interval in replicate light bottles. Negative rate of change = population decline; positive rate = growth. Curves show light levels expressed as % I_0 times measured PAR (photosynthetically available radiation) at Kunia Sugar Plantation, Oahu.

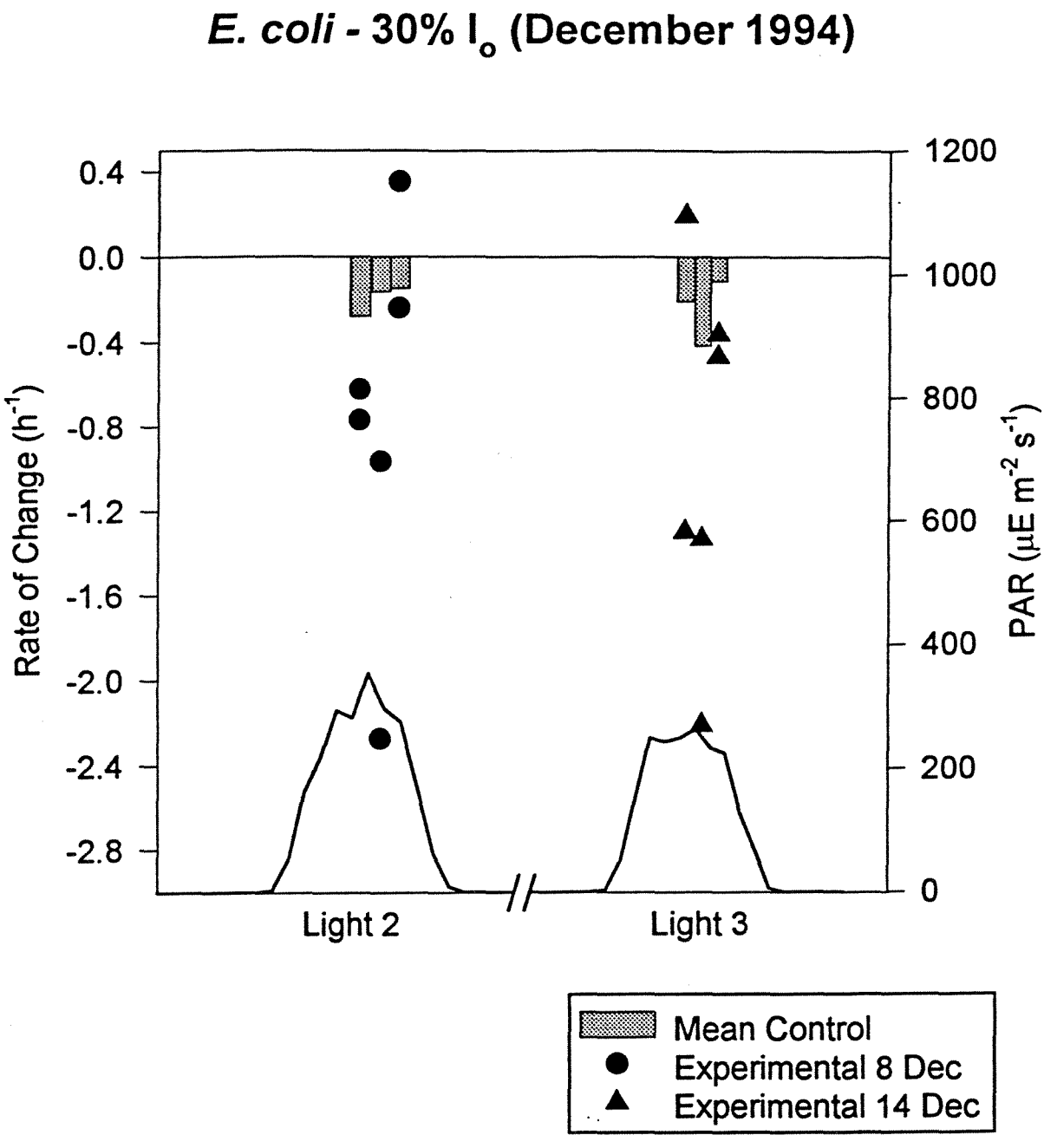

Fig. 4.37 Decay rate analysis for *E. coli* at 30% I_o during LIGHT 2 and 3 experiments. Symbols as in Fig. 4.36.

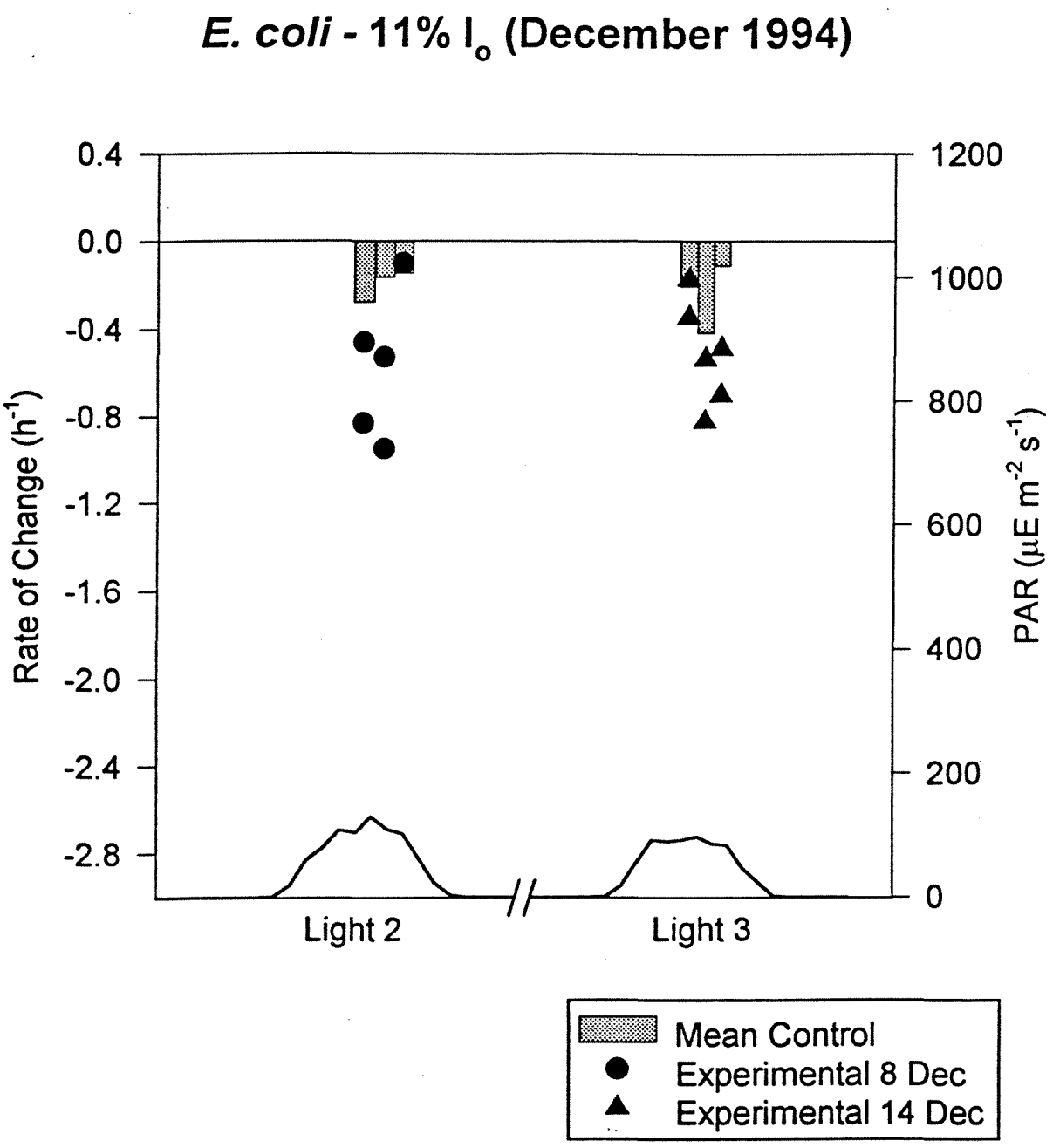

Fig. 4.38 Decay rate analysis for *E. coli* at 11% I_o during LIGHT 2 and 3 experiments. Symbols as in Fig. 4.36.

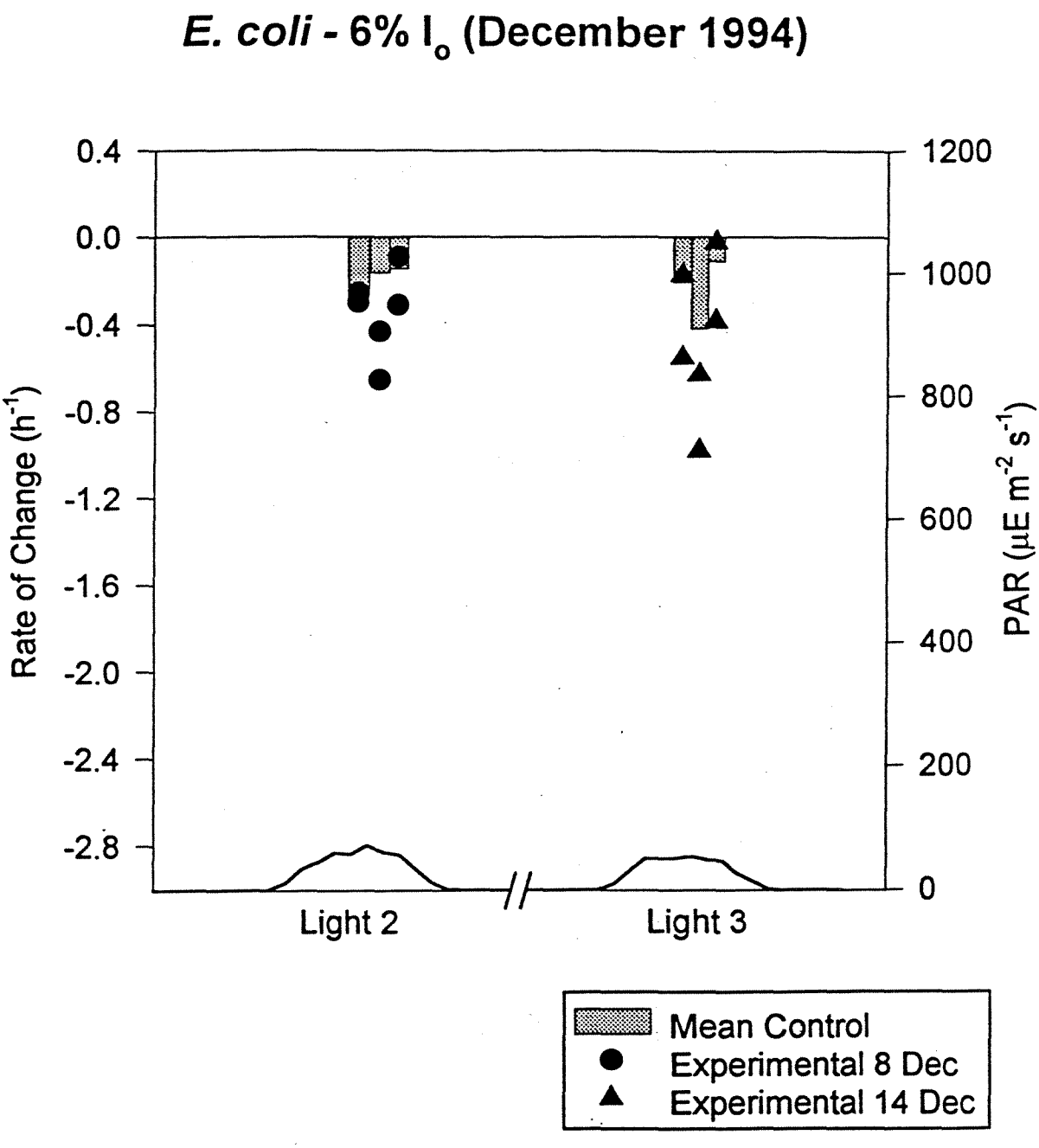

Fig. 4.39 Decay rate analysis for *E. coli* at 6% I_o during LIGHT 2 and 3 experiments. Symbols as in Fig. 4.36.

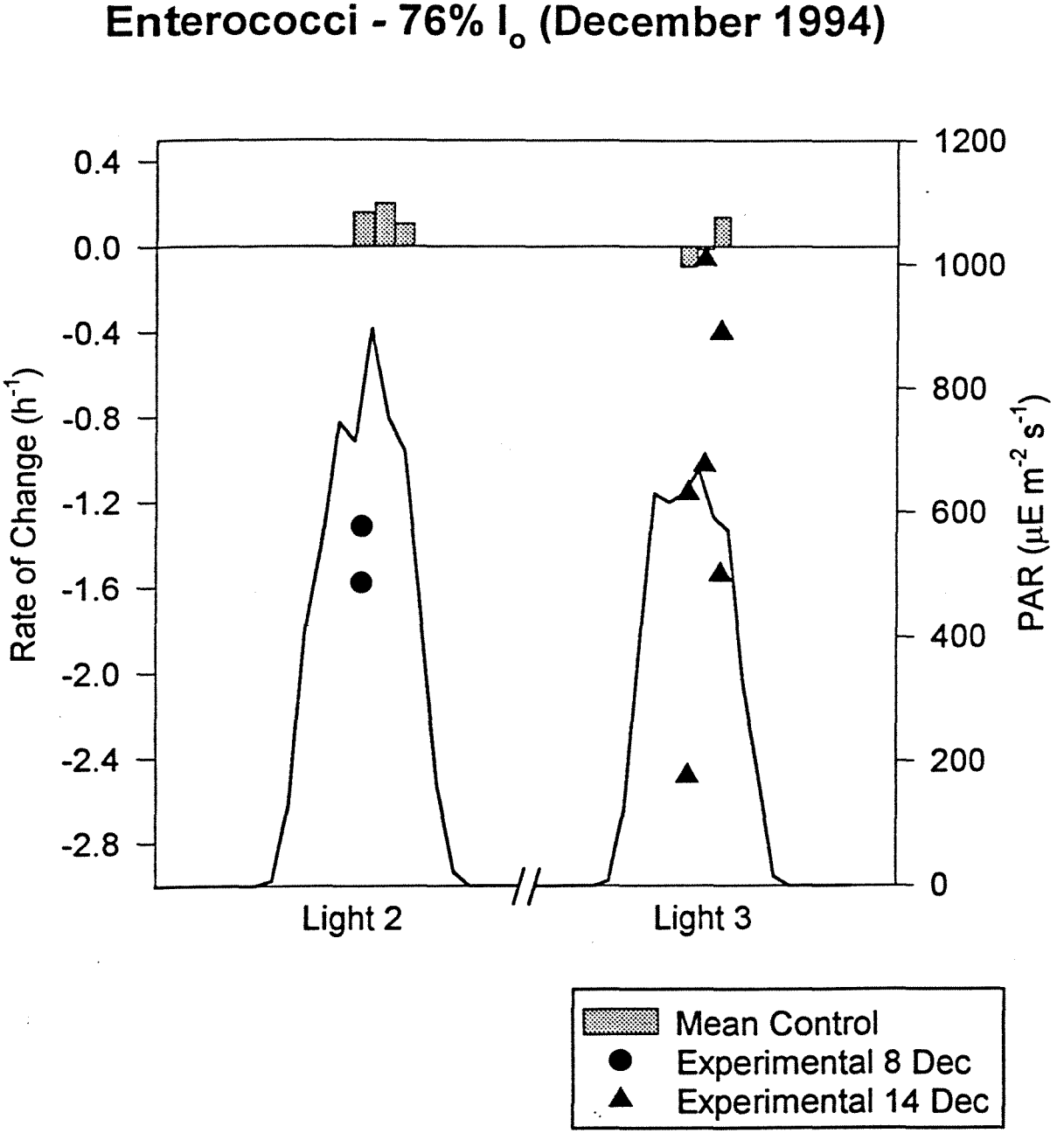

Fig. 4.40 Decay rate analysis for enterococci at 76% I_o during LIGHT 2 and 3 experiments. Symbols as in Fig. 4.36.

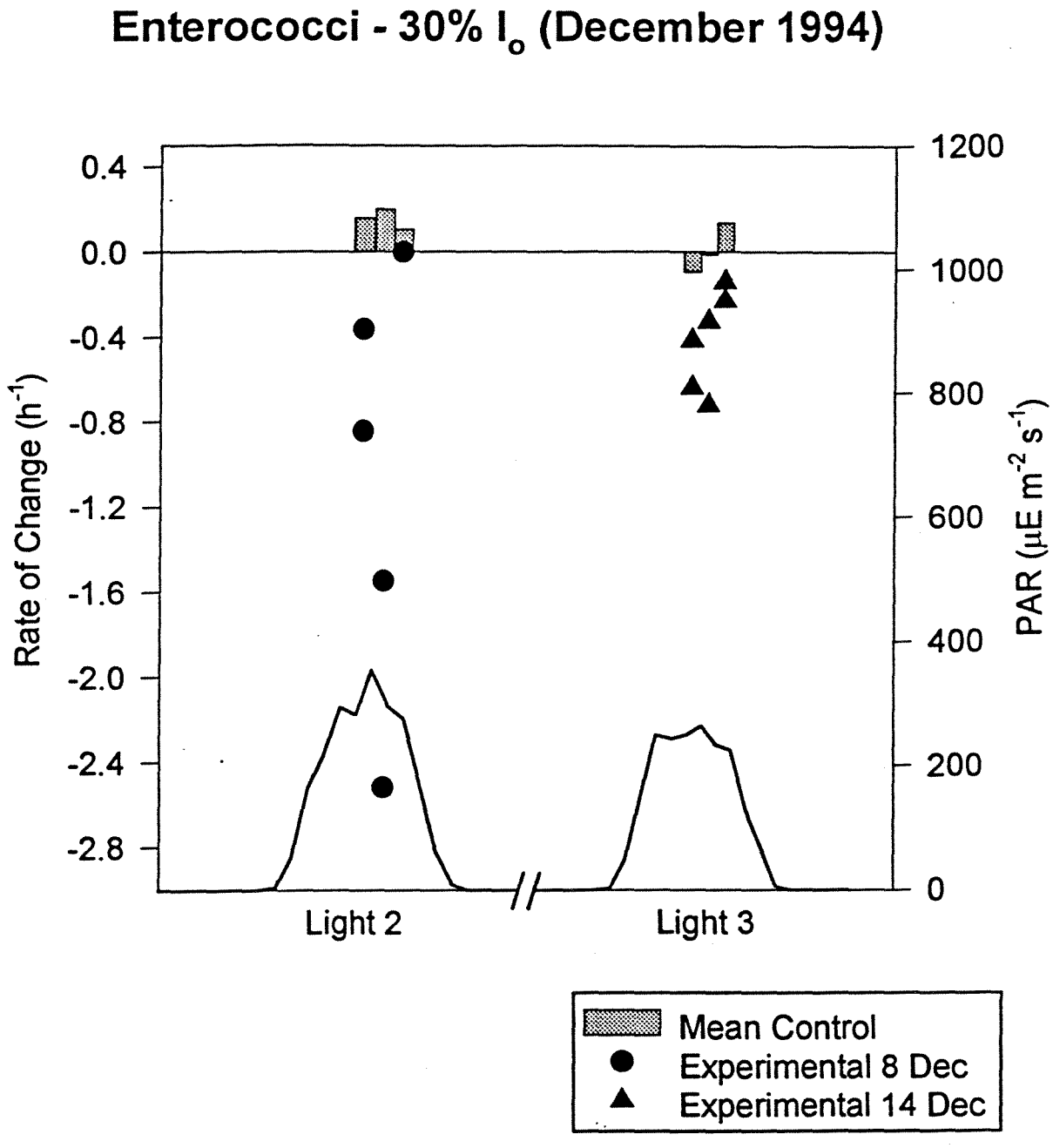

Fig. 4.41 Decay rate analysis for enterococci at 30% I_o during LIGHT 2 and 3 experiments. Symbols as in Fig. 4.36.

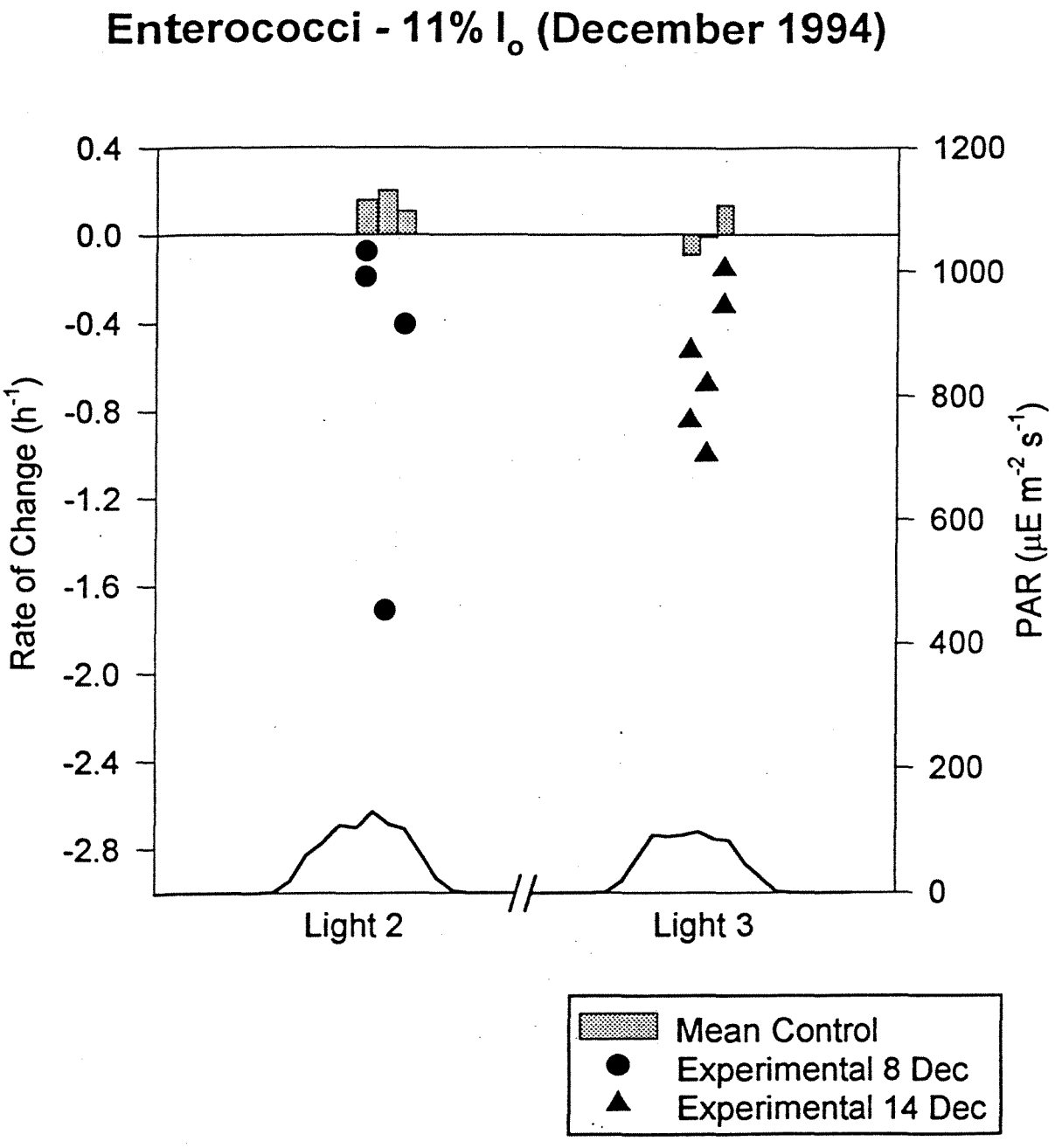

Fig. 4.42 Decay rate analysis for enterococci at 11% I_o during LIGHT 2 and 3 experiments. Symbols as in Fig. 4.36.

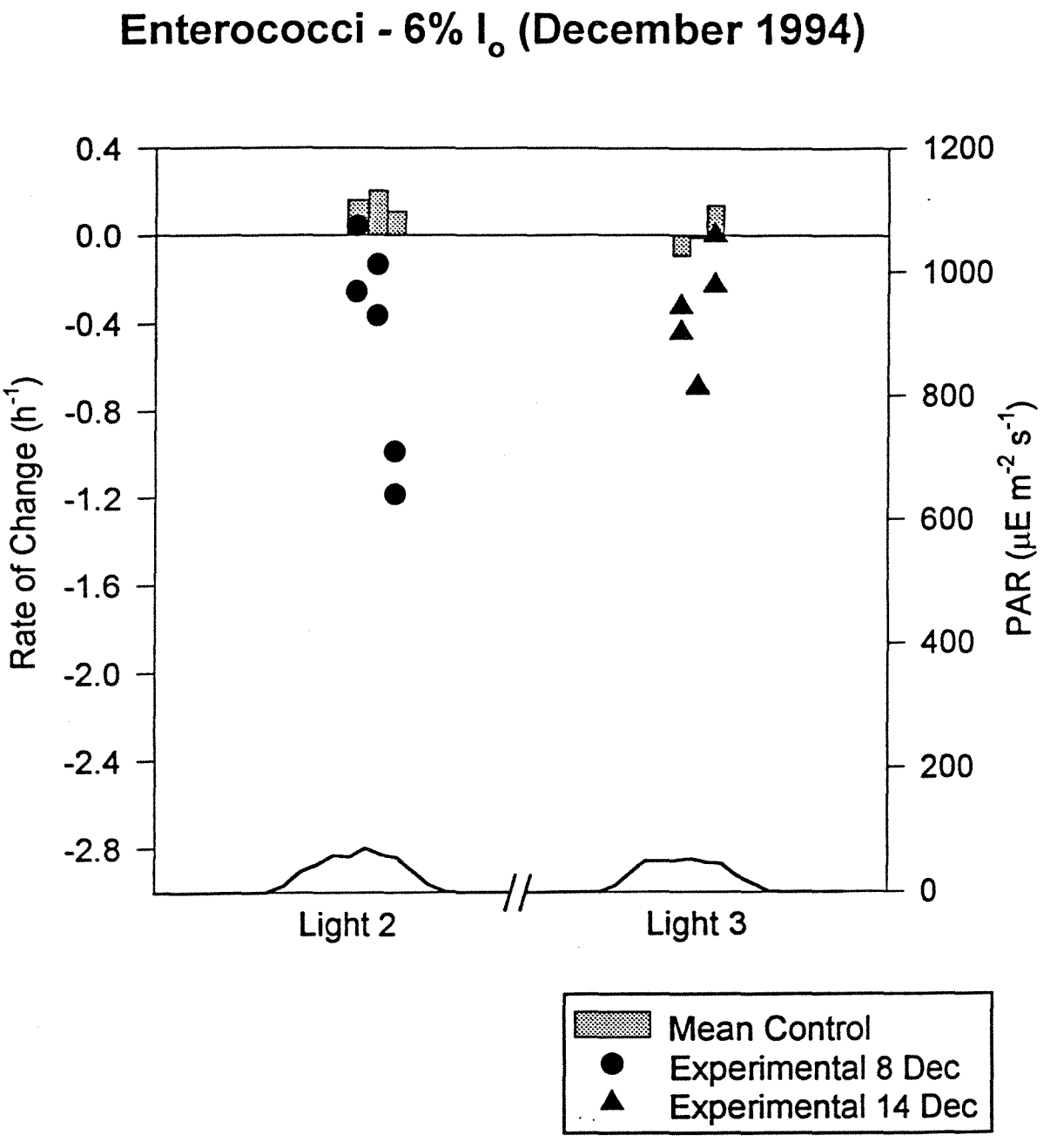

Fig. 4.43 Decay rate analysis for enterococci at 6% I_o during LIGHT 2 and 3 experiments. Symbols as in Fig. 4.36.

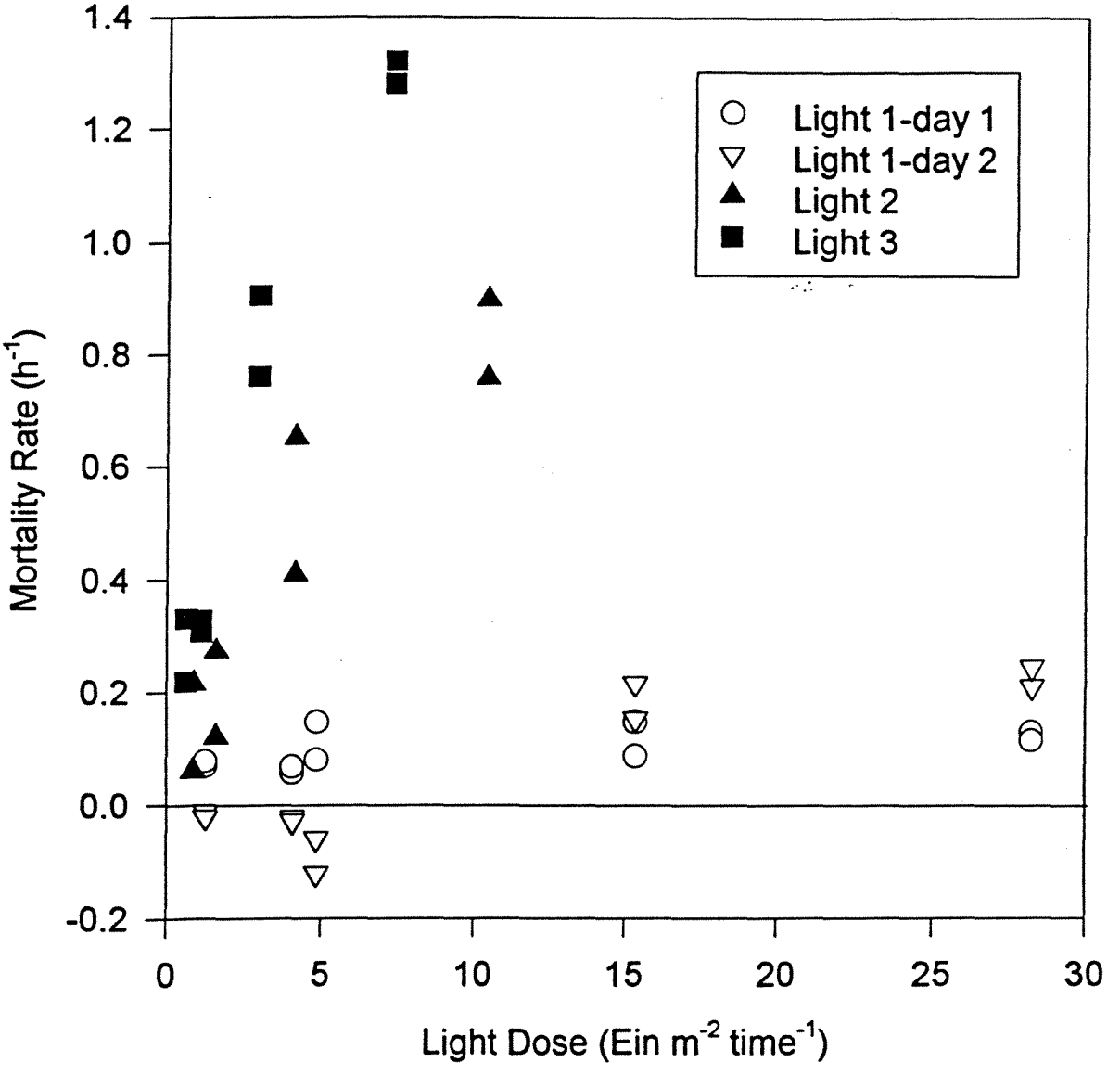

Fig. 4.44 Comparison of implied sunlight-induced mortality (decay) rates of *E. coli* as a function of light dose for incubation experiments involving short-term exposure to mid-day light intensities (LIGHT 2 and 3) and full-day exposure over the natural photoperiod (LIGHT 1). All rates are from individual treatment bottles less the mean rates of change for 4 to 5 dark controls incubated over the same period. Light dose is computed as the time-integrated exposure to light over the incubation period. Data from the first day and the second day of LIGHT 1 incubation are given separately.

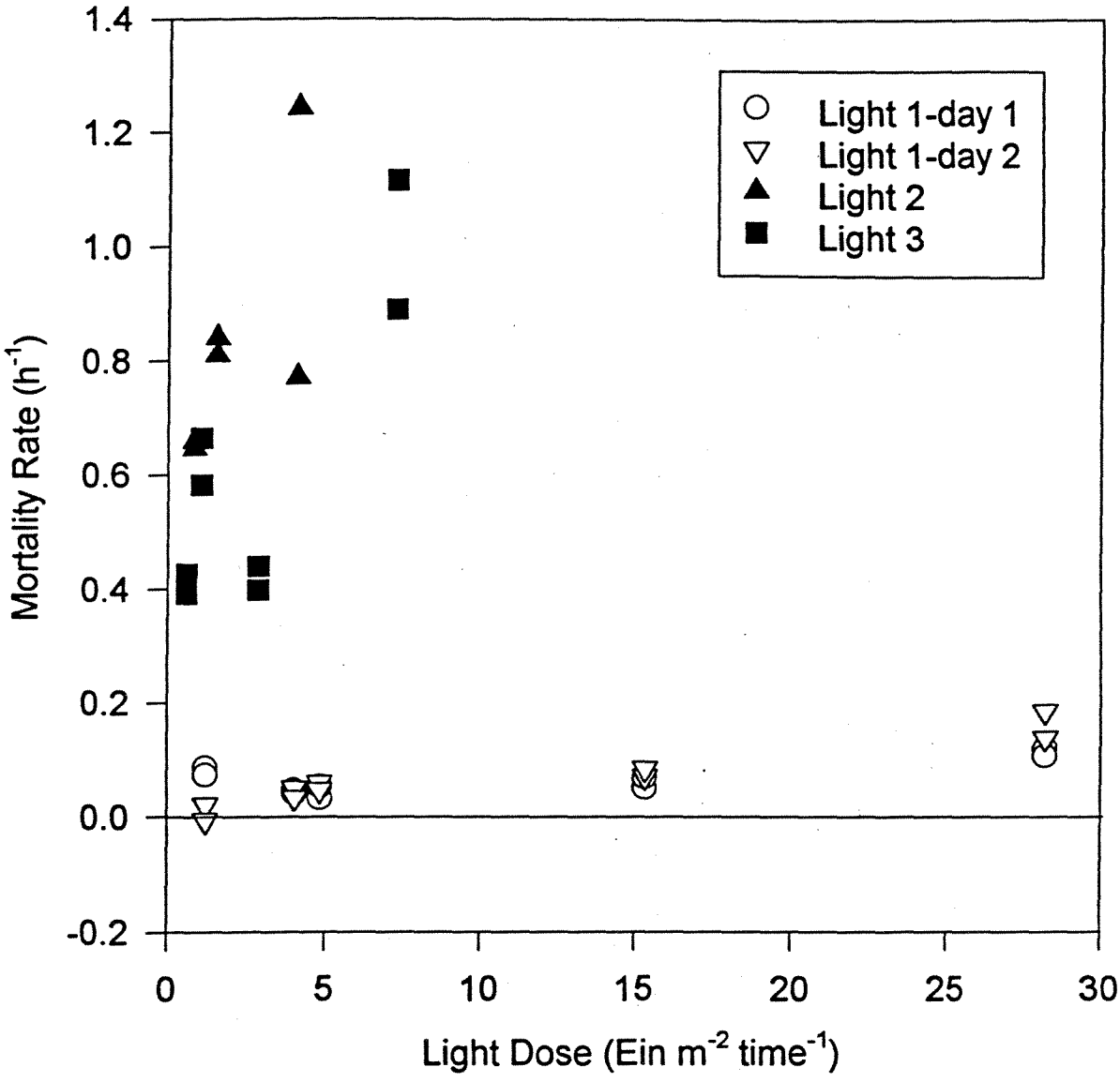

Fig. 4.45 Comparison of implied sunlight-induced mortality (decay) rates of enterococci as a function of light dose for incubation experiments involving short-term exposure to mid-day light intensities (LIGHT 2 and 3) and full-day exposure over the natural photoperiod (LIGHT 1).

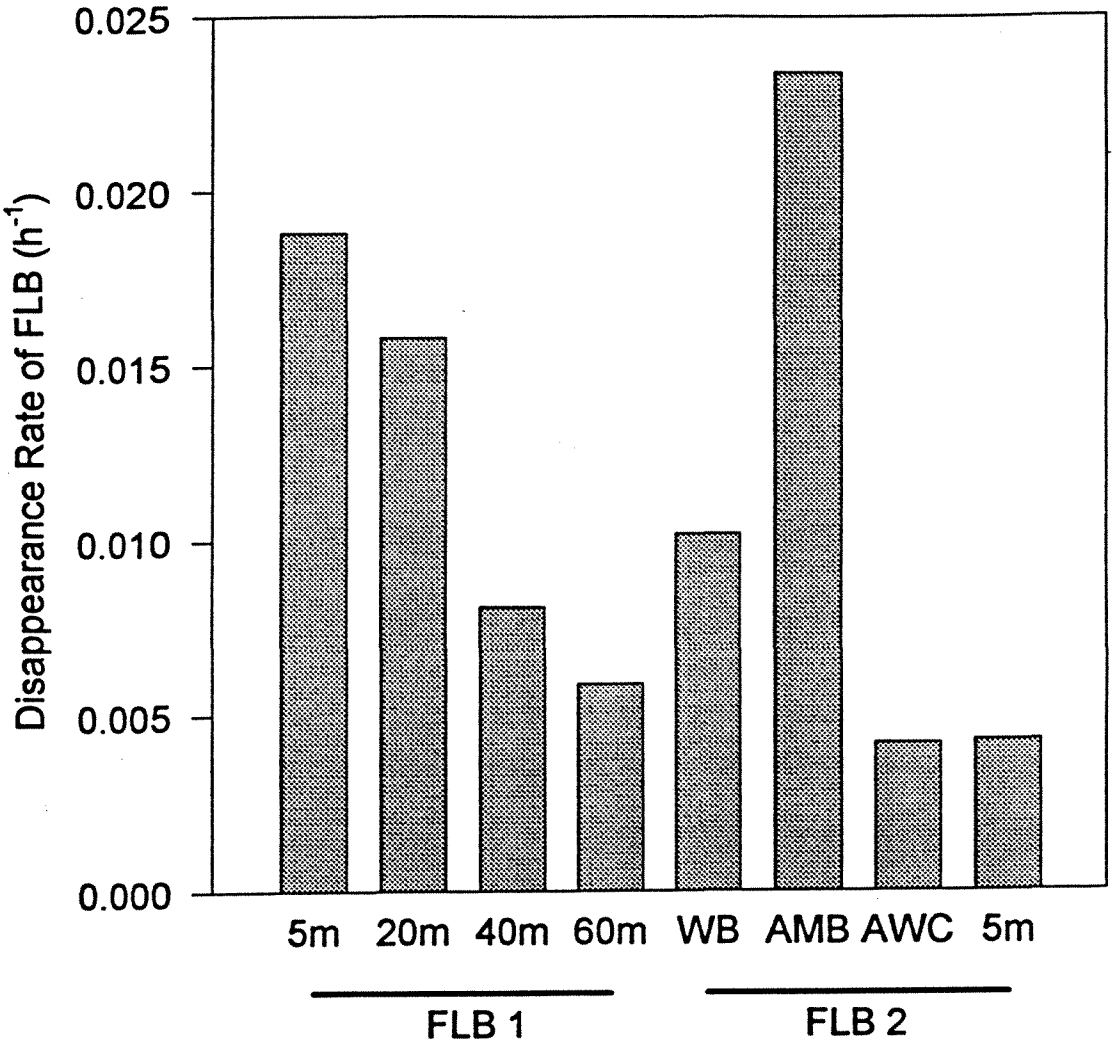

Fig. 4.46 Summary of bacterial mortality rate estimates from FLB disappearance experiments.

Table 4.4 compares protistan grazing rate estimates from studies in tropical open ocean Pacific and Hawaiian waters, including two in which FLB (*Vibrio damsella*, 1.1 μm) disappearance rates were measured concurrently with grazing mortality rates of comparably sized, natural photosynthetic bacteria (*Synechococcus* spp., 1.0 μm). Landry et al. (1995) found that grazing rates on FLB were slightly (20%) lower than on *Synechococcus*, presumably because some protozoans can discriminate between living and heat-killed prey (Landry et al. 1991). Nonetheless, rate estimates for both FLB and *Synechococcus*

Small flagellated protists are the dominant consumers of bacteria in the oceans, and particularly oligotrophic environments. Such consumers encounter their food by random contact at rates which decline approximately linearly with decreasing prey size (Monger & Landry 1990, 1991, 1992). This general size selective effect is of consequence in extrapolating FLB disappearance results to potential grazing impacts on smaller bacteria and viruses associated with the various pollution sources to Mamala Bay. Grazing of marine protists on virus-sized particles has been demonstrated using fluorescently labeled viruses (Gonzáles and Suttle 1993), but these rates are at least an order of magnitude lower than those on the smallest natural bacteria.

Overall, the natural "cleansing" of pollution-associated microbes from Mamala Bay waters due to protozoan grazing is limited with respect to the relatively short time scales over which potential pathogens may remain concentrated in areas where they can contact people. This is to say that the losses to protozoan predation are quite slow compared to dilution and transport process and the rates of light deactivation near the sea surface. Even if the rates are relatively slow, however, grazing may be the ultimate mechanism of removal of bacterial cells from the water (Gonzáles et al. 1992), particularly if they are not susceptible to light deactivation (e.g., *C. perfringens*) or if they remain viable, but not culturable after light deactivation. Thus, the decay rate estimates due to protozoan grazing impact provide the most conservative numbers to use for assessing public health risks from pathogens introduced at pollution sources.

5 CONCLUSIONS

The results of this component of the Mamala Bay Study can be summarized in the following conclusions:

a. Colony forming units of indicator bacteria appeared to vary with the daily pattern in numbers of bathers during the preliminary DIEL 1 and HOLIDAY sampling, but the relationship was not well demonstrated in the more intensive DIEL 2 and 3 studies. This may be due to spatial patchiness or to the fact that photodeactivation of bacterial culturability works against the trend for increasing bacterial densities during the early afternoon when bather density is greatest.

b. Despite the lack of a clear relationship between bacteria and bathers in fine scale temporal sampling, the "afternoon effect" of enhanced bacterial abundances was evident in morning and afternoon beach surveys for most of the popular Waikiki swimming beaches, and particularly those removed from strong influence by the Ala Wai Canal. Routine, early morning monitoring of water quality will consequently underrepresent levels of indicator bacteria during the times of the day when bathers are most likely to be exposed to public health hazards. On the other hand, because they are less apt to be confounded with bather effects or diminished by high sunlight, indicator counts from early morning samples (at or before sunrise) are likely to yield the most easily interpreted data for assessing advective inputs of pollution-related microbes to public beaches.

c. The morning and evening beach surveys were useful in revealing consistent "hot spots" of enhanced pollution indicator bacteria. That the Ala Wai Canal showed up as a significant pollution source was expected. However, the elevated concentrations of *E. coli* in the site 17 swimming area with restricted circulation, the high afternoon concentrations of enterococci on the eastern end of the survey (stations 18 to 21), and the peak in *C. perfringens* counts in the vicinity of the Waikiki Aquarium were not anticipated *a priori*. These results highlight areas for more intensive future studies.

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8 APPENDICES

Appendix 8.1 Data from DIEL 1 and HOLIDAY experiments.

DIEL 1

Time (h)	Number of People on Beach	Number of People in Water	Total Number of People	Fecal Coliform Rep. 1 CFU/100ml	Fecal Coliform Rep. 2 CFU/100ml	Enterococcus Rep. 1 CFU/100ml	Enterococcus Rep. 2 CFU/100ml
1600	37	31	68	160	162	26	28
2000	1	0	1	98	98	17	16
0	2	2	4	73	79	12	10
400	0	0	0	64	51	15	15
800	2	0	2	30	30	2	0.6
1200	46	39	85	81	71	12	12
1600	60	35	95	219	165	18	21

HOLIDAY

Date	Number of People on Beach	Number of People in Water	Total Number of People	Fecal Coliform Rep. 1 CFU/100ml	Fecal Coliform Rep. 2 CFU/100ml	Enterococcus Rep. 1 CFU/100ml	Enterococcus Rep. 2 CFU/100ml
1-Jul	60	16	76	22	21	49	36
2-Jul	54	17	71	30	30	36	15
3-Jul	48	28	76	51	35	41	36
4-Jul	79	33	112	74	60	49	41
5-Jul	60	18	78	49	26		38
6-Jul	37	16	53	20	21	43	45

Appendix 8.2 Data from DIEL 2 time-series sampling.

DIEL 2:

Waikiki Beach (WB)

Elapsed Time (h)	Clostridium CFU/100ml	*E. coli* CFU/100ml	Enterococcus CFU/100ml	Total Bacteria 10^5 cells/ml	Water Temp. (C)	Salinity (o/oo)	Number of People
0	0.1	3	58	6.4	26.2	34	0
2	0	1.5	89	5.9	26	34	2
4	0.3	1.8	54	5.3	28	34	15
6	0.1	23	76	5.6	28	33	7
8	0.8	4.5	71	7	29	33	20
10	0.3	14	100	9.5		33	29
12	0	18	106	10	29	33	12
14	0	12	407	9.9	27.5	33	2
16	0.7	4.5	325	8.6	27	33	0
18	0.9	5.5	237	9.2	27	33	0
20	0	9.8	138	5.5	27	33	0
22	1	1.8	148	5.5	27	33	0
24	0	0.5	31	6.7	26	33	2

Ala Moana Beach (AMB)

Elapsed Time (h)	Clostridium CFU/100ml	*E. coli* CFU/100ml	Enterococcus CFU/100ml	Total Bacteria 10^5 cells/ml	Water Temp. (C)	Salinity (o/oo)	Number of People
0	1.2	1.8		6.9	24.9	34	0
2	0	2	66	7.7	26.5	34	3
4	1.3	2.3	140	7.2	27	34	10
6	1.1	2	78	6.9	28	33	15
8	2	2.8	87	6.6	29	33	13
10	0	5.5	11	7.7	28	33	13
12	1.1	3	134	9.5	28	34	4
14	0.5	3	57	11.7		33	0
16	0.7	7.8	72	11.5	27.5	33	0
18	0.3	2.5	38	10	26	33	0
20	0.2	2	24	10	25.9	33	0
22	0.3	7.3	25	9	25.5	33	0
24	0.5		46	7.6	25.9	33	0

Ala Wai Canal (AWC)

Elapsed Time (h)	Clostridium CFU/100ml	*E. coli* CFU/100ml	Enterococcus CFU/100ml	Total Bacteria 10^5 cells/ml	Water Temp. (C)	Salinity (o/oo)
0	10	274	357	45.6	27	20
2	13	459	154	39.4	27	21
4	10	29	135	28.5	27.5	19
6	5.4	24	138	23.1	29	24
8	1.2	16	34	24.9	29	22
10	0.4	4	18	43.6	29	20
12	5.4	7.5	100	38	29	24
14	0	1040	122	55.5	28.2	23
16	6	3040	310	44.1	27.8	22
18	0	45	385	46.8	26	21
20	0	250	565	45.5	25.2	21
22	6	201	1270	46.4	25.2	19
24	12	335	725	35		15

Appendix 8.5 Summary of nearshore transect data.

DIEL 2 - 1 AUGUST 1994

1600 h Sampling

Distance	Transect A Clostridium	E. coli	Enterococcus	Total Bacteria	Transect B Clostridium	E. coli	Enterococcus	Total Bacteria
Offshore (m)	CFU/100 ml	CFU/100 ml	CFU/100 ml	10^5 cells/ml	CFU/100 ml	CFU/100 ml	CFU/100 ml	10^5 cells/ml
10	0	14	110	9.5	0.6	116	88	11.1
20	na	na	na	12	0	79	93	na
30	0.8	20	104	11	0	70	77	10.8
40	0	8.5	63	12	0	8	110	11.6
50	0	9.5	39	12	0.4	21	59	11.6
60	0	4	40	na	0	10	46	11.3

0600 h Sampling

Distance	Transect A Clostridium	E. coli	Enterococcus	Total Bacteria	Transect B Clostridium	E. coli	Enterococcus	Total Bacteria
Offshore (m)	CFU/100 ml	CFU/100 ml	CFU/100 ml	10^5 cells/ml	CFU/100 ml	CFU/100 ml	CFU/100 ml	10^5 cells/ml
10	0	1	20	6.7	0	0	42	7.2
20	0.2	0.5	34	6.4	0	1	16	7
30	0	2	18	6.9	0	0	17	6.6
40	0	1	38	6.9	0	1.5	7	6.9
50	0	0	30	6.6	0	1	28	6.8
60	0.2	1	280	6.8	0	1.5	29	6.5

DIEL 3 - 29 AUGUST 1994

1600 h Sampling

Distance	Transect A E. coli	Enterococcus	Total Bacteria	Transect B E. coli	Enterococcus	Total Bacteria
Offshore (m)	CFU/100 ml	CFU/100 ml	10^5 cells/ml	CFU/100 ml	CFU/100 ml	10^5 cells/ml
10	5	16	11.4	5.5	52	11.2
20	9	5	11.9	5	28	11.7
30	5.5	4.5	11.5	1	16	11.4
40	5.5	4.5	11.5	3.5	21	10.8
50	1	4	11.6	2.5	4.5	11.2
60	0	4.5	11.3	1	13	10.8

0600 h Sampling

Distance	Transect A E. coli	Enterococcus	Total Bacteria	Transect B E. coli	Enterococcus	Total Bacteria
Offshore (m)	CFU/100 ml	CFU/100 ml	10^5 cells/ml	CFU/100 ml	CFU/100 ml	10^5 cells/ml
10	5	50	9.9	3	16	10.2
20	4	148	9.8	1	12	9.9
30	4.5	63	10	0.5	12	9.7
40	4	10	10	2	5.5	9.6
50	3.5	136	9.9	1	2	9.8
60	1	138	9.8	0.5	7	9.6

Appendix 8.6 Data from LIGHT 1 experiment.

Treatments are 1 to 5 from highest to lowest light. For each treatment, bottles A and B are experimental replicates, and C is the dark control.

Time Elapsed (h)	1A CFU/ml	1B CFU/ml	1C CFU/ml	2A CFU/ml	2B CFU/ml	2C CFU/ml	3A CFU/ml	3B CFU/ml	3C CFU/ml	4A CFU/ml	4B CFU/ml	4C CFU/ml	5A CFU/ml	5B CFU/ml	5C CFU/ml
E. coli															
0	433	500	433	389	344	367	333	356	500	389	300	333	333	422	367
3.5	511	311	478	489	411	389	322	489	389	489	489	478	344	411	211
7.75	522	333	367	378	633	467	656	367	633	556	644		189	378	322
9.75	270	480		420	330		540	370		410	460		200	180	
11.25	210	130	420	300	220	410	300	350	340	280	300	210	100	160	170
12.75	30	43		94	46		191	176		270	250		50	120	
14.75	20	6	230	10	9	250	58	37	150	71	78	110	54	15	50
17.5	14	10		4	4		21	16		115	89		34	23	
19.25	10.8	9.5		27	7		22	28		79	62		37	32	
22.25	9	14.4	218			208	21.1	5.1	195	41	25	183	27.1	28	46
27.25	1.9	4.4	196	14.8	2.7	174	1.6	12.9	142	17.1	10	136			
31.75	0.5	0.48	69	1.4	0.7	179	7.9	7	45	8.5	14	61	8.5	11	8.5
38.25	0.04	0.03	46	0.73	0.61	115	9.7	11.1	30	6.9	8.4	32.7	8.8	5.1	11.9
45.5	0.01	0.01	23.3	0.02	0.02	0.2	1.1	3	14	9.6	7	16.3	5.2	6.7	10.2
Enterococci															
0	68	49	71	40	69	62	53	68	67	92	81	80	128	102	118
3.5	58	51	46	80	67	81	73	62	43	86	86	68	84	83	77
7.75	63	60	50	44	46	58	57	62	63	70	61	69	37	42	50
9.75	42	30		42	40		62	56		54	49		48	39	
11.25	21	21	44	24	20	50		39	39	50	56	46	36	31	38
12.75	7	11		18	20		11	20		24	16		27	29	
14.75	8.3	7.4	46	11.8	12.1	44	15.3	12.1	40	18.1	21.9	41	18.4	19.9	234
17.5	4.1	4.7		8.3	9.1		13	13		14.7	14.2		13.2	11.9	
19.25	3.1	2.8		7.2	8.3		11.2	15.5		13.2	14.4		12.7	9.1	
22.25	2.27	2.23	37	6.2	7.1	58	12.3	10	26	15.6	15.7	43	9	9.6	26.6
27.25	1.9	1.6	23.1	4.9	4.5	26.2	2.7	10.8	27	14.6	12.9	72	7.7	8.8	49
31.75	2.03	2.02	24	5.3	4.8	37	8.9	9.3	34	11.1	11	35	7	6.9	13.4
38.25	0.25	0.17	15.4	1.38	1.33	22.4	3.3	4	16.4	4.9	5.4	16.6	13.6	5.6	13.5
45.5	0.03	0.01	7.3	0.4	0.33	0.2	1.01	1.09	11.5	1.6	2.4	10.7	1.8	3.6	16

Appendix 8.6 Data from LIGHT 1 experiment. (cont.)

Treatments are 1 to 5 from highest to lowest light. For each treatment, bottles A and B are experimental replicates, and C is the dark control.

Time Elapsed (h)	1A CFU/ml	1B CFU/ml	1C CFU/ml	2A CFU/ml	2B CFU/ml	2C CFU/ml	3A CFU/ml	3B CFU/ml	3C CFU/ml	4A CFU/ml	4B CFU/ml	4C CFU/ml	5A CFU/ml	5B CFU/ml	5C CFU/ml
Clostridium															
0	18	22	12	16	5	14	15	9	23	20	23	21	39	34	50
7.75	13	24	17	13	8	18	12	0	21	10	0	17	24	0	31
11.25	18	18	19	19	16	20	15	15	27	16	18	10	37	36	26
14.75	13	0	6	0	22	13	13	16	14	20	0	17	19	25	0
22.25	16	11	22	15	4	11	13	16	8	13	19	23	28	0	25
27.25	9	4	13	16	13	18	22	23	16	16	24	18	34	32	15
31.75	9	0	6	6	0	11	26	12	7	11	16	10	0	3	19
38.25	0	7	8	7	13	12	16	9	13	14	15	10	21	17	6
45.5	0	5	0	2	0	8	6	8	0	0	0	0			

Appendix 8.7 Data from LIGHT 2 & 3 experiments.

Treatments are 1 to 4 from highest to lowest light. For each treatment, bottles A and B are experimental replicates, and C is the dark control.

LIGHT 2 - 8 December 1994

Time Elapsed (h)	1A CFU/ml	1B CFU/ml	1C CFU/ml	2A CFU/ml	2B CFU/ml	2C CFU/ml	3A CFU/ml	3B CFU/ml	3C CFU/ml	4A CFU/ml	4B CFU/ml	4C CFU/ml
E. Coli Counts												
0	320	300	250	350	230	150	210	240	230	220	220	230
1.5	30	30	160	110	90	110	60	120	150	140	150	140
2.6	7	12	190	9	31	114	21	67	68	68	93	
3.85	5	9	90	14	23	70	63	59	129	46	83	121
Enterococci Counts												
0	3.6	3.2	2.7	5.7	3.8	3.8	3.7	4.4	4.7	3.1	5.3	3.8
1.5	0.5	0.3	4.1	1.6	2.2	6.2	3.3	3.3	4.1	3.3	3.6	4.6
2.6	0	0	4.5	0.1	0.4	5.8	0.5	0	6	2.2	3.1	6.9
3.85	0	0.1	1	0.1	0.4	7	0.3	0.4	6.5	0.5	0.9	7.9

LIGHT 3 - 14 December 1994

Time Elapsed (h)	1A CFU/ml	1B CFU/ml	1C CFU/ml	2A CFU/ml	2B CFU/ml	2C CFU/ml	3A CFU/ml	3B CFU/ml	3C CFU/ml	4A CFU/ml	4B CFU/ml	4C CFU/ml
E. coli Counts												
0	210	280	280	260	450	260	190	230	230	310	290	230
1.08	10	30	300	320	110	0	130	190	310	170	240	130
2.25	4	3	136	24	23	129	69	72	158	81	76	158
3.25	2	2	142	15	16	109	34	44	137	55	74	131
Enterococci Counts												
0	44	21	21	20	22	46	39	40	39	42	47	35
1.08	3	6	38	10	14	25	22	16	34	26	33	27
2.25	2.8	1.8	28.8	6.8	6	32	6.8	7.2	27.4	11.6	14.6	32.6
3.25	0.6	1.2	33.6	5.4	5.2	37.2	5.8	5.2	36	11.6	11.6	31.6