OCS Study
MMS 99-0061

Ecological Responses to, and Recovery From, Produced Water Discharge: Application of a BACIPS Assessment Design

Final Technical Summary

Final Study Report

U.S. Department of the Interior
Minerals Management Service
Pacific OCS Region

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FINAL TECHNICAL SUMMARY

STUDY TITLES:
Study I. Effects of Produced Water on Demographic Rates
Study II. Spatial Scale of Produced Water Impacts as Indicated by Plume Dynamics and Biological Field Assays[†]
Study III. Environmental Recovery Following Cessation of a Produced Water Discharge

REPORT TITLE: Ecological Responses to, and Recovery From, Produced Water Discharge: Application of a BACIPS Assessment Design

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Study III: 14-35-0001-30761

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APPLICABLE PLANNING AREA(S): Southern and Central California

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Study I: 1989-90; 1990-91; 1991-92
Study II: 1992-93; 1993-94
Study III: 1994-95; 1995-96

COMPLETION DATE OF REPORT: March 1999

COSTS: FY 89-90 - $52,000; FY 90-91 - $58,044; FY 91-92 - $30,783; FY 92-93 - $18,814; FY 93-94 - $14,386; FY 94-95 - $39,207; FY 95-96 - $48,793

CUMULATIVE PROJECT COSTS: STUDY I: $140,827**; STUDY II:** $33,200**;**
STUDY III: $88,000

PROJECT MANAGERS: [1]C.W. Osenberg, [2]R.J. Schmitt

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PRINCIPAL INVESTIGATORS: R.J. Schmitt and C.W. Osenberg

[†] This report covers the biological field assay of this project. The plume dynamics results of this study are reported in the MMS Final Report OCS- 96-0001, *Spatial Scale of Produced Water Impacts as Indicated by Plume Dynamics*, by Sally MacIntyre and Libe Washburn

KEY WORDS: Santa Barbara Channel; produced water; Before-After-Control-Impact Paired Series assessment design; BACI; BACIPS; field assessment; assessment design; long-term monitoring; recovery from impacts; mussels; infauna; *Mytilus*; urchins; *Strongylocentrotus*; fertilization; barium.

BACKGROUND:

Produced water, an aqueous waste generated during oil and gas production, is commonly discharged into marine environments. Its environmental effects are poorly understood, and what is known, is based largely on studies conducted in the Gulf of Mexico and adjacent estuarine systems. Field assessments in the Gulf of Mexico have been complicated by other types of production activities that confound effects of produced water (Spies 1987, Carney 1987, Osenberg and Schmitt 1996). In addition, results from the Gulf, where discharge often occurs in sheltered, shallow embayments, may yield limited insights about expected effects in other systems, such as the more open, high energy coast of the western United States. Due to more rapid diffusion of the produced water plume in these open-coast systems, and the highly seasonal nature of the systems, effects of produced water may be reduced and/or more difficult to detect and quantify. Examination of individual-based parameters (such as growth, reproductive output, or fertilization success) might provide more sensitive indicators of environmental impacts than assessment of population density (Carney 1987; Osenberg *et al*. 1992b, 1994). This approach also might reveal a different spatial extent of biological effects than documented by variation in population densities. Furthermore, the examination of effects on individual growth and reproduction, allow a more production-based assessment than is possible by looking only at population density.

Because population densities and many other environmental variables of interest vary tremendously among different sites and at different times, powerful assessment designs are needed to document environmental impacts (Stewart-Oaten *et al*. 1986, 1992, Osenberg and Schmitt 1996). Although not part of our original proposal, we soon anticipated the opportunity to apply the Before-After-Control-Impact Paired Series assessment design to quantify effects of produced water on a nearshore, open-coast marine system located near Carpinteria, California.

OBJECTIVES:

The original goals of this project were three-fold: 1) to conduct field studies quantifying patterns of spatial distribution in physical-chemical, and individual, demographic and population-level biological parameters around an existing produced water outfall (Osenberg *et al*. 1992b, Krause 1994); 2) to use the spatial patterns to estimate the effects of produced water using a Control-Impact design, and to use these effects together with a time-series of Before data from another SCEI study to estimate the statistical power of BACIPS designs to detect impacts on chemical-physical, and individual-based and population-based biological parameters (Osenberg *et al*. 1992a, 1994); 3) to quantify effects on reproduction using laboratory studies (Krause *et al*. 1992, Krause 1994); and 4) to use the BACIPS design to quantify individual-based responses in conjunction with another SCEI project studying a planned produced water outfall at Gaviota, California. However, the outfall at Gaviota never went into full operation, whereas the existing plant at Carpinteria actually stopped discharging produced water during our studies. As a result, we modified the fourth objective: 4') to use a

BACIPS design to quantify the environmental effects of produced water on biological parameters at the Carpinteria site (this analysis was based upon comparisons of field data collected during produced water discharge to data collected following cessation of discharge). Our final two objectives focused on the long-term contributions of our program to environmental sciences: 5) to advance the theoretical developments and application of the BACIPS assessment design (Stewart-Oaten *et al*. 1992, Osenberg *et al*. 1994, Osenberg and Schmitt 1994, Schmitt and Osenberg 1996); and 6) to train students in the application of ecological concepts and statistical tools to environmental problems.

DESCRIPTION:

Our study site was located near Carpinteria, CA, USA (34^{o}23'N, 119^{o}30'W), where the subtidal environment consists of a sand bottom with little or no physical relief. This area is an open coast environment, although it is somewhat sheltered due to the presence of the northern Channel Islands and the east-west orientation of the coast. Discharge of produced water occurs approximately 200 - 300 m from shore at bottom depths of 10 - 12 m. The volume discharged is extremely consistent from month to month, averaging ~2.5 million liters/day. Produced water was discharged continuously (except for occasional daily interruptions) from 1978 through 1992. In July 1992, the plant reduced its activities and permanently stopped discharging produced water. We conducted studies at sites within 1 km of the outfall between 1990 and 1995, thus spanning the periods with "discharge" and "no discharge". Our fieldwork focused on several types of data: (1) performance of outplanted mussels and sea urchins at 6-8 sites that varied in their proximity to the diffuser; (2) barium content in these mussel shells (barium is a marker of the produced water plume that is incorporated into mussel shells: Higashi *et al.* 1992, Osenberg *et al.* 1992b), which permitted us to relate patterns of mussel performance to exposure; (3) infaunal densities at 20 sites that varied in their proximity to the diffuser (from a few meters out to 1 km upcoast and downcoast). Most of these data were collected both before and after July 1992, which enabled us to apply the BACIPS assessment design.

SYNOPSIS OF MAJOR FINDINGS AND RESULTS:

In our lab studies, we documented significant effects of produced water exposure on fertilization and early development rates of sea urchins (Krause *et al*. 1992, Krause 1995). Produced water reduced the apparent fertilization success in urchins: produced water inhibited the vitelline membrane from lifting off the egg surface in ~30% of the eggs, but otherwise did not affect syngamy. Survival of embryos was not affected; however, produced water did significantly retard developmental rates, and this effect varied depending on whether eggs or sperm were exposed. These effects were apparent even at the lowest concentration of produced water: 0.0001%.

Sea urchin apparent fertilization success (based on presence of a fertilization membrane), infaunal densities, and mussel performance showed considerable spatial variation that was correlated with distance from the diffuser (Osenberg *et al*. 1992b, Krause 1994, 1995). We used some of these data, together with time series data in the absence of produced water (from a companion SCEI project) to estimate the statistical power of BACIPS assessment designs (Osenberg *et al*. 1992a, 1994). Between-site differences in chemical - physical parameters (e.g., elemental concentration) and in individual-based and demographic parameters (e.g.,

body size and survival) were relatively consistent through time, whereas differences in population-based parameters (e.g., density) were more variable. The magnitude of effects was estimated to be greatest for population-based parameters and least for chemical - physical parameters, which tended to balance the statistical power associated with these two parameter groups. Individual-based parameters were intermediate in estimates of effect size. The ratio of effect size to variability (and thus statistical power) was greatest for individual-based parameters and least for population and chemical - physical parameters. The results suggested that relatively few of the population and chemical - physical parameters, but many of the individual-based/demographic parameters, could provide adequate power given time constraints of most studies.

Analysis of the complete data set, collected both during discharge and after cessation of discharge, revealed that spatial patterns in mussel performance, shell barium content, and infaunal density were almost entirely due to effects of produced water. More limited data on sea urchin fertilization also showed effects due to produced water (Krause 1995). Shortly after cessation of produced water discharge, spatial variation in these parameters declined considerably. Comparison of the Discharge data with the Non-discharge data showed strong deleterious effects of produced water on urchin fertilization (Krause 1995), mussel growth and condition, shell barium concentration and the density of several key infauna (some infauna, notably nematodes, showed positive responses to produced water discharge). For example, exposure of mussels to produced water reduced mussel tissue production by ~30% at the closest stations (within 10 m of the outfall) and by ~10% at 500 m. Shell barium content mirrored these patterns of performance and served as a reliable indicator of produced water exposure.

In addition to our laboratory and field work, we also expanded the conceptual development of the BACIPS design and encouraged the application of BACIPS to other environmental studies. We accomplished this through publications in peer reviewed journals and books, publication of a Special Feature in *Ecological Applications*, the organization of and participation in workshops and meetings, and the publication of a book (Schmitt, R.J. and C.W. Osenberg. 1996. *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press, San Diego. 401 pages). The book represented a major undertaking and comprised a large portion of our activities during the latter stages of this project. We also helped train over 30 students and field and laboratory assistants, and incorporated our results into several new courses that were developed at UC Santa Barbara and UC Berkeley.

STUDY PRODUCTS:

PUBLICATIONS (listed chronologically; * indicates most important publications: reprints included with the Final Study Report):

Osenberg, C.W., S.J. Holbrook and R.J. Schmitt. 1992a. Implications for the design of environmental assessment studies. Pages 75-90 *In* P.M. Grifman and S.E. Yoder (eds.) *Perspectives on the Marine Environment*. USC Sea Grant, Los Angeles, California.

*Krause, P.R., C.W. Osenberg, R.J. Schmitt. 1992. Effects of produced water on early life stages of a sea urchin: gender-specific responses and delayed expression. Pages 431-444 *in* J.P. Ray and F.R. Englehardt (eds.), *Produced water: technological/environmental issues and solutions*, Plenum Publishing Corp., New York.

*Osenberg, C.W., R.J. Schmitt, S.J. Holbrook and D. Canestro. 1992b. Spatial scale of ecological effects associated with an open coast discharge of produced water. Pages 387-402 *in* J.P. Ray and F.R. Englehardt (eds.), *Produced water: technological/environmental issues and solutions*, Plenum Publishing Corp., New York.

Osenberg, C.W. and R.J. Schmitt. 1994. Detecting human impacts in marine habitats. Ecological Applications 4:1-2.

*Osenberg, C.W., R.J. Schmitt, S.J. Holbrook, K. E. Abu-Saba, and A. R. Flegal. 1994. Detection of environmental impacts: natural variability, effect size, and power analysis. Ecological Applications 4:16-30.

*Krause, P.R. 1994. Effects of an oil production effluent on gametogenesis and gamete performance in the purple sea urchin (*Strongylocentrotus purpuratus* Stimpson). Env. Tox. and Chem. 13:1153-1161.

*Krause, P.R. 1995. Spatial and temporal variability in receiving water toxicity near an oil effluent discharge site. Archives of Environmental Contamination and Toxicology 29:523-529.

*Schmitt, R.J. and C.W. Osenberg (editors and contributing authors). 1996. *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press, San Diego.

Osenberg, C.W. and R.J. Schmitt. 1996. Detecting ecological impacts caused by human activities. Pages 3-16 *in* R.J. Schmitt and C.W. Osenberg (eds.) *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press, San Diego.

Osenberg, C.W., R.J. Schmitt, S.J. Holbrook, K. E. Abu-Saba, and A. R. Flegal. 1996. Detection of environmental impacts: natural variability, effect size, and power analysis. Pages 83-108 *in* R.J. Schmitt and C.W. Osenberg (eds.) *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press, San Diego. [Republication of Osenberg *et al*. 1994, Ecological Applications 4:16-30.]

Schmitt, R.J., C.W. Osenberg, W.J. Douros, and J. Chesson. 1996. The art and science of administrative environmental impact assessment. Pages 279-291 *in* R.J. Schmitt and C.W. Osenberg (eds.) *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press.

Ambrose, R.F., R.J. Schmitt, and C.W. Osenberg. 1996. Predicted and observed environmental impacts: can we foretell ecological change? Pages 343-367 *in* R.J. Schmitt and C.W. Osenberg (eds.) *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press, San Diego.

Canestro, D., P.T. Raimondi, D.C. Reed, R.J. Schmitt, and S.J. Holbrook. 1996. A study of methods and techniques for detecting ecological impacts. Pages 53-67 in *Methods and Techniques of Underwater Research, Proceedings of the American Academy of Underwater Scientists Symposium*, AAUS, Nahant, MA.

Steichen, D.J., Jr., S.J. Holbrook, and C.W. Osenberg. 1996. Distribution and abundance of benthic and demersal macrofauna within a natural hydrocarbon seep. Marine Ecology Progress Series 138:71-82.

RESEARCH PRESENTATIONS

Herrlinger, T.J. and C.W. Osenberg. 1989. Demographic and behavioral responses of benthic marine organisms to produced water discharge: sensitive indicators and links to population dynamics. Third Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, University of California, San Francisco (poster).

Osenberg, C.W., R.J. Schmitt and S.J. Holbrook. 1989. An impact assessment design for detecting ecological effects of discharged produced water. Third Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, University of California, San Francisco (poster).

Osenberg, C.W., A. Stewart-Oaten and J.R. Bence. 1990. The analysis of environmental impact data resulting from the Before-After-Control-Impact-Paired (BACIP) design. Fourth Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, University of California, Santa Barbara (poster).

Osenberg, C.W., R.J. Schmitt and S.J. Holbrook. 1990. Differential ability to detect environmental impacts arising in ecological systems. Fourth Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, University of California, Santa Barbara (poster).

Osenberg, C.W., R.J. Schmitt and S.J. Holbrook. 1991. Implications for the design of environmental assessment studies. Symposium on *The Marine Environment*. 100th Anniversary of the Southern California Academy of Sciences. University of Southern California, May 1991. (invited)

Osenberg, C.W., S.J. Holbrook and R.J. Schmitt. 1991. The power to detect unreplicated perturbations varies among physical, chemical and biological parameters. Ecological Society of America, San Antonio, Texas.

Osenberg, C.W., S.J. Holbrook, and R.J. Schmitt. 1991. The spatial scale of ecological effects associated with point-source discharges. Fifth Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, San Francisco, California. (poster)

Krause, P.R., C.W. Osenberg, R.J. Schmitt. 1992. Effects of produced water on early life stages of a sea urchin: gender-specific responses and delayed expression. International Produced Water Symposium, San Diego, California. (poster)

Osenberg, C.W., R.J. Schmitt, S.J. Holbrook and D. Canestro. 1992. Spatial scale of ecological effects associated with an open coast discharge of produced water. International Produced Water Symposium, San Diego, California.

Osenberg, C.W., R.J. Schmitt and S.J. Holbrook. 1992. Detection of environmental impacts: power analysis and spatial inference. Symposium on *The design of environmental impact assessment studies: Conceptual issues and application*, held at the Second International Temperate Reefs Symposium, Auckland, New Zealand, January 1992.

MacIntyre, S., L. Washburn, C. Osenberg. 1992. Spatial scale of produced water impacts: prediction of past exposure from density stratification and current velocities and duration of impacts in invertebrates. Sixth Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, University of California, Santa Barbara (poster).

Osenberg, C.W., A. Sberze, and J. Dai. 1993. Assessing ecological impacts of human activities in aquatic environments. Seventh Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, University of California, Santa Cruz. November 1993 (poster).

Stone, S.W., L. Washburn, S. MacIntyre, and C.W. Osenberg. 1993. Spatial scale of produced water impacts as indicated by plume dynamics and biological field assays. Seventh Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, University of California, Santa Cruz. November 1993 (poster).

Stone, S.W., L. Washburn, S. MacIntyre, and C.W. Osenberg. 1994. Seasonal variations of a buoyant plume in a stratified environment. Ocean Sciences Meeting, San Diego, California. February 1994.

Müller, E., C.W. Osenberg, R.J. Schmitt, and R.M. Nisbet. 1996. Modeling the toxic effects of produced water on blue and California mussels using dynamic energy budgets. Ninth Annual Research Symposium of the UC Toxic Substances Research and Teaching Program, University of California, Santa Cruz. March 1996 (poster).

Canestro, D., P.T. Raimondi, D.C. Reed, R.J. Schmitt, and S.J. Holbrook. 1996. A study of methods and techniques for detecting ecological impacts. Annual meeting of the *American Academy of Underwater Scientists*.

Osenberg, C.W., R.J. Schmitt, S.J. Holbrook, C.M. St. Mary, and T.W.-M. Fan. 1998. Effects of produced water on mussel growth and production: application of the BACIPS design. 27th Benthological Ecology Meetings, Melbourne, Florida, March 1998.

Other (Invited Seminars and Workshops)

Osenberg presented results of this research during invited seminars at:
Department of Geography, University of California at Santa Barbara, November 1991.
Department of Zoology, Oregon State University, January 1994.
Department of Wildlife Ecology and Conservation, University of Florida, March 1997.
Department of Biology, University of Michigan, Ann Arbor, September 1997.
Department of Biological Sciences, Dartmouth College, September 1997.

Department of Zoology, University of Florida, September 1998.

The Design *of Environmental Impact Assessment Studies: Conceptual Issues and Application*. Schmitt and Osenberg organized this symposium, which was held at the Second International Temperate Reef Symposium, in Auckland, New Zealand (January 1992) and featured speakers from Australia, New Zealand and the United States. The US contingent included several SCEI investigators and an MMS scientist.

UC Toxic Substances *Research and Teaching Program (Coastal Environmental Program)*. All Principal Investigators were intimately involved in the UC Toxic Substances Research and Teaching Program (Coastal Environmental Program), and attended annual workshops throughout the UC system between 1989 and the present. In addition to attendance at the workshops, MMS-sponsored research was often the focus of research presentations.

Coastal Toxicology Research *in California*. Schmitt organized this workshop involving University of California, local, state and federal agency personnel. Santa Barbara, CA. February 1993. (Osenberg was an invited participant)

Meta-analysis, interaction strength and effect size: application of biological models to the synthesis of experimental data. Osenberg designed and organized a working group at the National Center for Ecological Analysis and Synthesis, Santa Barbara, California, which met five times between July 1996 and May 1998. Applications of meta-analysis include the synthesis of environmental impact assessments.

Florida Big Bend coastal research workshop: toward a scientific basis for ecosystem management. Sponsored by Florida Sea Grant, UF Department of Fisheries and Aquatic Sciences, USGS (Florida Caribbean Science Center), and Suwannee River Water Management District. Steinhatchee, Florida, May 1997. (Osenberg was an invited participant)

Oral Presentations at Workshops

"Integrated study of environmental impacts: linking environmental changes with biological responses". Annual workshop of the UC Toxic Substances Research and Teaching Program (Coastal Environmental Program). Bodega Marine Laboratory, January 1990. (Osenberg)

"Statistical considerations in environmental assessment studies: the Before-After-Control-Impact-Paired design". Annual workshop of the UC Toxic Substances Research and Teaching Program (Coastal Environmental Program). Bodega Marine Laboratory, January 1990. (Osenberg)

Chair, working group on "Environmental effects of produced water discharge". Annual workshop of the UC Toxic Substances Research and Teaching Program (Coastal Environmental Program). Bodega Marine Laboratory, November 1991. (Osenberg)

"Eco-toxicological research at the University of California". Annual workshop of the UC Toxic Substances Research and Teaching Program (Coastal Environmental Program). Bodega Marine Laboratory, September 1994. (Osenberg)

Oral Presentations at Public Meetings

"Ecological and Demographic Effects of Produced Water". Southern California Educational Initiative Site Visit, University of California at Santa Barbara, April 1990. (Osenberg)

"Demographic Effects of Produced Water". Southern California Educational Initiative Site Visit, University of California at Santa Barbara, March 1991. (Osenberg)

"Ecological Effects of Produced Water". Southern California Educational Initiative Site Visit, University of California at Santa Barbara, March 1991. (Osenberg)

"Environmental Effects of Produced Water". Presentation to the Minerals Management Advisory Board Outer Continental Shelf Scientific Committee, Santa Barbara, California, March 2, 1995. (Osenberg)

FINAL STUDY REPORT

Ecological Responses to, and Recovery From, Produced Water Discharge: Application of a BACIPS Assessment Design

I. GENERAL INTRODUCTION

A. Motivation and Background

Produced water, an aqueous waste generated during oil and gas production, is commonly discharged into marine environments. Its environmental effects are poorly understood, and what is known, is based largely on studies conducted in the Gulf of Mexico and adjacent estuarine systems. Field assessments in the Gulf of Mexico have been complicated by other types of production activities that confound effects of produced water (Spies 1987, Carney 1987, Osenberg and Schmitt 1996). In addition, results from the Gulf, where discharge often occurs in sheltered, shallow embayments, may yield limited insights about expected effects in other systems, such as the more open, high energy coast of the western United States. Due to more rapid diffusion of the produced water plume in these open-coast systems, and the highly seasonal nature of the systems, effects of produced water may be even more difficult to detect and quantify. Examination of individual-based parameters (such as growth, reproductive output, or fertilization success) might provide more sensitive indicators of environmental impacts than assessment of population density (Carney 1987; Osenberg *et al*. 1992b, 1994). This approach also might reveal a different spatial extent of biological effects than documented by variation in population densities. Furthermore, the examination of effects on individual growth and reproduction, allow a more production-based assessment than is possible by looking only at population density. Because population densities and many other environmental variables of interest vary tremendously among different sites and at different times, powerful assessment designs are needed to document environmental impacts (Stewart-Oaten *et al*. 1986, 1992, Osenberg and Schmitt 1996). This is often not possible, however, because data are seldom available from periods prior to the discharge of produced water.

In 1989, we began a field (and laboratory) study of the environmental effects associated with an open coast discharge of produced water. The separation facility that discharged the produced water was located near Carpinteria, California, and since 1978 had discharged ~2.5 million liters/day of produced water through a diffuser located approximately 250 m offshore (at a depth of 11 m). We began this study, ~12 years after produced water was first discharged at the site. Our initial field studies documented patterns consistent with an environmental impact of produced water, which for some parameters appeared to extend out to at least 0.5 km from the diffuser (e.g., Krause *et al*. 1992, Osenberg *et al.* 1992, Raimondi and Schmitt 1992, Krause 1994). However, these studies lacked "Before" data to compare with the data obtained during operation, and thus were unable to unequivocally separate effects of produced water discharge from other sources of spatial variation.

Because the plant shutdown in 1992, we were in the enviable position to examine recovery of biota near the discharge as well as obtain data that would permit us to separate natural spatial variation from effects of the produced water via application of the BACIPS design (in which

the "Before" and "After" periods correspond to "Discharge" and "Post-shutdown" periods, respectively). BACIPS requires a series of samples collected at a several sites during both discharge and non-discharge periods. Because we anticipated the change in plant activity, we collected (and archived) field samples in the hope that we could procure funds to process and analyze these samples. We were able to do so through subsequent awards from the SCEI (in fact, this project was supported through several SCEI projects, which were amended to take advantage of these unique opportunities: see Piltz 1996 for a general discussion of the importance of flexible and adaptive planning in environmental research). Thus, a primary component of this project was to rigorously test for an environmental effect of produced water (by separating the effect of produced water from other sources of variation) and compare the time scale of recovery for a variety of benthic organisms as well as two species of mussels that had been transplanted to the study sites. Our BACIPS analyses focused on three types of ecological data: (1) performance of mussels outplanted to 8 sites that varied in their proximity to the diffuser; (2) barium content in these mussel shells (barium is a marker of the produced water plume that is incorporated into mussel shells: Higashi *et al.* 1992, Osenberg *et al.* 1992b); (3) infaunal densities assessed at 20 sites that varied in their proximity to the diffuser (from a few meters out to 1 km upcoast and downcoast).

B. Basic Approach

Sea Urchin Reproduction

Fertilization success of purple sea urchins was evaluated using serial dilutions of produced water collected from the discharge pipe at the separation facility just prior to its discharge into the ocean ("effluent") or from the ocean at different distances from the outfall ("receiving water"). Gametes were collected from adult urchins following intracoelomic injections of 0.5 M KCl. Adults either were collected from a rocky reef distant from the produced water outfall and maintained in the laboratory or taken from cages placed at different distances from the outfall (thus, using adults that had been exposed to variable degrees to produced water under field conditions). Eggs and sperm were stored on ice and all tests were conducted within one hour of collection. Most tests were conducted following brief exposure of eggs, sperm or zygotes (or combinations thereof) to produced water. Fertilization success was determined microscopically by the presence of a fertilization membrane. Survivorship and developmental rates were also assessed. Gonad mass and egg size were also determined for the adults maintained in cages at different distances from the outfall. See Krause *et al*. (1992) and Krause (1994, 1995) for greater description about the laboratory and field protocol.

Mussel Performance

Beginning in June 1990 and continuing until March 1994, we examined the performance of mussels transplanted to a subset of these study sites. Initially, 12 buoy arrays were deployed at six of these sites (two each at 1, 5, 10, 50, 100, 1000 m west of the diffusers); we later added arrays at 500 m west and 1000 m east of the diffusers. We assessed the performance of California mussels (*Mytilus californianus*) during 7 different periods during discharge and 6 different periods after shutdown (1 additional period straddled the discharge/shutdown period). Each outplant lasted 3-4 months, and enabled us to assess mussel growth, tissue production, and survival as a function of distance from the diffuser. One of the outplants was done in collaboration with colleagues at the Bodega Marine Laboratory (G. Cherr, G. Harman) and UC Davis (T. Fan, R. Higashi) who examined the mussels to assess their developmental

and physiological status. We also conducted outplants of the bay mussel (*M. edulis*) for a subset of these periods, depending on the availability of these mussels. Before being transplanted to the field, individual mussels were measured and marked so that their initial size could be estimated upon collection. Forty mussels from a size range (20 – 60 mm shell length) were put into a bag made of oyster netting. Two bags (per species) were placed ~4.5 m above the ocean floor at each study site. A control bag was immediately frozen without being transplanted to provide an estimate of initial condition. Mussels were recovered after 3-4 months and frozen until they could be processed. Each mussel was measured and dissected, and gonadal and somatic tissue was then separated, dried at 60^{o}C for 24 hours and weighed.

Shell Barium Content

For many of these outplants, we (in collaboration with T. Fan and R. Higashi at UC Davis) also quantified the amount of barium in the mussel shells. Barium is a marker of the produced water plume (Higashi *et al.* 1992) and can substitute for calcium in the carbonate matrix of bivalve shells. Mussel shell fragments from the growing edge of ~10 shells / distance were chipped off, dried, and pulverized into a fine powder. The shell powder (*ca*. 0 .5 g) was digested in 6 ml of concentrated nitric acid in a Taylor tube, which was left overnight at room temperature followed by heated digestion at 50°C for 90 min, 100°C for 90 min, 140°C for 90 min (tube opening covered with a glass funnel for recondensing nitric acid vapor), 160°C for 90 min (with funnel removed), and 220°C until all nitric acid was evaporated. The residue was redissolved in 8 ml of 2% nitric acid and centrifuged at 4000 rpm to remove particulates. An additional set of shell samples was spiked with 62.5 ppb Ba standard and processed identically as above. The digest was then subjected to inductively coupled plasma-atomic emission spectrometry (ICP-AES) for Ba and Ca (using wavelengths of 455.4 and 315 nm, respectively). A calibration curve for Ba was composed of 0, 10, 50, 100, and 1000 ppb Ba standards while that for Ca consisted of 20, 100, and 400 ppm Ca standards. Each set of standards was run every 15 to 30 samples to check instrument drift with time; all standard curves had a linear correlation coefficient of > 0.999. Barium content was expressed on both a dry mass basis and normalized to Ca content, since shell may also contain non-carbonate material.

Infaunal Organisms

Beginning in February 1990 and continuing through June 1995, we conducted 14 intensive spatial surveys near the produced water outfall. Benthic samples were collected at a bottom depth of 10 m and at distances of approximately 1, 2, 5, 10, 25, 50, 100, 250, 500, and 1000 m upcoast (West) and downcoast (East) from the diffuser. Eight core samples (78 cm^2/core) were collected at each of the 20 sites to characterize the infaunal assemblage; sediments were also collected for enumeration of grain size distribution and sediment organic matter. Buffered formalin was added to the infaunal cores to bring the total concentration to 10% formalin, and later washed through 2 mm, 1 mm, and 0.5 mm sieves and stored in alcohol. Organisms were subsequently picked from the sediments, identified to broad taxonomic categories, and counted. For additional detail, see Osenberg *et al*. (1992b)

C. Results

Results from our research are summarized in the following sections (II – VI).

II. R.J. SCHMITT AND C.W. OSENBERG (EDS.). 1996. *Detecting Ecological Impacts: Concepts and Applications in Coastal Habitats*. Academic Press, San Diego.

(Major conceptual advances stemming from our SCEI program's elaboration of the BACIPS design are available in this edited volume. The Table of Contents is provided below.)

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Krause, P.R., C.W. Osenberg, R.J. Schmitt. 1992. Effects of produced water on early life stages of a sea urchin: gender-specific responses and delayed expression. Pages 431-444 *in* J.P. Ray and F.R. Englehardt (eds.), *Produced water: technological / environmental issues and solutions*, Plenum Publishing Corp., New York.

EFFECTS OF PRODUCED WATER ON EARLY LIFE STAGES OF A SEA URCHIN: STAGE-SPECIFIC RESPONSES AND DELAYED EXPRESSION

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INTRODUCTION

Common objectives of many bioassays are to provide a measure of relative toxicity of various effluents (e.g., Allen, 1971; Kobayashi, 1980; National Research Council, 1983), or to identify components in a given effluent that are most responsible for biological effects (e.g., Higashi et al., 1992). There is an indisputable need for such perspectives for produced waters from different coastal formations (Neff, 1987). However, while such a focus can provide a useful toxicity benchmark, it is not particularly well suited for exploring the nature, extent and generality of biological responses. For example, there is an emerging paradigm that earlier developmental stages of a marine organism are more sensitive to a pollutant than later ones (e.g., National Research Council, 1983; Neff et al., 1976), yet no consistent pattern emerges from the relatively few studies that have examined different life stages of the same species (e.g., Neff et al., 1976; Rossi and Anderson, 1978; see Capuzzo, 1987). Even when multiple life stages of a species have been considered, it is difficult to extend laboratory-based results to natural situations, in part because of uncertainty as to whether the concentrations and exposure durations used are realistic (e.g., Castagna et al., 1981; Neff, 1987).

Another feature of bioassays, and one that has not received a great deal of attention, concerns the endpoint (response variable) chosen to measure an "effect." For a number of logical and logistical reasons, the response variable selected often is a biological "milepost" that coincides with the end of an exposure period. For example, fertilization success often is used as the response variable to estimate effects of toxicant exposure on gametes (e.g., Kobayashi, 1971; Dinnel et al., 1987). However, the extent of deleterious consequences of exposure of gametes will be underestimated if additional effects are manifested at a later developmental stage for successfully fertilized zygotes. Delayed expression of effects, while known to occur in marine organisms (e.g., Castagna et al., 1981; Pagano et al., 1982, 1983), has not been explored systematically, and we therefore lack an appreciation for its potential importance. For similar reasons, we know little about the combined effects that might arise from repeated exposure of an individual in different life stages. To facilitate meaningful biological interpretation of laboratory bioassays, the challenge is to match the laboratory protocol with reasonable

Produced Water, Edited by J.P. Ray
and F.R. Engelhart, Plenum Press, New York, 1992

estimates of which life stages are at risk, the concentrations of contaminant they are likely exposed to, and the duration of the exposure under field conditions.

With respect to general classes of lethal and sublethal effects, one of the most important from an ecological perspective is impaired reproductive success of adults. If early life history stages are relatively more sensitive to toxicants than adults, then major effects on the reproductive success of adults could be mediated through effects on early stages of their progeny. For many benthic marine organisms, gametes are released directly into the water column where they undergo syngamy and form zygotes. Thus, important impacts on reproductive success of adults living near a point source outfall might be mediated through early exposure of their gametes and developing progeny. In the present study, we explore whether and how brief exposure to a range of concentrations of produced water affect gametes and early larval stages of the purple sea urchin, *Strongylocentrotus purpuratus*. In particular, we exposed separately and together eggs, sperm, and zygotes to ascertain (a) the relative sensitivities of these life stages to produced water at durations and concentrations realistic to each stage, (b) the nature of the biological responses, and (c) the potential for delayed expression.

We chose the purple sea urchin as our model organism, in part, because there is an extensive body of toxicological work on various life stages of sea urchins (e.g., Cherr et al., 1987; Dinnel et al., 1981, 1982, 1987, 1989; Hagstrom and Lonning, 1973; Hose, 1985; Kobayashi, 1971; McGibbon and Moldan, 1986; Oshida et al., 1981; Pagano et al., 1982, 1983, 1986). Furthermore, the purple sea urchin is representative of many marine organisms in that benthic adults broadcast eggs and sperm into the water column where fertilization and subsequent larval development occur. Purple sea urchin larvae typically develop into the pluteus stage within 48 hours. Up through the late pluteus (~72 hr), larvae are sustained by egg reserves and do not feed (Strathmann, 1987).

METHODS

Adult purple sea urchins were collected from a pristine reef near Santa Barbara, CA, and maintained in large, continuous flow sea water tanks located outdoors. Animals were supplied *ad libitum* with freshly collected giant kelp (*Macrocystis pyrifera*), a favored food item. For each experiment, gametes were collected from 4 - 6 adults of each sex within 15 minutes following intracoelomic injection of 0.5 - 1.0 ml of 0.5 *M* KCl (Strathmann, 1987). Eggs were cleaned by passing them twice through a 200 μm Nitex mesh and allowing them to settle through 100 ml of filtered sea water. Sperm were collected "dry" (i.e., without sea water, which metabolically activates sperm [Timourian and Watchmaker, 1970]) by pipetting the gametes from the dried surface of a male (Dinnel et al., 1987). Both gamete types were stored on ice until used in a trial, which occurred no later than 3 hours after gamete collection. Separate "control" trials were conducted to determine whether the delay between collection and use (and therefore manner of storage) altered the viability of eggs or sperm; results indicated no difference in viability of eggs or "dry" collected sperm that were stored for three hours compared with freshly released gametes.

Undiluted produced water used for laboratory tests was collected from an oil processing facility in Carpinteria, CA. Samples were collected from an onshore test spigot located on the discharge pipe just after the effluent leaves the final settling tank and just prior to ocean discharge. Produced water was collected without head space in clean amber glass bottles with Teflon lined lids. Samples were maintained on ice and in the dark until experiments were begun within approximately six hours of collection.

The general laboratory protocol involved cross-designed experiments in which produced water concentration (1, 0.01 and 0.0001%) was crossed with a second factor designating the life stage(s) that was exposed (e.g., eggs only, sperm only, zygote only, and/or a combination of these treatments). A control treatment was included and consisted of unexposed life stages kept in filtered sea water but otherwise handled identically to the other treatments. Produced water concentrations were made by serial dilution of the raw effluent with filtered sea water in

volumetric labware (Allen, 1971). All sea water was filtered through a 0.45 μm autoclaved filter. In all experiments, there were three replicates of each treatment, and each replicate typically involved approximately 500 eggs. The incubation units were 15 ml plastic Falcon multi-well culture dishes into which the sperm and eggs were introduced. All experiments were conducted in a cold (15° C) room on a continuous shaker table.

Ten minute gamete exposures were accomplished by adding sperm (or eggs) to a glass test tube containing a given concentration of produced water. Unexposed gametes were added to filtered sea water for 10 minutes. Following the 10 minute exposures, gametes were incubated together in 10 ml of filtered sea water. To assess fertilization success, replicates were fixed with 10% formalin 25 minutes after gametes had been added to the incubation units. Fertilization success was estimated by microscopic identification of the fertilization membrane.

In experiments that examined zygote performance, we manipulated zygote exposure by varying the concentration of produced water in which sperm and eggs were incubated (i.e., exposed eggs and sperm were introduced into incubation units that contained a gradient of produced water concentrations). Developing zygotes remained in these units for up to 96 hours. Thus, our "zygote exposures" included brief exposures of gametes during the period between introduction into the incubation chamber and fertilization. After a specified time, replicates were fixed with 10% formalin, and 100 individuals from each replicate were inspected and their developmental stage noted. Modification to these general protocol are noted below.

To facilitate comparisons among experiments and to avoid polyspermy, we standardized fertilization rates by adjusting the ratio of sperm to eggs (Cherr et al., 1987; Dinnel et al., 1987). Prior to each experiment, batches of eggs were flooded with varying amounts of a stock sperm solution to establish the relationship between fertilization success and the ratio of sperm to eggs. Each experiment was then conducted at the sperm:egg ratio that produced 90% fertilization in filtered sea water.

To prevent microbial contamination, 0.05% Penicillin G was added to each replicate in all experiments. An initial experiment was conducted to establish whether the added antibiotic altered normal fertilization or development, and whether there was a synergistic response of penicillin and produced water. The experiment involved two factors: the presence/absence of penicillin (0 or 0.05%) was crossed with the presence/absence of produced water (0 or 10%). Eggs and sperm were separately exposed to produced water (or filtered sea water) for 10 minutes and then incubated together in filtered sea water. Two endpoints were measured: (a) the proportion of successfully fertilized eggs (as measured by the presence of the fertilization membrane) after 25 minutes of adding sperm to the eggs, and (b) the proportion of embryos that had reached the pluteus stage after 48 hours.

The first main experiment explored the effects of produced water on fertilization success. Produced water concentrations used were 1, 0.01, and 0.0001%. Three exposure regimes were crossed with the produced water treatment: sperm exposed but eggs unexposed, eggs exposed but sperm unexposed, and both sperm and egg exposed. A 0% gamete exposure (filtered sea water only) served as the control, and all incubations were done in filtered sea water.

The second main experiment addressed (a) whether effects from the exposure of gametes might be delayed to a later developmental stage, (b) the relative sensitivities of eggs, sperm and zygotes, and (c) the magnitude of effects arising from combined exposure of different life stages. Unlike the first experiment, the incubation period (following the 10 minute exposure of gametes) was increased from 25 minutes to 48 hours. In addition, there were five exposure treatments: 1) sperm (i.e., eggs and zygotes unexposed); 2) eggs; 3) zygotes; 4) sperm and eggs; 5) sperm, eggs and zygotes. After 48 hours, each replicate was fixed in 10% formalin and the proportion of embryos that had reached the pluteus stage was determined microscopically.

In this experiment, the response parameter (proportion of larvae in the pluteus stage) was based on the relative developmental states of only the eggs and larvae that were still intact after 48 hours. Furthermore, the endpoint of this experiment was time dependent (i.e., assessed after only 48 hours). Thus, it was not possible to separate the relative contributions of mortality from

delayed development. Consequently, a third experiment was conducted to estimate directly the separate effects of produced water on embryo survivorship and developmental rates. To do this, initial cohorts of eggs were counted and the fates of all initial eggs followed through time. Treatments consisted of exposure to 1% produced water of eggs only, sperm only, and of zygotes. A control treatment consisted of exposure to filtered sea water only. The number of eggs in each replicate was known at the beginning of the experiment, and eighteen "copies" of each treatment were initiated; at specified intervals, three replicates per treatment were fixed for later analysis. The intervals were 25 min, 12 hr, 24 hr, 48 hr, 72 hr and 96 hr. Mortality (1 minus survivorship) was estimated as the number of "dead" eggs or embryos divided by the number of eggs initially present. "Dead" eggs and embryos were defined as those missing or obviously dead (i.e., damaged or broken). The numbers of individuals in the pluteus stage were counted at each sampling time and developmental success expressed as the proportion of the initial cohort that had entered the pluteus stage.

In general, results of each experiment were analyzed by analysis of variance. Comparisons with the control treatment were made using Dunnett's two-tailed *t* test, and *a posteriori* comparisons among treatment main effects were made using the Ryan-Elinot-Gabriel-Welsch multiple *F* test (REGWF: SAS, 1988). All data were arcsine-square root transformed prior to analysis.

RESULTS

Methodological Tests of Penicillin Effects

Before considering effects of produced water, we first present evidence that the addition of penicillin to control bacterial contamination does not obscure interpretation of laboratory experiments. Penicillin G had no detectable effect on the proportion of eggs that were successfully fertilized (Table 1; $F_{1,8} = 1.30$; $P > 0.25$). More importantly, there was no interaction between the antibiotic and the effect of produced water on fertilization (Table 1; $F_{1,8} = 0.43$; $P > 0.50$). Similarly, the proportion of embryos that reached the pluteus developmental stage in 48 hrs was not affected by the antibiotic (Table 1; in sea water, $F_{1,4} = 1.47$; $P > 0.25$). There were, however, marked effects of 10% produced water on fertilization success ($F_{1,8} = 98.4$; $P < 0.0001$) and development (Table 1).

Table 1. The effect of 0.05% Penicillin G on fertilization success and embryo development in the presence and absence of 10% produced water; data are the mean (± 1 SE) proportion of eggs fertilized and of embryos that were in the pluteus developmental stage at 48 hours

	Fertilization Success		Pluteus Stage	
Produced Water	Penicillin		Penicillin	
	Absent	Present	Absent	Present
Absent	0.900 (0.040)	0.840 (0.056)	0.720 (0.034)	0.773 (0.026)
Present	0.434 (0.020)	0.409 (0.020)	0	0

Fertilization Assay for Effects of Produced Water

Ten minute exposure of eggs and/or sperm to produced water resulted in statistically significant depressions in the fraction of successful fertilizations (Fig. 1; $F_{2,18} = 83.23$; $P < 0.0001$). As expected, the magnitude of the depression increased with increasing concentrations of produced water (Fig. 1), although a substantial fraction (>50%) of eggs still were fertilized at the highest concentration used (1%). However, even at the lowest produced water concentration (0.0001%)

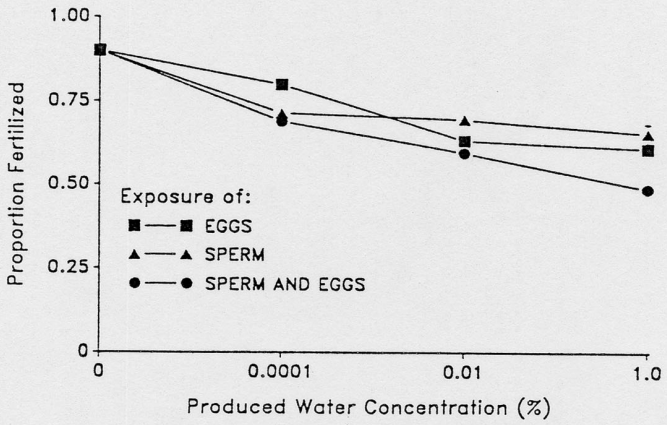

Figure 1. Fertilization success of gametes exposed for 10 minutes to a range in concentrations of produced water. Data are the mean (± 1 SE, n=3) proportion of eggs fertilized after 25 minute incubations of eggs and sperm in filtered sea water.

fertilization success was reduced by as much as ~10 - 20% (Fig. 1); each treatment response in the lowest concentrations differed significantly from the control (Dunnett's test, $P < 0.05$).

There was also a significant difference attributable to which gamete type(s) was exposed (Fig. 1; $F_{2,18} = 42.42$; $P < 0.0001$). *A posteriori* comparison of main effects (using REGWF) shows that exposure of sperm gave similar results as egg exposure, but that exposure of both sperm and eggs yielded a significantly lower fertilization rate. That is, based on fertilization success averaged across produced water concentrations, the sensitivity of sperm was about equal to that of eggs. Further, the greatest impairment occurred when both gamete types were exposed, but the combined effect appeared to be less than the sum of individual effects on each gamete type (Fig. 1). There was, however, an interaction between the gamete type exposed and concentration of produced water ($F_{4,18} = 11.27$; $P < 0.0001$), suggesting a cross-over in relative sensitivity of eggs and sperm to increasing produced water concentrations. It appears that sperm may be relatively more sensitive at low toxicant levels, whereas eggs may be more sensitive at higher concentrations (Fig. 1).

Embryonic Development Assay for Effects of Produced Water

Produced water reduced the fraction of larvae that were in the pluteus developmental stage after 48 hours (Fig. 2). For all gamete and zygote exposure combinations, the occurrence of pluteus larvae decreased with increasing concentration of produced water ($F_{2,30} = 919.37$; $P< 0.0001$). The reductions in pluteus larvae were statistically significant even at the exceedingly low concentration of 0.0001% (Dunnett's test, $P< 0.05$).

Within a concentration of produced water, the smallest reduction in pluteus development occurred when the zygote only was exposed, and the greatest reduction occurred when sperm, egg and the zygote stage all were exposed (Fig. 2; REGWF comparison shows all main effects are significantly different from one another). These data indicate that the brief 10 minute exposure of gametes reduced larval development to a greater degree than did the subsequent 48 hour exposure of zygotes. Thus, gametes were exceedingly more sensitive than zygotes to produced water. Furthermore, most of the effect arising from exposure of gametes was due to sperm, and very little from exposure of eggs (Fig. 2). Based on the pluteus development assay, the rank of sensitivity of each life stage to produced water was:

sperm > > egg > > zygote.

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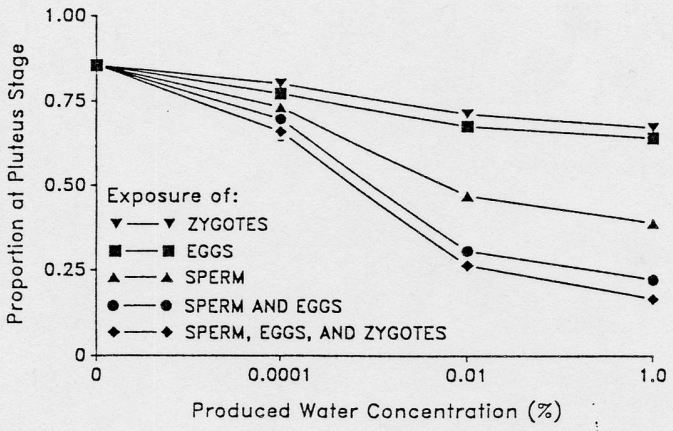

Figure 2. The mean (± 1 SE, n=3) proportion of embryos that are in the pluteus stage of development after 48 hours as a function of life stage exposed and produced water concentration. Gametes were exposed to produced water for 10 minutes prior to fertilization and zygotes were exposed for the entire 48 hour incubation period.

Cohort Analysis for Effects of Produced Water

The effect noted above could be due to a combination of increased mortality of developing embryos and/or a reduction in the development rate of survivors. To distinguish between direct mortality and reduced developmental rates, cohorts of eggs of known number were followed through time and the fraction of the initial number of eggs that eventually reached the pluteus larval stage was estimated. This was done using 1% produced water, which was the effluent concentration that yielded the greatest effects in both our fertilization (Fig. 1) and embryo development (Fig. 2) assays.

The results indicate that 1% produced water had no discernible effect on mortality (Fig. 3). Regardless of the life stage exposed, > 85% of the initial cohort survived to 96 hours, and there was no significant difference among the control or exposure treatments (Fig. 3; $F_{3,8} = 1.23$; $P > 0.35$). It is important to note that zygotes were exposed to produced water for the entire 96 hr period, yet still did not show an increased death rate.

By contrast with no affect on mortality, produced water greatly altered developmental rates of sea urchin embryos (Fig. 4). For example, the fraction of initial eggs that had reached the pluteus stage by 48 hours differed markedly among treatments ($F_{3,8} = 17.65$; $P<0.001$); Once again, zygote exposure yielded the smallest effect, while sperm exposure gave the greatest effect. These patterns at 48 hours (Fig. 4) were both qualitatively and quantitatively similar to those observed in the embryonic development bioassay reported above (Fig. 2). However, the effluent did not affect the number of eggs that ultimately reached the pluteus stage by 96 hours (Fig. 4; $F_{3,8} = 1.02$; $P> 0.40$). More than 70% of the initial cohort of eggs in each treatment had developed into pluteus larvae by 96 hours, and all treatments appeared to be asymptoting near 85%. Thus produced water slowed the developmental rates of sea urchin embryos but did not contribute directly to their mortality. Based on the slowing of developmental rates relative to exposure duration, this experiment indicates that the rank sensitivity of life stages was:

sperm > eggs >> zygotes.

The cohort analysis directly contradicted the finding in our fertilization assay regarding the fraction of eggs that ultimately underwent development: e.g., we observed only 61% of eggs fertilized after exposure of eggs to 1% produced water (Fig. 1), but we subsequently found that

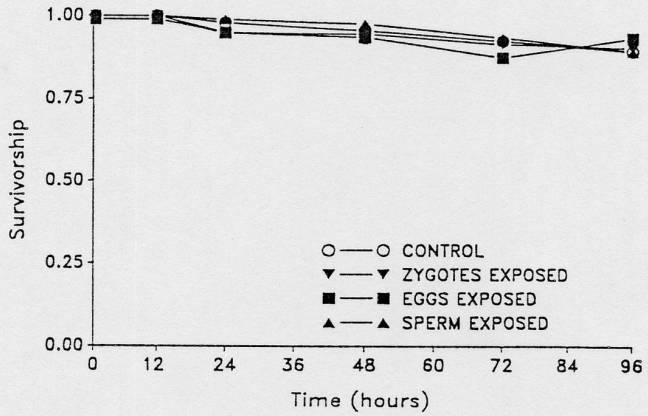

Figure 3. Survivorship of sea urchin eggs and embryos. Data are the mean proportion (± 1 SE, n=3) of cohorts of sea urchins (beginning at the egg stage) alive at each time interval. Gamete treatments involved exposure of eggs or sperm for 10 minutes prior to fertilization (no subsequent exposure of zygotes), and the zygote treatment involved exposure for the entire 96 hour period.

83% of all eggs developed to the pluteus stage (Fig. 4). It is possible that, in the cohort analysis, sperm continued to fertilize eggs long after the 35 minute exposure and incubation period used in the fertilization bioassay. A direct test of this hypothesis indicated that only ~10% of sperm remain viable 35 minutes after activation, and none were capable of fertilizing eggs after 45 minutes (Fig. 5). Exposure of gametes to 1% produced water lowered the fraction of viable sperm of a given age, and did not prolong the length of time that sperm remained capable of fertilization (Fig. 5). The estimate of age-specific sperm viability was independent of the endpoint (i.e., 25 or 120 mins) used to measure successful fertilization (Fig. 5). This experiment also indicated that the effect of produced water on viability of sperm was virtually instantaneous,

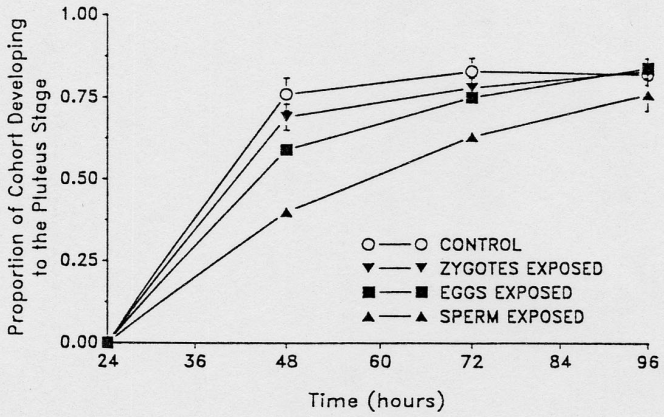

Figure 4. Mean proportion (± 1 SE, n=3) of initial sea urchin eggs that had entered the pluteus larval stage at different times. Gamete treatments involved exposure of eggs or sperm for 10 minutes prior to fertilization (no subsequent exposure of zygotes), and the zygote treatment involved exposure for the entire 96 hour period.

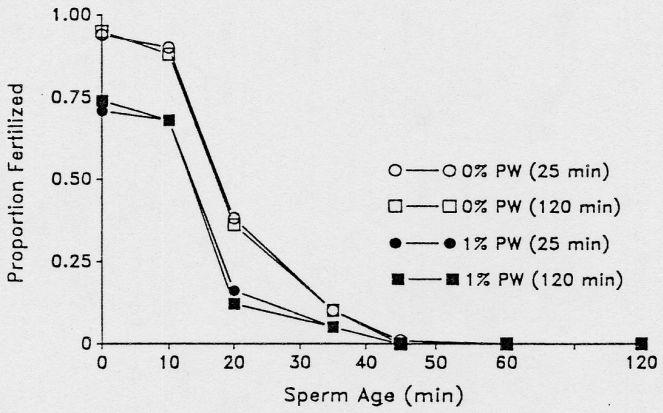

Figure 5. Viability of purple sea urchin sperm as a function of time since activation. Data are the mean (± 1 SE, n=3) proportion of eggs fertilized by sperm in the absence (circles) and presence (squares) of 1% produced attributable water. Only sperm were exposed to produced water: eggs and zygotes were kept in filtered sea water. Fertilization was assayed after 25 (open symbols) and 120 (closed symbols) minute incubations.

and suggests that at least part of the "zygote" sensitivity we have observed might be due to brief exposure of sperm that occurs when we exposure zygotes.

An alternative explanation to prolonged sperm viability is that the presence of a vitelline membrane underestimated successful syngamy when gametes had been exposed to produced water. This was supported by re-examining data from the cohort analysis and expressing fertilization success as the fraction of eggs that either had a fertilization membrane or had undergone cleavage. The initial depression of fertilization in the produced water treatment diminished through time such that by 36 hours there was no difference between treatments in

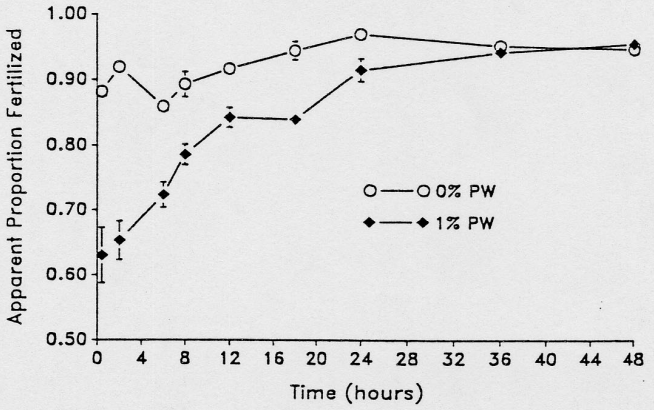

Figure 6. Apparent fertilization success as a function of time. Data are the mean (± 1 SE, n = 3) proportion of egg cohorts known to be fertilized based on the presence of either a vitelline membrane or cleavage at each interval in the absence (open circles) and presence (solid diamonds) of 1% produced water. Sperm, eggs, and zygotes were all exposed to produced water in this example.

the fraction of initial eggs known to have been successfully fertilized (Fig. 6). The results indicate that early indicators of fertilization (e.g., presence of the vitelline membrane) grossly underestimated the fraction of eggs that were actually fertilized and subsequently began embryonic development (Fig. 6).

DISCUSSION

Interpretation of Bioassay Studies

Bioassays typically use a response variable that coincides with the end of the planned exposure period. For example, tests of effects on gametes usually involve estimating changes in fertilization ability immediately after gamete exposure (e.g., Adams and Slaughter-Williams, 1988; Allen, 1971; Dinnel et al., 1981; Hagstrom and Lonning, 1973; Kobayashi, 1971; Pagano et al., 1982, 1983), and fertilization success has become a standard toxicity measure (e.g., Cherr et al., 1987; Dinnel et al., 1982, 1987; Dinnel and Stober, 1987; McGibbon and Moldan, 1986; Pagano et al., 1985, 1986). Post-fertilization assays often measure the fraction of young individuals that reach some developmental "milepost" in a specified time period (e.g., Adams and Slaughter-Williams, 1988; Byrne and Calder, 1977; Dinnel et al., 1989; Hose, 1985; Oshida et al., 1981; Pagano et al., 1985, 1986). However, our application of these event and time-dependent bioassays to produced water revealed several limitations of this general approach.

Our fertilization assay did not capture accurately the effect of produced water on syngamy. The apparent effect on gametes was to reduce successful fertilization (Fig. 1). However, our embryo cohort analyses revealed that the fraction of eggs eventually fertilized was unaffected by produced water (Fig. 4). Failure of the fertilization test to properly convey the lack of an overall affect on syngamy could have been due either to the particular design of the bioassay (i.e., sperm fertilized eggs long after the 35 minute exposure/incubation period used in our fertilization trials), or from the use of an inaccurate measure of syngamy. The first hypothesis can be rejected (Fig. 5). Other workers have also found that, once activated, sea urchin sperm remains viable for a very short period, usually < 25 minutes (e.g., Tyler, 1953; Timourian and Watchmaker, 1970; Pennington, 1985). By contrast, our measure of fertilization appeared to be imprecise. As is typical in these types of assays (e.g., Dinnel et al., 1987; McGibbon and Moldan, 1986; Oshida et al., 1981), we used the presence of a fertilization membrane to score successful fertilization. However, following exposure of gametes to produced water, the fraction of initial eggs that eventually underwent embryonic development was grossly underestimated by the fertilization membrane criterion (Fig. 6). It appears that produced water inhibited the vitelline membrane from lifting off the surface of some eggs, yet did not preclude syngamy. Alternative techniques to estimate fertilization success, such as nuclear staining, may be more reliable (though less practical) measures of syngamy.

With respect to differential sensitivity of gametes, our fertilization assay also incorrectly suggested that effects arising from pre-fertilization exposure on eggs and sperm were roughly equivalent (Fig. 1). However, our embryo development tests demonstrated that effects arising from sperm exposure were far greater than those from egg exposure (Figs. 2 & 4). The inconsistency resulted from an effect from sperm exposure that was manifested much later in development, and which was not evident at the time of fertilization. These results cogently illustrate that a delay in expression of an effect cannot be discovered when the assay endpoint, such as fertilization success, coincides with the end of the exposure period. The scope and magnitude of biological responses from a toxicant can be underestimated by failing to determine whether exposure at one life stage of an individual has an effect that is expressed in a later stage, yet this aspect is largely unexplored (but see Adams and Slaughter-Williams, 1988). In the case of fertilization, this limitation was perhaps presaged by the suggestion of certain authors that fertilization assays may be less sensitive indicators of effects from gamete exposure than exploration of abnormalities later in development (e.g., Allen, 1971; Hose, 1985).

However, the interpretation of bioassays that examine delayed effects can still be problematic. For example, we found that the fraction of sea urchin embryos reaching the pluteus larval stage in 48 hours was greatly impaired by produced water (Fig. 2). A logical interpretation of these results is that produced water exposure killed sperm, eggs, or embryos in some developmental phase prior to the pluteus stage. Our cohort analyses revealed that this interpretation was incorrect. The only effect of produced water was to impede developmental rates of embryos (Fig. 4): it did not directly increase mortality of developing zygotes (Fig. 3) or reduce fertilization of eggs (Fig. 6).

These results underscore a crucial but little appreciated difficulty with standard bioassay approaches; without additional evidence, the existence and contribution of various biological effects (e.g., delayed expression, reduced developmental rates, increased mortality) cannot be inferred safely from bioassays with fixed-time endpoints. While such techniques are useful in ranking the relative toxicity of various effluents, their utility in providing an understanding of the nature of biological responses is more limited, and alternative approaches should be sought. Endpoints and exposure regimes should be selected that enhance biological insight and therefore either help pinpoint mechanisms of toxicity or permit extrapolation to field situations where biological effects arise from exposure of particular life stages (e.g., Somerville et al., 1987; Higashi et al., 1992; Raimondi and Schmitt, 1992).

Biological Effects of Produced Water

Predicting or understanding the potential for long-term environmental impacts arising from the discharge of produced water into coastal waters is impeded because biological effects of whole produced waters have received little attention (Neff, 1987). To the extent that laboratory bioassays have been applied to produced waters, most produced waters do not appear to be acutely toxic (for review see Neff, 1987). Results of our study support this notion for produced water from oil fields in the Santa Barbara Channel; brief (10 minute) exposure of sea urchin gametes and prolonged (96 hr) exposure of zygotes did not result in increased mortality, even at the moderately high concentration of 1% produced water (Fig. 3). We did, however, detect sublethal responses to produced water at substantially lower levels; statistically significant responses of young purple sea urchins were detected at produced water concentrations as low as 1 part per million, with the magnitude of the response(s) increasing with increased concentration of the effluent.

It has been argued that such sublethal or chronic effects may be a better gauge of potential environmental significance than the acute lethality of an effluent (e.g., Capuzzo, 1987; Neff, 1987). Extrapolation from laboratory demonstration of sublethal effects to assessment of ecological consequences under natural conditions is not, however, straightforward. For example, exposure concentrations and durations used in a laboratory study do not necessarily bear any relationship to exposure regimes that arise under field conditions. Two lines of evidence suggest that our laboratory exposures spanned conditions likely to occur in the field. First, our results show that the effect of produced water on gametes, particularly sperm, was virtually instantaneous (Fig. 5). Therefore, adult urchins that spawn in the vicinity of the produced water outfall are likely to produce offspring with delayed developmental rates even though those developing larvae may soon be transported away from the outfall. Second, we have examined effects under field conditions by exposing urchin gametes and larvae to water collected at different distances from the produced water diffuser and compared these results with those obtained from known concentrations of produced water (Krause, unpublished data). These results suggest that although produced water is rapidly diluted (the diffuser is designed for an initial seawater:effluent dilution of 125:1), detectable developmental effects can persist out to 100 - 500 m, where produced water concentrations drop to approximately 1 ppm. Thus, the produced water concentrations we used in our laboratory experiments nicely matched with the concentrations that can occur in the field at sites within 500 m of the outfall.

Another potential difficulty in extrapolating laboratory findings of developmental delays to field consequences is that delayed development can lead to an indirect increase in mortality: e.g., by increasing the length of time that early life stages remain vulnerable to natural sources of mortality, such as size-selective predators (e.g., Werner et al., 1983). Thus, slight sublethal effects, such as delayed development, could indirectly contribute to significant reductions in the number of larvae available to recruit into benthic populations. However, processes linking larval and benthic dynamics are poorly understood and therefore the ecological significance of larval mortality is largely unknown (e.g., Capuzzo, 1987; Nisbet et al., 1993; Raimondi and Schmitt, 1992). Much work remains before impacts on benthic populations can be predicted based on sublethal responses of larval stages.

Because so little is known regarding sublethal effects of produced water, it is not yet possible to assess whether a reduction in developmental rate is a common response of early life stages. Lowered growth or developmental rates have been observed for individuals exposed to certain constituents of produced water. For example, petroleum hydrocarbons can retard individual growth of adult copepod crustaceans (Hay et al., 1988), larval decapod crustaceans (Caldwell, 1977; Cucci and Epifano, 1979; Katz, 1973; Laughlin et al., 1978; Laughlin and Neff, 1979; Wells, 1972; Wells and Sprauge, 1976) and larval bivalve mollusks (Byrne and Calder, 1977). In each of these cases, the life stage exposed was capable of feeding, and hydrocarbons are known to reduce feeding rates of planktotrophic larvae (e.g., Johns and Pechenik, 1980; Wells and Sprauge, 1976). Hence, reduced growth or developmental rates could have resulted primarily from an indirect effect on food intake. This process was not involved in the reduced developmental rates of purple sea urchins; larvae of this species do not feed until the late pluteus stage (Strathmann, 1987), which is not attained until ~72 hours after fertilization in normally developing embryos. The response of young purple sea urchins, especially that arising from exposure of gametes, must have involved direct alteration of normal cellular activity. Delayed development of embryos mediated through exposure of gametes to PCBs has been observed for the sea urchin *Arbacia punctulata* (Adams and Slaughter-Williams, 1988). Like produced water, the actual mechanism producing this effect of PCB exposure is unknown, but like certain petroleum hydrocarbons (Capuzzo, 1987), PCBs are highly lipophilic. Lipid reserves are of primary importance in embryonic development (e.g., Holland, 1978), and it has been suggested that larvae may shunt energy reserves away from differentiation to be used to detoxify such lipophilic compounds as petroleum hydrocarbons (e.g., Sharp et al., 1979).

Produced water, of course, contains numerous potential toxicants in addition to petroleum hydrocarbons (e.g., Neff, 1987), and the particular constituents of the Carpinteria effluent responsible for the observed effect on purple sea urchins remains to be determined. Higashi et al. (1992) explored "toxicity" of various fractions of produced water from Carpinteria, and found that the majority of biological effects arose from the water-only soluble fraction that contained divalent cations. Interestingly, Pagano et al. (1982) found results for other sea urchin species exposed to cadmium that were qualitatively similar to ours. Although cadmium is not common in the Carpinteria produced water, analogous elements, such as barium and strontium, are present (Higashi et al., 1992). Furthermore, barium and strontium are known to impair development (Conrad and Davis, 1980), and recent work suggests that these ions might mediate their effects through modification of microtubule function (Tamm, 1989; Tamm and Tamm, 1990). Because we observed that sperm exposure yielded the greatest effect on development, and because this effect appeared to arise very early in development, we suggest that the toxicological mechanisms most likely involve a feature specific to sperm that involves microtubule function. One likely candidate is the sperm centriole, which is responsible for the transport of the sperm pronucleus to the egg pronucleus (Bestor and Schatten, 1981). If the function of the centriole were impaired, this could lead to an early retardation of development by delaying nuclear fusion. Other studies using produced water from this same source have found responses that might involve microtubule-mediated effects: e.g., swimming and chemoreception of abalone larvae (Raimondi and Schmitt, 1992), swimming of kelp spores (Reed, 1992), migration of kelp nuclei (Pillai et al.,

1990), and growth (possibly feeding) of mussels (Osenberg et al., 1992). If these diverse biological effects of produced water were mediated through a similar toxicological mechanism, it would provide a strong unifying theme to the study of this complex effluent.

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REFERENCES

Adams, J.J., and Slaughter-Williams, S., 1988, The effects of PCB's (Aroclors 1254 and 1016) on fertilization and morphology in *Arbacia punctulata*, Water, Air, and Soil Pollut. 38:299-310.

Allen, H., 1971, Effects of petroleum fractions on the early development of a sea urchin, Mar. Poll. Bull. 2:138-140.

Bestor, T.M., and Schatten, G., 1981, Anti-tubilin immunofluorescence microscopy of microtubules present during the pronuclear movements of sea urchin fertilization, Dev. Biol. 88:80-91.

Byrne, C.J., and Calder, J.A., 1977, Effects of the water-soluble fractions of crude, refined, and waste oils on the embryonic and larval stages of the Quahog clam *Mercenaria* sp, Mar. Biol. 40:225-231.

Caldwell, R.S., Calderone, E.M., and Mallon, M.H, 1977, Effects of seawater-soluble fraction of Cook Inlet crude oil and its major aromatic components on larval stages of the Dungeness crab, *Cancer magister* (Dana), *in*: "Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms," D.A. Wolfe, ed., Pergamon Press, New York.

Capuzzo, J.M., 1987, Biological effects of petroleum hydrocarbons: assessments from experimental results, *in*: "Long-Term Environmental Effects of Offshore Oil and Gas Development," D.F. Boesch and N.N. Rabalais, eds., Elsevier Applied Science, NY.

Castagna, A., Sinatra, F., Scalia, M., and Capodicasa, V., 1981, Observations of the effect of zinc on the gametes and various development phases of *Arbacia lixula*, Mar. Biol. 64:285-289.

Cherr, G.N., Shenker, J.M., Lundmark, C., and Turner, K.O., 1987, Toxic effects of selected bleached kraft mill effluent constituents on the sea urchin sperm cell, Env. Toxicol. Chem. 6:561-569.

Conrad, G.W., and Davis, S.E., 1980, Polar lobe formation and cytokinesis in fertilized eggs of *Ilyanassa obsoleta*, Devel. Biol. 74:152-172.

Cucci, T.L. and Epifano, C.E., 1979, Long term effects of water- soluble fractions of Kuwait oil on the larval and juvenile development of the mud crab *Eurypanopeus depressus*, Mar. Biol. 55:215-220.

Dinnel, P.A., Stober, Q.J., and DiJulio, D.H., 1981, Sea urchin sperm bioassay for sewage and chlorinated seawater and its relation to fish bioassays, Mar Env. Res. 5:29-39.

Dinnel, P.A., Stober, Q.J., Crumley, S.C., and Nakatani, R.E., 1982, Development of a sperm cell toxicity test for marine waters, *in*: "Aquatic toxicology and hazard assessment: Fifth Conference, ASTM STP 766," J.G. Pearson, R.B. Foster, and W.E. Bishop, eds., Amer. Soc. Testing and Materials, Philadelphia, PA.

Dinnel, P.A., Link, J.M., and Stober, Q.J., 1987, Improved methodology for sea urchin sperm cell bioassay in marine waters, Arch. Environ. Contam. Toxicol. 16:23-32.

Dinnel, P.A., and Stober, Q.J., 1987, Application of the sea urchin sperm bioassay to sewage treatment efficiency and toxicity in marine waters, Mar. Env. Res. 21:121-133.

Dinnel, P.A., Link, J.M., Stober, Q.J., Letourneau, M.W., and Roberts, W.E., 1989, Comparative sensitivity of sea urchin sperm bioassay to metals and pesticides, Arch. Environ. Contam. Toxicol. 18:748-755.

Hagstrom, B.E., and Lonning, S., 1973, The sea urchin egg as a testing object in toxicology, Acta. Pharm. et Toxicol. 32(Supp. I):1-49.

Hay, S.J., Evans, G.T., and Gamble, J.C., 1988, Birth, growth and death rates for enclosed populations of calanoid copepods, J Plank. Res. 10(3):431-454.

Higashi, R., Cherr, G., Bergens, C., Fan, T., and Crosby, D., 1992, Toxicant isolation from a produced water source in the Santa Barbara Channel, *in*: "Produced Water: Technological/ Environmental Issues and Solutions," J.P. Ray and F.R. Engelhardt, eds., Plenum Publishing Corp., New York, New York, USA.

Holland, D.L., 1978, Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates, *in*: "Biochemical and Biophysical Perspectives in Marine Biology, Volume 4," D.C. Malins and J.R. Sargent, eds., Academic Press, New York.

Hose, J.E, 1985, Potential uses of sea urchin embryos for identifying toxic chemicals: Description of a bioassay incorporating cytologic, cytogenetic and embryonic endpoints, *J.* Appl. Toxicol. 5:245-254.

Johns, D.M., and Pechenik, J.A., 1980, Influence of the water accommodated fraction of No. 2 fuel oil on energetics of *Cancer irroratus* larvae, Mar. Biol. 55:247-254.

Katz, L.M., 1973, The effects of water-soluble fractions of crude oil on larvae of the decapod crustacean *Neopanope texana* (Say), Environ. Pollut. 5:199-204.

Kobayashi, N., 1971, Fertilized sea urchin eggs as an indicatory material for marine pollution bioassay, preliminary experiment, Publs. Seto Mar. Biol. Lab. 18:379-406.

Kobayashi, N., 1980, Comparative sensitivity of various developmental stages of sea urchins to some chemicals, Mar. Biol. 58:163-171.

Laughlin, R.B. Jr., and Neff, J.M., 1979, Interactive effects of salinity, temperature and polycyclic aromatic hydrocarbons on the survival and development rate of larvae of the mud crab *Rhithropanopeus harrisii*, Mar. Biol. 53:281-291.

Laughlin, R.B., Jr., Young, L.G.L., and Neff, J.M., 1978, A long-term study of the effect of water-soluble fractions of No.2 fuel oil on the survival, development rate, and growth of the mud crab *Rhithropanopeus harrisii*, Mar. Biol. 47:87-89.

McGibbon, S., and Moldan, A.G.S., 1986, Routine toxicity testing of toxicants using a sea urchin gamete bioassay, Mar. Pollut. Bull. 17(2):68-72.

National Research Council, 1983, "Drilling Discharges in the Marine Environment," National Academy Press, Washington, D.C.

Neff, J.M., Anderson, J.W., Cox, B.A., Laughlin, R.B., Jr., Rossi, S.S., and Tatum, H.E., 1976, Effects of petroleum on survival, respiration and growth of marine animals, *in*: "Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment," American Institute of Biological Sciences, Washington, D.C.

Neff, J.M., 1987, Biological effects of drilling fluids, drill cuttings and produced waters, *in*: "Long-Term Environmental Effects of Offshore Oil and Gas Development," D.F. Boesch and N.N. Rabalais, eds., Elsevier Applied Science, New York.

Nisbet, R. M., Murdoch, W.W., and Stewart-Oaten, A., 1993, Consequences for Adult Fish Stocks of Human Induced Mortality on Immatures. Ecol. Appl. in review.

Oshida, P.S., Goochey, T.K., and Mearns, A.J., 1981, Effects of municipal wastewater on fertilization, survival, and development of the sea urchin, *Strongylocentrotus purpuratus*, *in*: "Biological Monitoring of Marine Pollutants," F.J. Vernberg, A. Calabrese, F.P. Thurnberg, and W.B. Vernberg eds., Academic Press, New York.

Osenberg, C.W., Schmitt, R.J., Holbrook, S.J., and Canestro, D. 1992, Spatial scale of ecological effects associated with an open coast discharge of produced water, *in*: "Produced Water: Technological/ Environmental Issues and Solutions," J.P. Ray and F.R. Engelhardt, eds., Plenum Publishing Corp., New York, New York, USA.

Pagano, G., Esposito, A., and Giordano, G.G., 1982, Fertilization and larval development in sea urchins following exposure of gametes and embryos to cadmium, Arch. Env. Contam. Toxicol. *11:47-55.*

Pagano, G., Esposito, A., Bove, P., DeAngelis, M., Rota, A., and Giordano, G.G., 1983, The effects of hexavalent and trivalent chromium on fertilization and development in sea urchins, Env. Res. 30:442-452.

Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Ragucci, E., Giordano, G.G., and Trieff, N.M., 1985, Comparative toxicities of chlorinated biphenyls on sea urchin egg fertilization and embryogenesis, Mar. Env. Res. 17:240-244.

Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Ragucci, E., Giordano, G.G., and Trieff, N.M., 1986, The sea urchin: Bioassay for the assessment of damage from environmental contaminants, *in*: "Community toxicity testing, ASTM STP-920," J. Cairns ed., Amer. Soc. Testing and Materials, Philadelphia, PA.

Pennington, J.T., 1985, The ecology of fertilization of echinoid eggs: The consequences of sperm dilution, adult aggregation, and synchronous spawning, Biol. Bull. 169(10):417-430.

Pillai, M.C., Baldwin, J.D., and Cherr, G.N., 1990, Role of cytoskeleton in germtube elongation and nuclear translocation in a marine algal gametophyte, J. Cell Biol. 111:(No.5, Pt. 2) 415a.

Raimondi, P.T., and Schmitt, R.J., 1992, Effects of produced water on settlement of larvae: field tests using red abalone, *in*: "Produced Water: Technological/ Environmental Issues and Solutions," J.P. Ray and F.R. Engelhardt, eds., Plenum Publishing Corp., New York, New York, USA.

Reed, D.C., and Lewis, R.J., 1992, Water column effects of produced water discharges on the production and dispersive potential of giant kelp (*Macrocystis pyrifera*). In preparation.

Rossi, S.S., and Anderson, J.W., 1978, Petroleum hydrocarbon resistance in the marine worm *Neanthes arenaceodentata* (Polychaeta: Annelida) induced by chronic exposure to No. 2 fuel oil, *B*ull. Environ. Contam. Toxicol. 20:515-521.

SAS Institute Inc, 1988, "SAS/STAT User's Guide, Release 6.03 Edition," SAS Institute Inc, Cary, North Carolina.

Sharp, J.R., Fucik, K.W., and Neff, J.M., 1979, Physiological bases of differential sensitivity of fish embryonic stages to oil pollution, *in*: "Biological Monitoring of Marine Pollutants," F.J. Vernberg, A. Calabrese, F.P. Thurnberg, and W.B. Vernberg, eds., Academic Press, New York.

Somerville, H.J., Bennett, D., Davenport, J.N., Holt, M.S., Lynes, A., Mahieu, A., McCourt, B., Parker, J.G., Stephenson, R.R., Watkinson, R.J., and Wilkinson, T.G., 1987, Environmental effect of produced water from North Sea oil operations, Mar. Poll. Bull. 18(10):549-558.

Strathmann, M.F., 1987, Phylum Echinodermata, Class Echinoidea, *in*: "Reproduction and Development of the Marine Invertebrates of the Northern Pacific Coast: Data and Methods for the Study of Eggs, Embryos, and Larvae," M.F. Strathman ed., University of Washington Press, Seattle, Washington.

Tamm, S.L., 1989, Control of reactivation and microtubule sliding by calcium, strontium and barium in detergent-extracted macrocilia of *Beroe*, Cell Motil. Cytoskel. 12:104-112.

Tamm, S.L., and Tamm, S., 1990, Ca/Ba/Sr-induced conformational changes of ciliary axonemes, Cell Motil. Cytoskel. 17:187-196.

Timourian, H., and Watchmaker, G., 1970, Determination of spermatozoan motility, Dev. Biol. 21:62-72.

Tyler, A., 1953, Prolongation of life span of sea urchin spermatozoa and improvement of the fertilization reaction by treatment of the spermatozoa and eggs with metal chelating agents, Biol. Bull. 104:224-239.

Wells, P.G., 1972, Influence of Venezuelan crude oil on lobster larvae, Mar. Pollut. Bull. 3:105-106.

Wells, P.G., and Sprague, J.B., 1976, Effects of crude oil on American lobster (*Homarus americanus*) larvae in the laboratory, J. Fish. Res. Board Can. 33:1604-1614.

Werner, E.E., Gilliam, J.F., Hall, D.J., and Mittelbach, G.G., 1983, An experimental test of the effects of predation risk on habitat use in fish, Ecology 64(6):1540-1548.

IV. PRODUCED WATER EFFECTS ON SEA URCHIN REPRODUCTION, MUSSEL PERFORMANCE, AND INFAUNAL PATTERNS: FIELD STUDIES

A. EFFECTS OF AN OIL PRODUCTION EFFLUENT ON GAMETOGENESIS AND GAMETE PERFORMANCE IN THE PURPLE SEA URCHIN (*STRONGYLOCENTROTUS PURPURATUS* STIMPSON)

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Abstract — Adult organisms subjected to chronic discharges from a point source of pollution may exhibit several sublethal responses. One such response is the impairment of gamete production. This may be expressed in the amount and/or quality of gametes produced by adults. In this study the effects of chronic exposure to produced water (an oil production effluent) on the gametogenesis and gamete performance of the purple sea urchin (*Strongylocentrotus purpuratus* Stimpson) were examined using an in situ caging experiment. Adult purple sea urchins were kept in benthic cages arrayed down-field from a discharging diffuser at 13 sites, with distances ranging from 5 to 1,000 m. Cage exposures were maintained in the field for eight weeks, and each cage held 25 animals. Gametogenesis was examined for each sex by comparing a size-independent measure of relative gonad mass as determined by analysis of covariance. Results showed that there was a significant negative relationship between these estimates of relative gonad mass and distance from the outfall for both sexes, indicating that sea urchins living closer to the outfall produced significantly larger gonads. Gamete performance was measured through a fertilization kinetics bioassay that held the concentration of eggs constant and varied the amount of sperm added. The proportion of eggs fertilized under each sperm concentration was determined and the response fit to a model of fertilization kinetics. This experiment showed significant differences in the fertilizability of eggs between cages, and egg fertilizability showed a positive relationship with distance away from the outfall. These findings indicate that although adult sea urchins exposed to a produced water outfall exhibit larger gonads, they suffer a marked decrease in gamete performance.

Keywords — Produced water *Strongylocentrotus purpuratus* Gametogenesis Reproductive toxicity Bioassay

INTRODUCTION

The introduction of anthroprogenic discharges of various toxicants into the coastal marine environment of southern California has stimulated interest in understanding their fate and effects. In recent years, interest in the effects of oil-related effluents has increased with oil production in the Santa Barbara Channel. One type of discharge that has received little research attention in the western United States is produced water. During oil production, water pumped from the formation must be separated and discarded. This aqueous fraction, commonly called "produced water" or "oil-field brine," is often discharged into the marine environment. The effluent contains a diverse array of contaminants, including hydrocarbons, heavy metals, and chemical additives such as surfactants and corrosion inhibitors. Produced water is possibly the single largest source of contaminants that result from offshore oil production [1].

From an ecological perspective, one of the most important sublethal effects of toxicant exposure is the impairment of reproduction in adult organisms. To date, few studies have explored the sublethal effects of produced water on reproduction in marine organisms, either in the field [2–4] or in the lab [5,6]. One potential sublethal effect on reproduction is the impairment of gamete production in adult organisms. This may be expressed in the quantity of gametes produced by adults or in the quality of those gametes that are produced. In this study adult purple sea urchins (*Strongylocentrotus purpuratus* Stimpson) were used to test the effects of chronic exposure to produced water discharge on gametogenesis and gamete quality through the use of an in situ caging experiment. Specifically, this experiment tested how sea urchin gonad sizes and fertilization kinetics of gametes varied with proximity to an outfall following an eight-week field exposure to discharges of produced water.

Fertilization kinetics

A model of sea urchin fertilization kinetics was proposed by Vogel et al. [7] to predict fertilization based on gamete concentrations; sperm life span; and two rate constants, β and β_0. The Vogel–Czihak–Chang–Wolf (VCCW) model is described by the following equation [7; Eq. 13]:

$$F = 1 - \exp\left(-\frac{\beta S_0}{\beta_0 E_0}\left(1 - \exp(-\beta_0 E_0 \tau)\right)\right),$$

where F is the proportion of eggs fertilized, β is the rate constant of fertilization, β_0 is the rate constant of sperm–egg encounter, S_0 is the sperm concentration, E_0 is the egg concentration, and τ is the sperm life span [7]. The fertilizable

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surface fraction of the egg is described by the ratio of β/β_0 [7]. This model assumes that a sperm cell will attach to the first egg that it encounters. It also accounts for "sperm scavenging" by eggs, whereby one egg may have several sperm attached to it, yet only one sperm cell will eventually fertilize the egg. Under this model, with all eggs capable of being fertilized, all eggs will become fertilized if enough sperm are available and the model saturates at 100% fertilization (Fig. 1, curve A).

Exposure to a toxic pollutant may have several effects on fertilization kinetics and thus alter the shape of the kinetics model [7]. First, if all the eggs are capable of becoming fertilized but there is an effect on the potency of the sperm, the model will still saturate at 100% fertilization, but the initial slope of the curve will be lower (Fig. 1, curve B). All the eggs will eventually become fertilized, but it will take a much higher sperm concentration to achieve saturation. The initial rate of fertilization as it approaches saturation can be thought of as the attack rate of sperm on eggs. That is, the initial slope is a function of the rate at which sperm find and fertilize eggs. Second, if the major effect is on the fertilizability of eggs and all the sperm remain healthy, the curve should be similar to the first case, but saturation should occur at fertilization levels <100% (Fig. 1, curve C). This assumes that unfertilizable eggs will continue to attract sperm in the same manner as fertilizable eggs. Third, if the effect of the pollutant is a combination of effects on fertilizability of eggs and potency of sperm, the model should be represented by a curve that is even more depressed. It will saturate at <100% fertilization, with an even lower initial slope (Fig. 1, curve D).

Study organism

The organism selected for this experiment was the purple sea urchin, *S. purpuratus* Stimpson (Echinodermata: Echinoidea). Purple sea urchins are common invertebrates of the rocky intertidal and submerged reefs along the California coast. They become sexually mature when they reach a test diameter of about 25 mm [8] and typically spawn between December and May [9], although subtidal populations may be gravid throughout much of the year [10; P. Krause, personal observations]. Mature eggs of the purple sea urchin are typically between 78 and 80 μm in diameter [9]. Lab populations may be sustained in spawning condition, and ripe gametes may be collected several times over a reproductive cycle if the adults are maintained in a well-fed condition [11].

MATERIALS AND METHODS

Study site

The produced water discharge and study site are located near Carpinteria, California, (34°23'N, 119°30'W), approximately 20 km east of Santa Barbara. The general area consists of a sandy bottom environment with little relief. The coastline at Carpinteria can be characterized as an open-coast, high-energy environment. Currents run essentially parallel to the coastline in an east–west direction, with westerly flow (i.e., east to west) more common than easterly flow. Current flow from depth profiles at the outfall diffuser measured on 30 sampling dates was from the north (flow offshore), south (flow onshore), east, and west 25, 6, 42, and 27% of the time, respectively [2]. The north–south currents were driven primarily by changing tidal cycles on sampling dates.

The Carpinteria produced water outfall has operated for approximately 13 years. The subtidal diffuser is located approximately 200 to 300 m offshore at a depth of approximately 11 to 12 m. The diffuser is made up of 10 T-shaped discharge ports located along the final 25 m of the pipe. The discharge ports are oriented perpendicularly to the pipe and rise about 0.75 m above the sand substrate. The outfall discharges approximately 16,000 bbl/d (2.6×10^6 L/d) at a calculated minimum initial dilution of 125 to one (CRWQCB

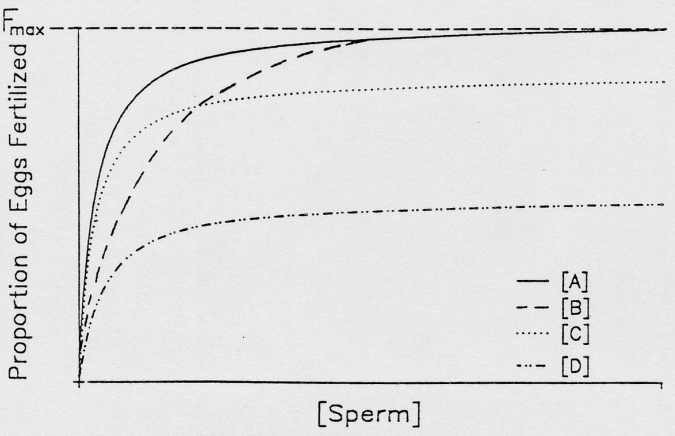

Fig. 1. Idealized curves for the fertilization kinetic responses of sea urchin eggs. [A] Response with all fertilizable eggs and all sperm capable of fertilizing eggs; [B] response with all fertilizable eggs but decreased sperm potency; [C] response with only a portion of fertilizable eggs but all sperm capable of fertilizing eggs; [D] response with a combination of effects reducing both fertilizability of eggs and potency of sperm. F_{max} = maximum fertilizability of eggs. Sperm attack rate is the initial slope of the response.

NPDES permit CA0000230). The effluent is approximately 5°C warmer than the receiving waters, and previous studies have shown that there is no detectable thermal gradient beyond 0.5 m of the discharge ports [2]. The effluent has a lower salinity (about 18 ppt) than the receiving waters, and salinity gradients rapidly reach background levels (32 ppt) within 5 to 10 m from the outfall (P. Krause, in preparation). The chemical characteristics of the effluent from the Carpinteria outfall have been summarized by Higashi et al. [12]. Briefly, they reported the effluent to be a complex mixture of both metal and organic pollutants. Due to the highly variable nature of the effluent [1; J. Wallace, personal communication], to date no definitive measurement exists of the extent of the physical or chemical plume produced by the outfall, although previous studies have shown biological effects may be detected out to approximately 100 m from the outfall [2,3]. This experiment was, in part, designed to determine the spatial extent of biological responses resulting from the plume.

Experimental urchin cages were deployed at 13 sites to the west of the outfall. Cage sites were located at 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 1,000 m from the produced water diffuser (Fig. 2). These sites were chosen for several reasons. First, the predominant current direction is alongshore from the east. The most probable plume direction is to the west of the outfall. Second, the bottom characteristics in many areas to the east of the outfall would have precluded attachment of cages to the substrate. Last, and most important, a priori information from previous field studies at this outfall has indicated that biological effects are likely to be found along the western transect out to approximately 100 m from the outfall [2,3]. The distant site at 1,000 m can, therefore, be thought of as a far-field control.

As this study was designed to detect biological responses along the western transect, the statistical design was that of a regression analysis. That is, it was determined that intersite variability was more important to detect than intrasite variation. Therefore, intrasite variation was sacrificed for additional sites, and only one cage was deployed at each location. This resulted in a better estimate of the overall response curve at the expense of an estimate of within-site variability.

Cages measured 1 × 0.5 × 0.25 m and were constructed of 13 mm PVC plastic pipe frame covered with 13 mm mesh plastic netting. The top of the cage was hinged to provide access in the field. Cages were fixed to the substrate with 1 m earth anchors at a water depth of approximately 11 to 12 m along a transect line bearing 180° magnetic. The cage anchors were checked several times throughout the deployment period and tightened as needed to maintain the cages securely on the substrate.

Field exposure of study organisms

Adult purple sea urchins were collected from a subtidal rocky reef near Santa Barbara, California. Animals were maintained in large, continuous-flow seawater tanks located outdoors for the period between collection and field deployment. During this period the sea urchins were fed with freshly collected giant kelp (*Macrocystis pyrifera*) blades. Four days before deployment in the field, all animals were induced to spawn artificially by injecting them with of 0.5 to 1.0 ml of 0.5 M KCl [9]. This procedure ensured that sea urchins deployed in field enclosures began the experimental period with few ripe gametes, therefore subsequent gametogenesis must have occurred during the exposure period. Of the 400 sea urchins collected, only 14 died between capture and deployment.

On April 8, 1992, 25 sea urchins were outplanted into each cage. The animals were supplied with 2 kg giant kelp blades. At two-week intervals throughout the exposure period, each of the cages was serviced. At this time any uneaten kelp ma-

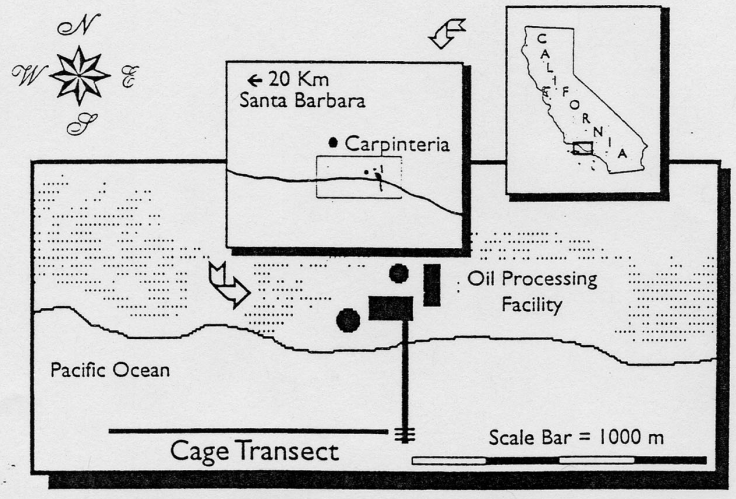

Fig. 2. Diagrammatic map of experimental site at Carpinteria, California. Experimental cage locations were along the cage transect at distances of 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 1,000 m. Water depth at the diffuser was approximately 11 m.

terial was removed, the cages cleaned of fouling organisms, and the animals fed 2 kg giant kelp blades. At no time during the exposure period did the sea urchins go without food, as there was always uneaten kelp material within each cage. There was no mortality of urchins during the experimental period. After an eight-week field exposure all sea urchins were recovered from each cage on June 3, 1992. Recovered sea urchins were collected into marked bags, maintained in coolers, and returned to the lab.

To determine if gametogenesis differed between cages, the gonads from a subsample of sea urchins from each cage were examined. Upon reaching the lab, 10 sea urchins from each cage were picked haphazardly and sacrificed for gonadal analysis. To avoid spontaneous gamete release, the animals were first frozen at −20°C for 2 h and then fixed in 10% buffered formalin. The remainder of the sea urchins from the field cages were transferred to marked cages and maintained in continuous-flow seawater until fertilization analysis. To test for effects on gamete performance, a fertilization bioassay was performed on gametes spawned from sea urchins of each cage.

Gonadal analysis

Fixed sea urchins were drained of excess formalin, their test diameters measured, and their gonads removed. Gonad material was dried for 24 h at 55°C and weighed. To facilitate comparisons between cages, size-independent measures of relative gonad mass were obtained for each sex using analysis of covariance (ANCOVA) of log(gonad mass) with log(test diameter) as the covariate [2]. From this analysis adjusted means of log(gonad mass) for each sex were determined from each cage. The relationship between these adjusted means, for both males and females, and cage distance from the outfall were determined by regression analysis.

Fertilization bioassay

For each cage, the remaining 15 urchins were spawned for 15 min using the method previously described and the gametes were collected. The sex ratio of spawned organisms varied from cage to cage, but each test involved at least four individuals of each sex. Eggs were collected and cleaned by passing them twice through a 200-μm Nitex® mesh (E.A. Case, Andover, NJ) and allowing them to settle through 100 ml of filtered sea water. Sperm were collected "dry" by pipetting the gametes from the dried surface of male sea urchins [13]. Both gamete types were collected separately and stored on ice. All bioassays were conducted no later than 1 h after gamete collection. Previous studies have shown that this delay between spawning and assay does not alter viability of eggs or sperm [6].

The general protocol for the fertilization bioassay involved exposing a fixed concentration of eggs to several different sperm concentrations and determining the proportion of eggs fertilized under these different concentrations. After determining the concentration of eggs in the stock suspension, a working suspension of 25,000 eggs ml^{-1} was made in a glass test tube. Of this, approximately 500 eggs were transferred to 10 ml seawater for each of 12 fertilization incubations. Sperm concentration was determined for the dry sperm stock. The sperm stock suspension was diluted with seawater and sperm transferred to each fertilization incubation to final sperm concentrations of 2,500, 5,000, 10,000, 12,500, 15,000, 17,500, 20,000, 25,000 30,000, 37,500, 50,000, and 250,000 sperm ml^{-1}. In all experiments, there were three replicates of each treatment. To assess fertilization success, replicates were fixed with 10% formalin 25 min after gametes had been added to the incubation units. Fertilization success was estimated by microscopic examination of the fertilization membrane. The incubation chambers were 15-ml plastic Falcon® (Becton Dickinson, Lincoln Park, NJ), multiwell culture dishes. All seawater was filtered through a 0.45-μm autoclaved filter, and all experiments were conducted in a cold (15°C) room on a continuous shaker table. The cage order for performing the bioassays was determined randomly before running the experiments.

Data analysis involved linear regressions of various parameters of the fertilization kinetic response against distance from the outfall. First, to test for effects on eggs, the maximum asymptotic fertilization (F_{max}) was regressed against log(distance) from the diffuser. Second, to test for effects on sperm, the sperm attack rate was similarly regressed against log(distance) from the diffuser. The attack rate of sperm was defined by the initial slope of the fertilization kinetic response. All fertilization proportions were arcsine square-root transformed before analysis. The maximum asymptotic fertilization was determined by fitting the data to a modified VCCW model [7] of sea urchin fertilization kinetics. The initial slope of the response (sperm attack rate) was determined by linear regression of the proportion fertilized against the log(sperm concentration) for the first seven sperm concentrations with the intercept forced through the origin. This criterion was chosen because it covered the initial linear portion of the response and provided a good linear fit ($r^2 > 0.9$ for all distances).

The VCCW model was modified to account for asymptotic fertilization below 100% by multiplying the right side of the equation by the maximum fertilizability of eggs (F_{max}). The modified model becomes

$$F = F_{max}\left(1 - \exp\left\{-\frac{\beta S_0}{\beta_o E_0}\left[1 - \exp(-\beta_0 E_0 \tau)\right]\right\}\right),$$

where F is the proportion of eggs fertilized, F_{max} is the maximum fertilizability of eggs, β is the rate constant of fertilization, β_0 is the rate constant of sperm–egg encounter; S_0 is the sperm concentration; E_0 is the egg concentration; and τ is the sperm life span [7,14]. Substituting

$$k = \left(\frac{\beta}{\beta_0}\right)\left[1 - \exp(-\beta_0 E_0 \tau)\right],$$

the expression simplifies to

$$F = F_{max}\left\{1 - \exp\left[-k\left(\frac{S_0}{E_0}\right)\right]\right\}$$

Thus, since E_0, and τ are held constant throughout the experiments and the ratio of β/β_0 is a constant [7,14], k becomes an overall rate constant of the fertilization response.

Data from the fertilization bioassay were fit to the modified VCCW model with a nonlinear regression by iterating the DUD method in SAS® [15] to find the best fitted value of F_{max} for each cage. To verify that the modified model accurately fit the experimental data, the maximum fertilizability of eggs was also determined from the proportion of eggs fertilized under the highest (250,000 sperm ml^{-1}) sperm concentration. An ANCOVA was then used to examine how the two estimates of maximum fertilizability of eggs varied with distance from the produced water outfall.

To determine if spawned eggs were of a mature size, an analysis of eggs collected from sea urchins in each cage was made. For each cage, 100 eggs were measured at 40× on a compound microscope equipped with an optical micrometer. The egg diameter and the proportion of eggs <80 μm in diameter were determined. The presence or absence of germinal vesicles in eggs was also determined, as this is a good indicator of egg maturity [16]. ANOVA was performed on the mean egg diameter between cages. A similar ANOVA was done on the proportion of small eggs between cages.

RESULTS

Analysis of gonads

Examination of gonad size data revealed a striking pattern with distance from the outfall. ANCOVA indicated a significant effect of log(test diameter) on log(gonad mass) in both genders (males: $F_{12, 50} = 65.27$, $P = 0.001$; females: $F_{13, 53} = 79.61$, $P = 0.001$). Analysis of size-independent relative mean gonad masses indicated that sea urchins caged closer to the produced-water discharge had larger gonads than urchins caged farther away (Fig. 3). This pattern was similar for both males and females. Male sea urchins showed a weak but significant relationship between gonad size and cage distance (Fig. 3A; $r^2 = 0.44$, $F_{1, 10} = 7.93$, $P = 0.018$). A similar and slightly stronger pattern was found for female sea urchins (Fig. 3B; $r^2 = 0.54$, $F_{1, 10} = 13.37$; $P = 0.004$). ANCOVA revealed that there was not a significant difference between male and female responses with distance ($F_{22, 24} = 11.24$, $P = 0.0004$).

Fertilization bioassay

The pattern of response in the fertilization bioassay was similar for all groups of sea urchins regardless of distance from the outfall. Each test showed an increasing function that saturated with an asymptotic number of eggs fertilized with between 50,000 and 250,000 sperm ml^{-1}. The modified VCCW model fit the data well ($r^2 > 0.9$ for all distances). The asymptotic maximum fertilizations (F_{max}) estimated from the model reflected accurately the maximum attainable fertilizations observed. There was no significant difference in the estimates of maximum fertilization either with the model fit or using the maxima from the raw data (Fig. 4A; $F_{1, 25} = 0.11$, $P = 0.754$). This indicates that the modifications to the VCCW model increased the accuracy of the model in describing the pattern found in the data. For all

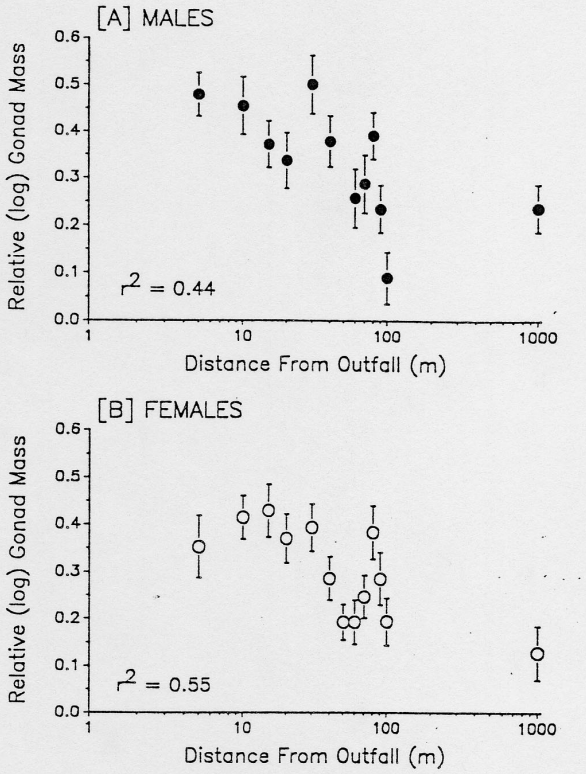

Fig. 3. Relationship between relative gonad mass and distance from the outfall. Data are mean (±SE, $n = 10$) gonad mass for each sex. (**A**) males; (**B**) females. Size-independent measures of gonad mass were determined by ANCOVA using log(test diameter) as covariate.

cages, the fraction of fertilizable eggs differed dramatically over the tested range of distances and increased with distance from the diffuser (Fig. 4A). For example, only about 38% of eggs from the 5-m cage were fertilizable, whereas 87% of eggs from the 100-m cage were fertilizable. There was a significant linear relationship between F_{max} and log(distance) from 5 m out to 80 m (Fig. 4A; $r^2 = 0.984$, $F_{8, 9} = 486$, $P = 0.0001$). The effect on F_{max} diminished between 90 and 100 m and was no longer evident at the 1,000-m site, where eggs were 100% fertilizable (Fig. 4A).

The pattern of the sperm attack rate with distance from the diffuser was similar to that of the fertilizability of eggs. The relationship between fertilization and log(sperm concentration) was linear and showed good fit for sperm concentrations between 2,000 and 20,000 sperm ml^{-1} ($r^2 > 0.9$ for all cages). Only sperm concentrations of 20,000 or less were used to determine sperm attack rates because linearity decreased at successively higher sperm concentrations. The slowest (lowest initial slope) attack rate was observed at the 5-m site, and rates increased through the 80-m site. The effect on sperm attack rate was similar at cages between 90 and 100 m and greatest (steepest initial slope) at the 1,000-m site

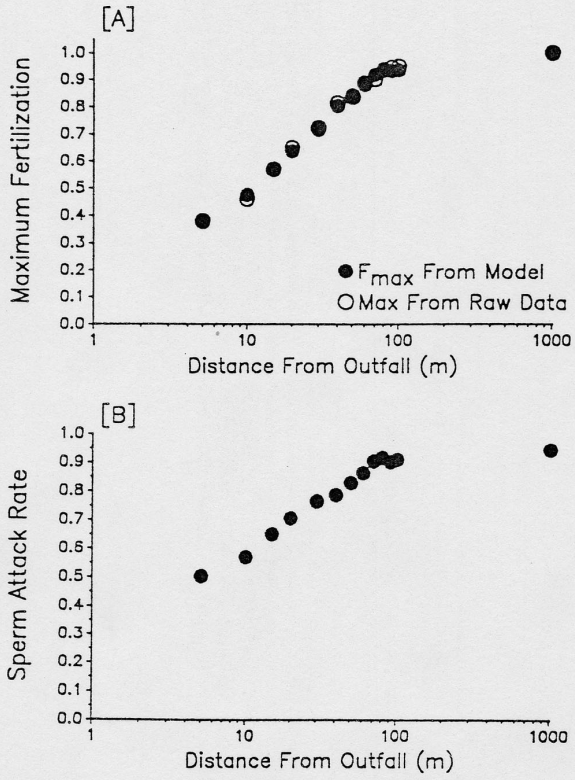

Fig. 4. Fertilization kinetic parameters (F_{max} and sperm attack rates) as a function of distance from an active produced water outfall. (**A**) Relationship between maximum fertilization (F_{max}; ●) as determined from the fit of the modified VCCW model [7] and the maximum fertilization attained under the highest sperm concentration (○) with distance from outfall. Data are back-transformed values obtained from arcsine-transformed fertilization. (**B**) Relationship between sperm attack rates and distance from outfall. Sperm attack rate is defined as the initial slope of the fertilization response (see "Methods").

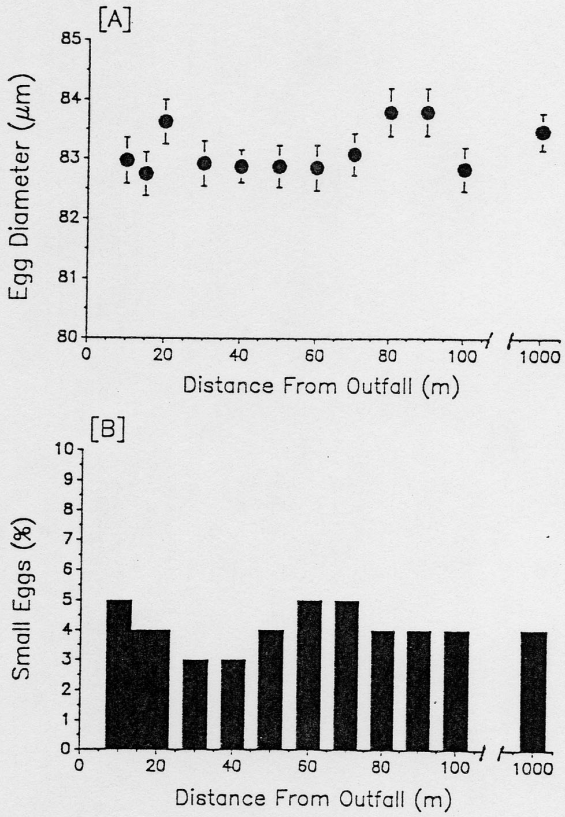

Fig. 5. Relationship between urchin egg size and distance from an active produced water outfall. (**A**) Diameter of eggs from caged urchins. Data are mean (±SE, $n = 100$) egg diameter (μm). (**B**) Percentage of eggs from caged urchins that had diameters <80 μm.

(Fig. 4B). There was a significant relationship between sperm attack rate and log(distance) from the produced water outfall over the first 80 m (Fig. 4B; $r^2 = 0.991$, $F_{8,9} = 927$, $P = 0.0001$).

The size of eggs released by females did not vary significantly with distance from the outfall (Fig. 5A; $F_{11,1,198} = 0.95$, $P = 0.330$). The mean egg diameter ranged from 82.80 μm (SE = 0.36) from the 100-m cage to 83.78 μm (SE = 0.40) from the cage at 80 m. There were no observable morphological abnormalities in the egg morphology in any of the examined eggs, and no eggs had germinal vesicles. For all batches of eggs spawned from caged females, no more than 5% of the eggs were <80 μm in diameter, which did not vary significantly with distance (Fig. 5B; $F_{1,12} = 0.01$, $P = 0.929$).

DISCUSSION

Field exposure of adult purple sea urchins down-current from an active produced water outfall resulted in toxic effects on both gametogenesis and subsequent gamete performance. Observed effects were manifested in decreased egg fertilizability and decreased sperm attack rates as determined by analysis of fertilization kinetics. The magnitude of the observed effects on both eggs and sperm is directly related to the distance from the outfall, with greater effects found nearer the discharge (Fig. 4). If the results of the regressions of both kinetic parameters (F_{max} and sperm attack rate) are extrapolated to their maxima, effects associated with the produced water outfall would be expected to occur out to approximately 100 m from the discharge (Fig. 4).

The observed effects differed between eggs and sperm. Effects on eggs resulted in decreased fertilizability, whereas effects on sperm resulted in decreased attack rates. Although not directly tested here, decreased egg fertilizability may be related to the accumulation of toxicants within the developing eggs. This has been hypothesized as a possible passive detoxification mechanism for biologically soluble hydrocarbons and metals [17–21]. Lipophilic organic pollutants have been shown to accumulate in the eggs of mussels [5], amphipods [22], fish [21,23–25], birds [26], and mammals [27]. Such a

detoxification mechanism could serve to reduce the toxic burdens of adult females but with an associated cost of reduced effective fecundity. This passive detoxification mechanism is probably more active in females that produce large quantities of lipid-rich eggs than in males with relatively lipid-poor sperm. Another possible explanation is that produced water directly affects the synthesis of the egg surface receptor for the attachment of sperm during oogenesis [28]. Thus, sperm may no longer be able to attach to and fertilize the eggs, that is, eggs would become essentially unfertilizable.

There are several possible explanations for the decreased attack rates of sperm on eggs. First, it is possible that produced water exposure during spermatogenesis resulted in the development of less capable sperm. This could come about through any of several biochemical mechanisms: changes in the ability of a sperm to undergo the acrosome reaction [29], decreasing binding and/or penetrance of eggs [16], or decreased motility of sperm cells [30]. To the extent that it was observed, there did not appear to be any gross difference in the motility of sperm cells from the different cages. Second, the decreased attack rates could be an artifact of decreased egg fertilizability. Thus, it may simply be that more sperm are required to find the smaller proportion of eggs that are capable of becoming fertilized; with a smaller proportion of fertilizable eggs, greater amounts of sperm would be required to fertilize the same proportion of eggs. This interpretation is likely if the unfertilizable eggs continue to attract sperm to the same degree as healthy eggs. Scavenging of sperm by unfertilizable eggs would decrease the effective sperm concentration available to fertilize the healthy eggs. The VCCW model takes into account the fact that eggs will attract sperm and many sperm will become stuck in the jelly layer of the egg [7]. Microscopic observations could not discern any differences in the number of sperm cells attached to the fertilized or unfertilized eggs throughout this experiment. Third, there may be a combination of both mechanisms (sperm controlled and egg controlled) operating to decrease the apparent attack rates of sperm.

In addition, gametogenesis in adult sea urchins was affected by exposure to produced water discharge. Like the effects on fertilization kinetics, the relative mass of gonads from urchins varied with distance from the outfall. Sea urchins caged closer to the outfall had substantially larger relative gonad masses than sea urchins from farther away (Fig. 3). This was consistent for both males and females. These results were similar to those found for mussels caged at the same outfall by Garman and Cherr [31], who found that mussels deployed closer to the outfall became gravid earlier and had larger gonads than those further away. They also found significant decreases in embryo performance from mussels nearer the outfall [31]. Their studies, however, differed from those of Osenberg et al. [2], who found that mussels caged near the same outfall showed larger gonadal masses as related to distance from the outfall. Osenberg et al. [2] did not, however, measure subsequent performance of gametes or embryos. They also did not measure the physiological state of the gonads, and it remains possible that in their study mussels caged nearer the outfall became ripe and spawned in the field before recovery. It is unlikely that sea urchins in this study spawned in the field because the animals were spent before being outplanted and they were subjected to a relatively short (eight-week) exposure time. If the experimental animals had spawned in the field, it is unlikely that even moderate quantities of ripe gametes would have been recovered from those individuals. After the exposure period urchins caged 1,000 m away from the produced water outfall released gametes with the highest performance (Fig. 5) yet exhibited the smallest gonads. This would indicate that these individuals had not spawned out in the field before recovery. These results suggest that increased gonad size, and possibly increased gamete output, may compensate for decreased performance of gametes. The effective per-capita fertilization rates may be similar among sites because urchins that had the lowest gamete performance characteristics had the largest gonad masses.

Until recently, field studies of ecological effects resulting from the chronic discharge of produced waters have emphasized effects on the structure of benthic communities [32–35]. Many of these studies have addressed the spatial scale of effects by quantifying distributional patterns of infaunal benthic communities in relatively shallow embayments. Armstrong et al. [32] found that infaunal densities in Trinity Bay were depressed out to a distance of 1 km from the discharge point. Similarly, Rabalais et al. [35] found that benthic microfaunal communities in Louisiana coastal environments were affected between 500 m to 1 km from the discharge source. It is difficult to extrapolate these results to coastal discharges into open high-energy environments similar to those typically found in southern California. Dilution rates are probably higher, transport mechanisms different, and the general oceanography of the area may result in more localized effects [2,32,33].

Osenberg et al. [2] studied the spatial scale of effects on benthic organisms at the Carpinteria outfall and found that effects were limited to areas close (<100 m) to the outfall. However, they also found that effects on the growth of outplanted mussels were detectable to between 100 m and 1 km [2]. Raimondi and Schmitt [3] found that effects on swimming and settlement performance of abalone larvae occurred over a similar spatial scale at the same site. These studies indicate that effects from produced water discharges in open coastal environments may not be as localized as previously believed. Results presented here indicate that effects on sea urchin gametogenesis and gamete performance are distributed over a similar spatial scale. The observed decrease in egg fertilizability was most pronounced at the 5-m station, with only about 38% of the eggs capable of being fertilized. This station also showed the lowest sperm attack rates. The effects on fertilization kinetics increased linearly with log(distance) out to roughly 80 m from the outfall (Fig. 5). The effect continued to be measurable out to at least 100 m, although the magnitude of effect lessened with distance after 80 m. As there were no cages between 10 and 1,000 m, it was impossible to determine the exact extent of the effect. At the furthest site (1,000 m) fertilization kinetics were more normal, with 100% of eggs fertilizable and sperm attack rates the highest measured.

Recent lab studies have shown that produced waters from

the Carpinteria outfall may exhibit a variety of ecologically important sublethal effects. Krause et al. [6] demonstrated that exposure of purple sea urchin gametes and zygotes significantly decreased fertilization rates and slowed embryonic development rates. Reed and Lewis [4] found that brief exposures of giant kelp (*M. pyrifera*) spores resulted in decreased swimming rates. Raimondi and Schmitt [3] have similarly shown that when red abalone (*Haliotis rufesens*) larvae are exposed to produced water, they stop swimming. Despite these recent studies, lab investigations of produced water toxicity have been difficult because of the complex nature of the toxicant and highly variable composition of chemical components [1,12,33]. Such lab toxicity tests are usually limited to a relatively short (<96 h) period because of problems associated with loss of constituents through volatilization, adsorption, or biological breakdown [36]. Although these relatively short-term toxicity tests are useful for elucidating immediate biological responses to environmental pollutants, longer term exposure studies are needed to identify chronic or sublethal responses [34,36].

This study was essentially a long-term bioassay maintained in the natural environment. The use of such in situ investigations is essential to the further understanding of the effects of chronic exposures of anthropogenic inputs to environmental systems. This type of study does not suffer from the disadvantages of testing for responses in an artificial system in a lab. In the field, organisms are exposed to naturally variable effluent concentrations at environmentally realistic levels. They can be maintained in the field for long periods, and many ecologically important species are suitable as study organisms. The general design presented here can be expanded in both time and space. Biological data from such in situ studies can not only provide new insights into the spatial and temporal distribution of ecological impacts, but also, combined with specific physical and chemical effluent data, may help determine the mechanism of the toxic responses found in the field.

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REFERENCES

1. **Neff, J.M., N.N. Rabalais** and **D.F. Boesch.** 1987. Offshore oil and gas development activities potentially causing long-term environmental effects. In D.F. Boesch and N.N. Rabalais, eds., *Long Term Environmental Impacts of Offshore Oil and Gas Development*. Elsevier, New York, NY, pp. 149–174.
2. **Osenberg, C.O., R.J. Schmitt, S.J. Holbrook** and **D. Canestro.** 1992. Spatial scale of ecological effects associated with an open coast discharge of produced water. In J.P. Ray and F.R. Englehardt, eds., *Produced Water: Technological/Environmental Issues*. Plenum, New York, NY, pp. 387–402.
3. **Raimondi, P.T.** and **R.J. Schmitt.** 1992. Effects of produced water on settlement of larvae: Field tests using red abalone. In J.P. Ray and F.R. Englehardt, eds., *Produced Water: Technological/Environmental Issues*. Plenum, New York, NY, pp. 415–430.
4. **Reed, D.C.** and **R.J. Lewis.** 1992. Water column effects of produced water discharges on the production and dispersive potential of giant kelp (*Macrocystis pyrifera*). *Abstracts*, International Produced Water Symposium, San Diego, CA, February 4–7, p. 5.
5. **Fan, T.W.-M., R.M. Higashi, G.N. Cherr** and **M.C. Pillai.** 1992. Use of noninvasive NMR spectroscopy and imaging for assessing produced water effects on mussel reproduction. In J.P. Ray and F.R. Englehardt, eds., *Produced Water: Technological/Environmental Issues*. Plenum, New York, NY, pp. 403–414.
6. **Karuse, P.R., C.O. Osenberg** and **R.J. Schmitt.** 1992. Effects of produced water on early life stages of a sea urchin: Stage specific responses and delayed expression. In J.P. Ray and F.R. Englehardt, eds., *Produced Water: Technological/Environmental Issues*. Plenum, New York, NY, pp. 431–444.
7. **Vogel, H., G. Czihak, P. Chang** and **W. Wolf.** 1982. Fertilization kinetics of sea urchin eggs. *Math. Biosci.* **58**:189–216.
8. **Morris, R.H., D.P. Abbot** and **E.C. Haderlie.** 1980. *Intertidal Invertebrates of California*. Stanford University Press, Stanford, CA.
9. **Strathman, M.F.** 1987. Phylum Echinodermata, Class Echinoidea. In M.F. Strathman, ed., *Reproduction and Development of the Marine Invertebrates of the Northern Pacific Coast: Data and Methods for the Study of Eggs, Embryos, and Larvae*. University of Washington Press, Seattle, WA, pp. 511–534.
10. **Rowley, R.J.** 1989. The settlement, recruitment, and early growth and mortality of the purple sea urchin (*Strongylocentrotus purpuratus*), and the red sea urchin (*S. franciscanus*) in a kelp bed and urchin barren ground. Ph.D. thesis. University of California, Santa Barbara, CA.
11. **Lehay, P.S., T.C. Tutschulte, R.J. Britten** and **E.H. Davidson.** 1978. A large-scale laboratory maintenance system for gravid purple sea urchins (*Strongylocentrotus purpuratus*). *J. Exp. Zool.* **204**:369–380.
12. **Higashi, R., G. Cherr, C. Bergens** and **T. Fan.** 1992. An approach to toxicant isolation from a produced water source in the Santa Barbara Channel. In J.P. Ray and F.R. Englehardt, eds., *Produced Water: Technological/Environmental Issues*. Plenum, New York, NY, pp. 223–234.
13. **Dinnel, P.A., J.M. Link** and **Q.J. Stober.** 1987. Improved methodology for sea urchin sperm cell bioassay in marine waters. *Arch. Environ. Contam. Toxicol.* **16**:23–32.
14. **Levitan, D.R., M.A. Sewell** and **F.-S. Chia.** 1991. Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: Interaction of gamete dilution, age, and contact time. *Biol. Bull.* **181**:371–378.
15. **SAS Institute.** 1988 *SAS®/STAT User's Guide, Release 6.03 Edition*. Cary, NC.
16. **Giudice, G.** 1986. *The Sea Urchin Embryo: A Developmental Biological System*. Springer-Verlag, Berlin, West Germany.
17. **Kenaga, E.E.** 1980. Correlation of bioconcentration factors of chemicals in aquatic and terrestrial organisms with their physical and chemical properties. *Environ. Sci. Technol.* **35**:213–219.
18. **Brown, D.A., R.W. Gossett, G.P. Hershelman, H.A. Schafer, K.D. Jenkins** and **E.M. Perkins.** 1983. Bioaccumulation and detoxification of contaminants in marine organisms from southern California coastal waters. In D.F. Soule and D. Walsh, eds., *Waste Disposal in the Oceans*. Westview Press, Boulder, CO, pp. 171–193.
19. **Brown, M.P., M.B. Werner, R.J. Sloan** and **K.W. Simpson.** 1985. Polychlorinated biphenyls in the Hudson River. *Environ. Sci. Technol.* **19**:656–661.
20. **Aguilar, A.** and **A. Borrell.** 1988. Age- and sex-related changes in organochlorine compound levels in fin whales (*Balenoptera*

physalus) from the eastern North Atlantic. *Mar. Environ. Res.* **25**:195–211.
21. **Larsson, P., L. Okla** and **L. Collvin.** 1993. Reproductive status and lipid content as factors in PCB, DDT, and HCH contamination of a population of pike (*Esox lucius* L.). *Environ. Toxicol. Chem.* **12**:855–861.
22. **Linden, O.** 1976. Effects of oil on the reproduction of the amphipod *Gammarus oceanicus*. *Ambio* **5**:36–37.
23. **Hogan, J.W.** and **J.L. Brauhn.** 1975. Abnormal rainbow trout fry from eggs containing high residues of PCB (Aroclor 1242). *Prog. Fish. Cult.* **37**:229–230.
24. **von Westernhagen, H.R., V. Dethlefsen, W. Ernest, U. Harms** and **P.D. Hansen.** 1981. Bioaccumulating substances and the reproductive success in Baltic flounder *Platichthyes flesus*. *Aquat. Toxicol.* **1**:85–99.
25. **Daniels, C.B.** and **J.C. Means.** 1989. Assessment of the genotoxicity of produced water discharges associated with oil and gas production using a fish embryo and larval test. *Mar. Environ. Res.* **28**:303–307.
26. **Ratcliffe, D.A.** 1967. Decrease in egg shell weight in certain birds of prey. *Nature* **215**:208–210.
27. **Britt, J.O.** and **E.B. Howard.** 1983. Tissue residues of selected environmental contaminants in marine mammals. In E.B. Howard, ed., *Pathobiology of Marine Mammal Diseases*. CRC, Boca Raton, FL, pp. 47–67.
28. **Foltz, K.R., J.S. Partin** and **W.J. Lennarz.** 1993. Sea urchin egg receptor for sperm: Sequence similarity of binding domain and hsp 70. *Science* **259**:1421–1425.
29. **Vaquier, V.D.** 1979. The fertilizing capacity of sea urchin sperm rapidly decreases after induction of the acrosome reaction. *Growth and Differ.* **21**:61–69.
30. **Timourian, H.** and **G. Watchmaker.** 1970. Determination of spermatozoan motility. *Dev. Biol.* **21**:62–72.
31. **Garman, G.** and **G.H. Cherr.** 1990. Effect of produced water on mussel development. *Proceedings*, 11th SETAC Conference, Arlington, VA, Nov. 11–15, p. 186.
32. **Armstrong, H.W., K. Fucik, J. Anderson** and **J.M. Neff.** 1979. Effects of oilfield brine effluent on sediments and benthic organisms of Trinity Bay, Texas. *Mar. Environ. Res.* **2**:55–69.
33. **Middleditch, B.S.** 1984. Ecological effects of produced water discharges from offshore oil and gas production platforms. API Project 248. Final Report. American Petroleum Institute, Washington, DC.
34. **Neff, J.M.** 1987. Biological effects of drilling fluids, drill cuttings and produced waters. In D.F. Boesch and N.N. Rabalais, eds., *Long-Term Environmental Impacts of Offshore Oil and Gas Development*. Elsevier, New York, NY, pp. 469–538.
35. **Rabalais, N.N., B.A. McKee, D.J. Reed** and **J.C. Means.** 1992. Fate and effects of produced water discharges in coastal Louisiana, Gulf of Mexico, USA. In J.P. Ray and F.R. Englehardt, eds., *Produced Water: Technological/Environmental Issues*. Plenum, New York, NY, pp. 355-370.
36. **Capuzzo, J.M.** 1987. Biological effects of petroleum hydrocarbons: Assessments from experimental results. In D.F. Boesch and N.N. Rabalais, eds., *Long-Term Environmental Effects of Offshore Oil and Gas Development*. Elsevier, New York, NY, pp. 343–410.

B. Krause, P.R. 1995. Spatial and temporal variability in receiving water toxicity near an oil effluent discharge site. *Archives of Environmental Contamination and Toxicology* **29**:523-529.

Spatial and Temporal Variability in Receiving Water Toxicity Near an Oil Effluent Discharge Site

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Abstract. The distribution of point-source pollutants in the field can vary in both time and space. This study examined spatial and temporal patterns of toxicity from a produced water (an oil production effluent) discharge plume using a sea urchin fertilization toxicity test. Specifically, the sensitivity and response patterns of sea urchin gametes exposed to receiving waters sampled along a 1 Km transect near an active produced water outfall were tested. Fertilization success varied significantly with proximity to the outfall, with reduced fertilization found closer to the outfall. Although toxicity in receiving water samples, based on fertilization success, was variable in time—perhaps responding to variation in the quantity or make-up of produced water discharges—the general spatial pattern of toxicity along the transect remained relatively constant. The discharge plume was well established in the westerly direction throughout the experimental period. Toxicity data from samples of effluent and receiving waters, which were collected simultaneously, were used to determine the effective plume concentrations of produced water at seven sampling stations along a 1 km transect down-field from an active outfall. Strong evidence that field toxicity was directly attributable to the presence of produced water was provided by sampling the discharge plume during a period while the produced water discharge was not operating. During this period, no toxicity was found at any of the field sites.

During oil production, large quantities of aqueous waste effluents are generated. Water pumped from oil formations, along with water associated with the production process, must be separated from the oil product and discarded. These aqueous effluents, commonly called "produced waters," contain a diverse array of contaminants including hydrocarbons, heavy metals, and chemical additives such as surfactants and corrosion inhibitors. In offshore or near-shore oil operations, these effluents are often discharged directly into the marine environment. Produced water has been identified as the single largest waste stream associated with the exploration and production of oil (Neff *et al.* 1987). For example, Reilly *et al.* (1991) pointed out that in 1990, oilfield operations in the Gulf of Mexico generated over 850 million barrels (1 bbl = 159 L) of produced water effluents. With such large volumes being discharged, the fate and effects of released produced water have become significant environmental issues. It has been suggested recently that there is a need for a better understanding of the spatial and temporal distribution of impacts from chronic discharges of produced water (Neff *et al.* 1987; Payne *et al.* 1987; Osenberg *et al.* 1992; Rabalais *et al.* 1992). This is true especially for effects associated with the water column.

Until recently, there have been few investigations into the fate or effects of produced water discharges in the water column (Gamble *et al.* 1987; Neff 1987; Payne *et al.* 1987; Spies 1987). This was, in part, because it was thought that the ocean discharge of produced waters would result in rapid dilution of the effluent to relatively non-toxic levels (Lysyj 1981; Middleditch 1984; Rose and Ward 1981; Payne *et al.* 1987). However, recent field studies have suggested that significant ecological impacts can be detected in the water column, and that impacts may occur over a relatively large spatial scale (Krause 1994; Osenberg *et al.* 1992; Raimondi and Schmitt 1992). For example, Krause (1994) found that caged sea urchins (*Strongylocentrotus purpuratus*) were reproductively impaired up to 100 m from an outfall. In a similar study, Osenberg *et al.* (1992) found that growth rates and reproductive performance of mussels (*Mytilus californicus* and *M. edulis*) caged in the water column were depressed at distances between 100 m and 1 km from an active discharge. Raimondi and Schmitt (1992) also found that swimming behavior, settlement, and metamorphosis of larval abalone (*Haliotis rufescens*) were impaired over a similar spatial scale.

Effects in the water column may also be important because many marine species spend part or all of their life cycle in the water column. For many marine organisms, gametes are released directly into the water column where they undergo syngamy to form zygotes. These early life stages may be more sensitive to toxicants than adults (Capuzzo 1987; Neff 1987; Krause *et al.* 1992), and previous work has shown that toxicity testing procedures that utilize early life stages are capable of

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detecting produced water toxicity at low levels (Krause *et al.* 1992). In this investigation, a series of experiments were performed to identify: (a) the sensitivity of sea urchin gametes to toxicity within the receiving waters; (b) the spatial and temporal variation in toxicity within the receiving waters; (c) the variability of toxicity within the produced water effluent itself; and (d) to explore the origin of the toxic responses elicited by receiving water samples. In addition to these objectives, further experiments were conducted to derive an estimate of produced water concentrations within the discharge plume found in the field.

Materials and Methods

Study Site

The study site was located near Carpinteria, CA, USA (34°23′N, 119°30′W), approximately 20 Km east of Santa Barbara, CA. The general physical environment has been described previously (Osenberg *et al.* 1992; Krause 1994). Briefly, the produced water was discharged from an on-shore processing facility through a diffuser pipe located approximately 200–300 m offshore at a depth of about 11–12 m. The diffuser was made up of 10 T-shaped discharge ports along the final 25 m of the pipe. The specific design of the diffusers on the outfall provided a final produced water dilution of 125:1 (0.8%; CRWQCB NPDES Permit #CA0000230). Throughout this study, the outfall discharged approximately 16,000 bbl/d (2.6×10^6 L/d) of produced water. The discharge ports were oriented perpendicular to the pipe and rose about 0.75 m above the bottom. The predominant long-shore currents in the region flow from the east of the diffuser and parallel to the shoreline (Osenberg *et al.* 1992). The chemical characteristics of the effluent have been summarized by Higashi *et al.* (1992) and the complex effluent contains both metal and organic toxicants.

Sample Collection and Handling

Receiving Water: To determine both the spatial and temporal patterns of toxicity in the field, and the relative sensitivities of the early life stages of the purple sea urchin to receiving waters, samples of receiving water were collected by SCUBA divers around the produced water discharge during times of effluent release. Receiving water samples were collected at five field stations located 5, 10, 100, and 1,000 m to the west of the discharge, and a reference station located 1000 m to the east of the outfall. On three separate dates, two replicate samples of receiving water were collected from approximately 1 m above the substrate (the depth of the diffuser ports) into clean 0.5 L amber glass bottles and sealed with Teflon-lined lids. On two dates, sampling was expanded to include additional stations at 15, 20, and 30 m, to the west of the discharge.

After collection, all bottles were kept on ice in the dark, and returned to the laboratory for immediate processing to determine the toxicity in the samples. Toxicity experiments were begun within approximately 6 h of collection. All sample bottles were pre-cleaned using 20% HCl and rinsed in de-ionized water prior to use in the field. Upon return to the laboratory, all samples were filtered through Whatman-40 paper filters to remove gross (8 μm) particulates. Replicate bottles of sterile filtered (0.45 μm) sea water were used as a control and diluent. The control sea water was also passed through paper filters to control for filter effects. Previous studies have shown that filtration does not influence the measured toxicity using the fertilization assay (Krause 1994). Salinity was measured to the nearest 1 ppt on all samples using a Bausch and Lomb refractometer.

Produced Water Effluent: Samples of produced water effluent were collected from the oil processing facility on four separate dates. On three dates, three replicate samples were collected from an onshore test spigot located on the discharge pipe just after the effluent leaves the final settling tank, and immediately prior to ocean discharge. Produced water was collected without head space into pre-cleaned amber glass bottles and sealed with Teflon-lined lids. On one date, three replicate samples of produced water were collected from a subsurface discharge port. These samples were collected by a SCUBA diver directly from the discharge port during the active release of produced water from the outfall. Divers used a sampling device connected to the discharge port to ensure collection of undiluted effluent. After collection, all samples were kept on ice in the dark, and returned to the laboratory for immediate processing to determine toxicity. All samples and control sea water were passed through paper filters to remove gross (8 μm) particulates, and the salinity was recorded.

Toxicity Determinations

All toxicity determinations were made by a sea urchin fertilization test described by Krause *et al.* (1992). For each experiment, gametes of the purple sea urchin (*Strongylocentrotus purpuratus* Stimpson) were collected from 4–6 adults of each sex following intracoelomic injections of 0.5 M KCl. Urchins used in all experiments were collected from a subtidal rocky reef near Santa Barbara, California and maintained in large, continuous flow seawater tanks. Both gamete types were collected separately in plastic centrifuge tubes and stored on ice. All toxicity tests were conducted no later than 1 h after gamete collection. Previous studies have shown that this delay between spawning and use does not significantly alter the viability of eggs or sperm (Krause *et al.* 1992).

To facilitate comparisons among experiments and to avoid polyspermy, fertilization rates were standardized by adjusting the ratio of sperm to eggs (Cherr *et al.* 1987; Dinnel *et al.* 1987; Krause *et al.* 1992). Prior to each experiment, batches of eggs were flooded with varying amounts of stock sperm solution to establish the relationship between fertilization success and the ratio of sperm to eggs. Each experiment was then conducted at the sperm to egg ratio that produced 90% fertilization in the filtered sea water control. All fertilization test incubations were conducted in 15 ml plastic Falcon multi-well culture dishes, and were conducted in a cold (15°C) room on a continuous shaker table (15 oscillations per min). In all experiments, three separate subsamples of each of the two replicate samples were tested, and statistical analyses were made using the mean of the subsamples as the test measure.

The toxicity test method involved a brief (10 min) pre-fertilization exposure of both eggs and sperm to the given test solution (receiving water, effluent, or control seawater), followed by co-incubation in the test solution (Krause *et al.* 1992). Ten-minute gamete exposures were accomplished by adding sperm (or eggs) to a glass test tube containing a given test solution. Following the 10 min exposures, gametes were incubated together in 10 mls of test solution for 25 min. After the 25 min co-incubation period, the samples were fixed with 10% formalin, and fertilization success was determined under a microscope.

Salinity of all samples, including diluted produced water effluents, showed salinites between 30 ppt and 32 ppt. The control seawater used throughout the experiment had a salinity of 32 ppt. Since previous experiments (Krause 1992, 1994) have shown that the fertilization test method used here is not sensitive to changes in salinity of this magnitude, salinity adjustments were not made to the test solutions.

Relative Sensitivity of Early Life Stages: The first experiment was designed to determine the relative sensitivity of sea urchin early life stages to toxicants in receiving waters. This was a cross-designed experiment in which receiving water distance (e.g., 5, 10, 100, 1000 m west, and 1000 m east) was crossed with a second factor designating the life stage(s) that was exposed (e.g. eggs only, sperm only, or a combination of eggs and sperm). This experiment was conducted using

one set of receiving water samples collected on August 6, 1991. A control treatment was included and consisted of life stages exposed to filtered sea water rather than receiving waters, but otherwise handled identically to the other treatments.

Spatial and Temporal Variation in Toxicity: The second experiment was designed to determine the spatial and temporal variation in toxicity in receiving water samples. The basic design of this experiment was similar to the early life stage sensitivity experiments, except that it incorporated additional samples collected over time along the same spatial gradient and a minor modification to the protocol. Samples for this experiment were collected on September 6, 1991, March 19, 1992, and May 13, 1992. Therefore, along with data from the August 6, 1991 sample, this experiment examined a total of four sets of receiving water samples collected over a two-year period. The experimental protocol from the sensitivity experiment was modified to include only one treatment in which both eggs and sperm were exposed to the given test solutions. This modification was made because it was determined that this exposure provided the best resolution of effects.

To determine if the temporal variation in toxicity of the receiving waters was similar to temporal variation in toxicity of the produced water effluent, the fertilization experiment was repeated with five effluent samples. Effluent was tested at a dilution concentration of 1%. These samples were collected on February 22, 1991, May 15, 1991, July 1, 1991, and March 11, 1992.

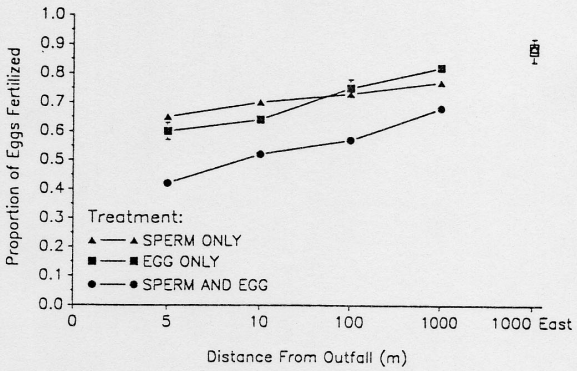

Fig. 1. Fertilization success of gametes exposed for 10 min to receiving water samples collected at a range of distances from an active produced water outfall. Data are mean (± 1 SE, n = 2) proportion of eggs fertilized after 25 minute incubations. For "Egg" and "Sperm" treatments, either the eggs or sperm were first exposed, then incubated in filtered sea water before fertilization was measured. For the "Egg and Sperm" treatment, fertilization and incubation occurred in the given receiving water test solution. Open symbols represent samples collected at a reference site to the east of the outfall

Estimated Effluent Concentrations in Receiving Waters

The receiving water and effluent samples collected on May 13, 1992 presented a unique opportunity to further explore the relationship between receiving water and effluent toxicity. Since both the receiving water and effluent samples were collected simultaneously in the field, the receiving waters represented an actual field dilution of the effluent sample. To determine the fertilization response pattern over a probable dilution range in effluent, each replicate of undiluted produced water was diluted with filtered seawater to obtain nine final effluent concentrations of 10, 5, 1, 0.25, 0.1, 0.01, 0.001, 0.0001, and 0%. A fertilization response pattern to these effluent dilutions was determined using the toxicity test described above. The relationship between the proportion of eggs fertilized and (log) produced water concentration was estimated by fitting the data to a quadratic polynomial function using the GLM procedure of SAS (SAS Institute 1988). To eliminate the undefinable log of zero, all produced water concentrations were augmented by 0.00001% before taking the log.

A one-dimensional plume map of "effective" produced water concentrations was developed using the toxicity data from the May 13, 1992 receiving water stations and the fertilization response curve from the same date. The "effective concentration" is defined as that effluent concentration that will elicit the same level of response as is observed in a given receiving water sample. The mean proportion of eggs fertilized at each of the replicate receiving water samples was used to estimate the "effective concentrations" at each of the receiving water stations by back-estimating from the fertilization response curve.

Origin of Effects in Receiving Waters

A final experiment was designed to establish that the pattern of toxicity present in the receiving water samples was actually attributable to the presence of produced water, and not some natural spatial gradient independent of the discharge. Another set of receiving water samples was collected and tested on October 29, 1993, during a period when produced water was not being discharged, and had not been discharged for over 4 weeks (J. Wallace, Carpinteria Oil and Gas Plant, pers. commun.). These samples were collected in the same manner as above, and were from the same five sampling stations. The protocol for this experiment was identical to that of the variability study above.

In general, results of each experiment were analyzed by analysis of variance. Comparisons with the control treatment were made using Dunnett's two-tailed t test, and *a posteriori* comparisons among treatment main effects were made using the Ryan-Elinot-Gabriel-Welsch multiple F test (REGWF: SAS 1988). All proportional fertilization data were arcsine-square root transformed prior to analysis.

Results

Sensitivity of Early Life Stages

Fertilization Assay: Exposure of sea urchin gametes to receiving waters resulted in statistically significant depressions in the fraction of successful fertilization (Figure 1; $F_{4,15} = 212.76$; $P < 0.0001$). The magnitude of depression of fertilization increased with proximity to the outfall (Figure 1), although a substantial fraction of eggs (> 50 %) were still fertilized in samples from the closest station tested (5 m). Fertilization success was significantly reduced compared to controls (Dunnett's test, $P < 0.05$) in receiving waters from all stations to the west of the outfall, and depressed by as much as 10–20% in samples collected up to 1000 m from the outfall. Fertilization success at the reference station 1000 m east of the outfall was not significantly different from the sea water control (Dunnett's test, $P > 0.05$).

Gamete treatments manipulated in the fertilization assay responded differently to exposure with receiving waters (Figure 1; $F_{2,15} = 95.29$; $P < 0.0001$). *A posterori* comparison of main effects show that pre-fertilization exposures of sperm gave similar results to those of egg exposures, but exposures of a combination of both eggs and sperm resulted in significantly lower fertilization success. In other words, based on fertilization success averaged across station distance, the sensitivity of sperm was about the same as that of eggs, while the greatest effect was

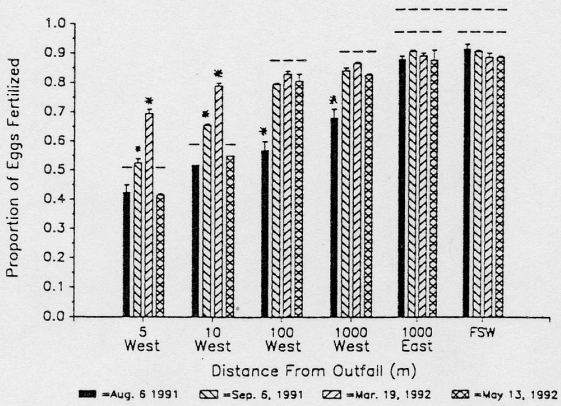

Fig. 2. Fertilization success in receiving waters collected on four dates around an active produced water outfall. The data are mean (± 1 SE, n = 2) proportion of eggs fertilized following a 10 min pre-fertilization exposure of eggs and sperm, and a 25 min incubation in the respective receiving water test solution. FSW treatments represent controls in which gametes were exposed to filtered sea water only. Asterisks (*) indicate significantly different treatments at a = 0.05 using REGWF (see methods). Bars (---) connect treatments that were not significantly different

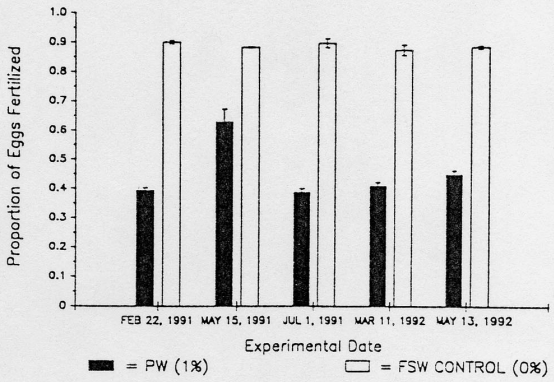

Fig. 3. Fertilization success in produced water collected on five dates. The data are mean (± 1 SE, n = 2) proportion of eggs fertilized following a 10 min pre-fertilization exposure of eggs and sperm, and a 25 min incubation in the respective produced water test solution. Treatments are in 1% produced water, or filtered sea water (FSW) controls

observed when both eggs and sperm were exposed. There was a significant interaction between the gamete type and distance from the diffuser (Figure 1; $F_{8,15} = 8.15$; $P < 0.0005$), suggesting that sperm may be more sensitive at greater distances (lower toxicant levels), while eggs may be relatively more sensitive closer to the outfall (higher toxicant levels; Figure 1).

Spatial and Temporal Variation in Toxicity

Receiving Water: Toxicity based on fertilization success was similar in scope to that found above, with greater toxicity found in samples collected closer to the outfall than from farther away on dates when samples were collected during active discharge of produced water (Figure 2; $F_{5,24} = 357.71$; $P < 0.0001$). The observed spatial pattern of toxicity was similar in extent on all four sampling dates. For example, statistically significant toxicity was measured from samples collected up to 1 Km away from the diffuser on all dates (Figure 2; Dunnett's test, $P < 0.05$), and toxicity was never observed in any samples collected at the reference station 1 Km to the east of the outfall (Figure 2; Dunnett's test, $P < 0.05$).

Fertilization success in receiving water samples exhibited a significant temporal component (Figure 2; $F_{3,24} = 96.26$; $P < 0.0001$), and there was a significant interaction between sampling date and distance from the outfall (Figure 2; $F_{15,24} = 17.92$; $P < 0.0001$); the plume toxicity varied from date to date, and exhibited different magnitudes of variation. For example, all water samples collected at sampling stations to the west of the outfall on August 6, 1991 described a gradient of relatively high toxicity. However, the receiving waters collected on September 6, 1991, March 19, 1992, and May 13, 1992 showed toxicity gradients that were stronger closer to the outfall than at greater distances (Figure 2).

Produced Water: Analysis of fertilization success in five samples of produced water effluent. revealed that the effluent too, can be variable on a temporal scale (Figure 3; $F_{4,20} = 10.95$; $P < 0.0001$). This pattern was driven primarily by the sample collected on May 15, 1991. Analysis of main effects showed that toxicity on this date was significantly lower than the rest of the samples, which were not different from each other. This resulted in a significant interaction between sampling date and treatment (Figure 3; $F_{4,20} = 15.01$; $P < 0.0001$). There was no difference in fertilization success among the control treatments (Figure 3; Dunnett's test, $P > 0.05$).

Estimated Effluent Concentrations in Discharge Plume

The exposure of sea urchin gametes to produced water dilutions collected May 13, 1992 resulted in a typical fertilization dose-response curve over the range of dilutions tested (Figure 4A). At the highest produced water concentration tested (10%), only about 20% of the eggs became fertilized, while at the lowest produced water concentration tested (0.0001%), fertilization was reduced by as much as 10% (Figure 4A) compared to the control values (Dunnett's test, $P > 0.05$). The relationship between the proportion of eggs fertilized and (log) produced water concentration fit the following quadratic function (Figure 2; $r^2 = 0.968$):

$$F = -0.015\,X^2 - 0.163\,X + 0.429$$

where F is the proportion of eggs fertilized and X is the log of the transformed produced water concentration.

As indicated above, the fertilization test revealed that samples of receiving waters collected May 13, 1992 varied in toxicity with distance from the outfall, with the lowest fertilization values being found in samples closer to the outfall (Figure 4B). Effective concentrations of produced water, as back-estimated values from the above quadratic data fit, showed decreasing effluent concentrations with distance from the outfall (Figure 4C). The one-dimensional map of effective produced water concentrations revealed that the effluent was detectable in

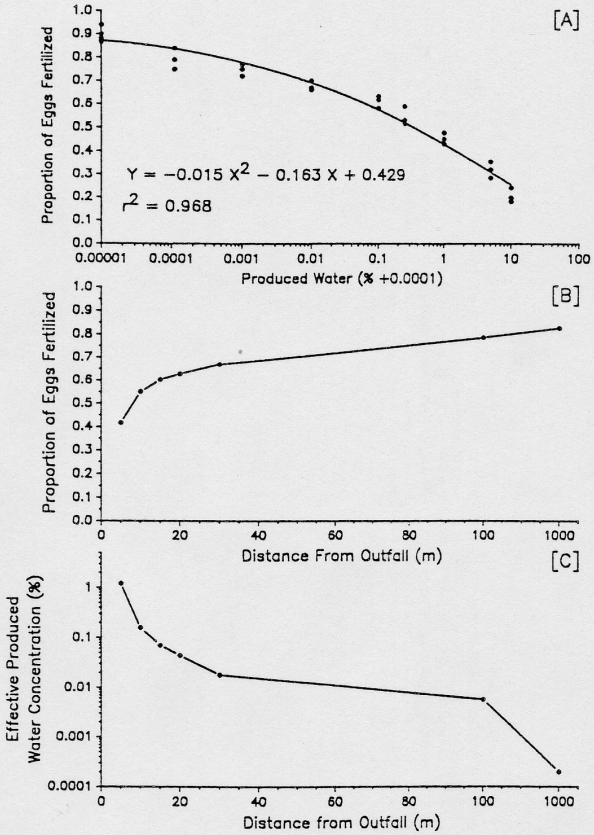

Fig. 4. [A] Fertilization response curve of gametes exposed for 10 min to a range in concentrations of produced water collected May 13, 1992 at the Carpinteria outfall. Data are proportion of eggs fertilized after 25 min co-incubations of eggs and sperm in each of three replicate samples of produced water. Data were fit to the quadratic expression. [B] Fertilization success of gametes in receiving water samples collected May 13, 1992 to the west of the Carpinteria outfall. Data are mean proportion of eggs fertilized after 25 minute incubations of gametes in receiving water samples. [C] One-dimensional plume map of effective produced water concentrations in the discharge around an active outfall as determined from receiving water toxicity (see Methods)

relatively dilute amounts at distances up to 1 Km from the discharge (Figure 4C). Effective concentrations at the 1000 m site (down-current from the source) were estimated to be approximately 2 ppm (0.0002%).

The undiluted produced water sample had a characteristically low salinity of 17 ppt. Analysis of receiving water samples indicated that salinity was depressed near the outfall (26 ppt at 1 m), probably resulting from mixing of effluent and entrained sea water. Salinity measurements in samples ≥ 10 m away from the outfall were indistinguishable from salinity measured in samples of control filtered sea water (32 ppt).

Toxicity of Receiving Waters in the Absence of Discharge

Receiving water samples collected at a time when produced water was not being discharged on October 29, 1992 showed no pattern of effect on fertilization success with distance (Figure 5;

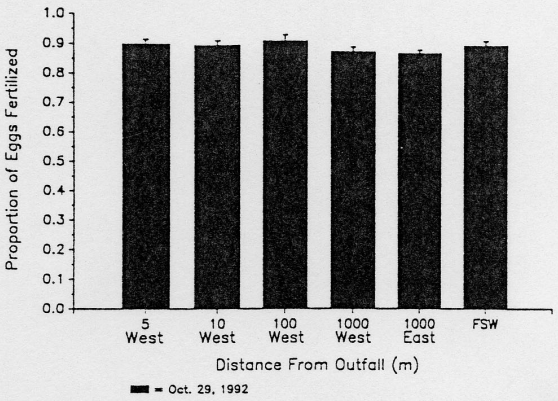

Fig. 5. Fertilization success in receiving water collected October 29, 1992, during a period when there was no active discharge of produced water. Data are mean (± 1 SE, n = 2) proportion of eggs fertilized following a 10 min pre-fertilization exposure of eggs and sperm, and a 25 min incubation in the respective receiving water test solution

$F_{5,6} = 1.16$; $P > 0.42$). Indeed, there was no detectable difference between any of the observed frequencies of fertilization and frequencies observed from the filtered sea water controls (Dunnett's test, $P > 0.05$ for all pairwise comparisons). In all receiving water samples, nearly 90% of all eggs became fertilized.

Discussion

The fertilization test provided a sensitive measure of toxicity in both receiving water samples and produced water effluents. The fertilization assay showed that eggs and sperm were of similar sensitivity, but the most sensitive treatment was one in which both eggs and sperm were exposed to toxicant (Figure 1). These results were both qualitatively and quantitatively similar to those reported earlier using produced water effluents (Krause *et al.* 1992). The resolution of this short-term test was great enough to detect differences, not only among receiving water samples collected at various distances from the outfall, but also among samples collected on different dates. Although longer term toxicity tests, such as embryo development assays, may be more sensitive (Kobayashi 1980; Carr 1993) and appropriate for detecting effects at the ecological level or investigating the mechanisms of toxicity (Krause *et al.* 1992), the fertilization test provides a relatively quick and reliable comparison of toxicity.

Results of this study show that toxicity in waters receiving produced water effluents can be variable in both space and time. The plume of discharged produced water at the Carpinteria discharge site was always found to the west of the outfall in the direction of the prevailing long-shore current. There was no evidence of the discharge at the eastern reference station, where water samples never displayed toxicity on any sampling date. Furthermore, fertilization success data for this station was not different from the controls, again indicating the lack of toxicity to the east of the outfall. In addition, a gradient of toxicity occurred down current as receiving waters were more toxic

closer to the outfall than farther away. However, the magnitude of this gradient varied in time (Figure 2).

The fertilization response of sea urchin gametes provided a sensitive measure to compare toxicity values between known effluent concentrations and those observed from the field. This study represents the first attempt to generate a distributional map of effluent concentrations based solely on toxicity determinations (Figure 4). It provided a good indicator of effluent diffusion throughout the westerly discharge gradient. The design of the Carpinteria outfall's diffuser is such that at the edge of the zone of initial dilution, the effluent would be diluted with entrained seawater in a ratio of 1:125 (0.8%; CRWQCB NPDES Permit #CA0000230). The results of this study show that the effective concentrations of this magnitude are indeed located within the region extending 5 m from the diffuser (Figure 4C).

Causation of Observed Field Effects

Although the possibility always remains that natural environmental gradients may actually cause the observed patterns attributable to the discharge (Raimondi and Schmitt 1992), several lines of evidence support the fact that the observed toxicity gradients were due to produced water in the water column. First, the gradients were consistently established to the west in the direction of the long-shore current (Osenberg *et al.* 1992; Krause 1994), while toxicity was never detected at the reference station 1 Km to the east (Figure 2). Second, the temporal variability of plume toxicity resembled the pattern of variation observed for toxicity in dilutions of produced water effluent (Figure 3). Several mechanisms could account for the observed patterns of temporal variability of plume toxicity in the field. Rates of effluent discharge are known to fluctuate in time (J. Wallace, Carpinteria Oil and Gas Plant, pers. commun.), while both the velocity and direction of local currents may vary among sampling dates and thereby alter dilution rates in the field. Third, effects observed in the field were always greatest near the outfall (Figure 2, 4B) and resembled those observed from dilutions of produced water effluents (Krause *et al.* 1992).

Nevertheless, the most compelling evidence that the presence of produced water effluents in the field caused toxicity in the receiving water samples was provided by the total lack of toxicity in samples collected during the period when the outfall was not discharging effluent (Figure 5). Receiving waters were toxic only when effluents were being discharged. This also indicates the transient nature of pollutant gradients in the water column of high energy environments such as that near the Carpinteria outfall. Receiving waters lost all traces of toxicity after only 4 weeks of non-operation (J. Wallace, Carpinteria Oil and Gas Plant, pers. commun.). This rapid recovery period suggests that the periodicity of discharge may be critical in determining ecological effects. For instance, if release of discharge occurs at the same time as spawning, or at times when high numbers of larvae are present in the water column, greater ecological impacts may be expected. Raimondi and Schmitt (1992) provided evidence that marine larvae exposed to produced water effluents in the vicinity of the Carpinteria outfall suffered decreased swimming, settlement, and metamorphosis, but that these sublethal effects were not evident during a similar period when the outfall was not operating. However, processes linking larval and benthic dynamics are poorly understood, and therefore the ecological significance of such sublethal effects on larvae are largely unknown (Capuzzo 1987).

Implications and Recommendations for Future Research

To date, few studies have characterized the behavior of plumes of discharged produced water (Payne *et al.* 1987). In order to fully understand the potential impacts associated with a point source of pollution, however, one must measure both the spatial and temporal variation of the discharge plume. Several studies have attempted to model discharge plumes emanating from point sources using a variety of dispersion models (Brandsma *et al.* 1992; Runchal 1983); with the conclusion that toxicant concentrations should be reduced to background levels within a few hundred meters of the source. However, none of these studies have attempted to describe discharge plumes by measuring toxicity directly.

Since produced waters are complex chemical mixtures (Middleditch 1984; Neff *et al.* 1992; Shepherd *et al.* 1992) and the fractionation and analysis of such complex mixtures is expensive and time consuming (Higashi *et al.* 1992; Middleditch 1984; Shepherd *et al.* 1992), it may be more practical to measure discharge plumes using short-term, relatively inexpensive toxicity tests. Because we are ultimately interested in describing the distribution of potential ecological impacts around pollution sources, the pattern of toxicity in the field may be a more reliable indication of pollution than the distribution of certain physical and chemical characteristics of the plume. Indeed, several recent studies have shown that the distribution of these physical and chemical parameters may not accurately predict the distribution of biological effects (Osenberg *et al.* 1992; Rabalais *et al.* 1992). Clearly, more data are needed to determine the nature and extent of such uncoupling of physical and biological parameters associated with pollutant discharges.

Data presented in this study demonstrate the potential of using toxicity data to describe a discharge plume. Future field studies should be planned that will incorporate actual samples of receiving water compared to diluted samples of the effluent collected over large spatial and temporal scales; the responses of sea urchin eggs and sperm to both receiving waters and effluent dilutions clearly indicates that used together, they adequately describe the pattern of "effective concentrations" of effluents in the field. Thus, effective concentration data from a sample array in space and time could be used efficiently to describe a discharge in detail and create probabilistic maps of toxicant distributions around point sources of pollution.

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References

Brandsma MG, Smith JP, O'Reilly JE, Ayers RC Jr, Holmquist AL (1992) Modeling offshore discharges of produced water. In: Ray JP Englehardt FR (eds) Produced water: Technological/ environmental issues. Plenum Publishing, NY, pp 59–71

Capuzzo JM (1987) Biological effects of petroleum hydrocarbons: Assessments from experimental results, In: Boesch DF, Rabalais NN (eds) Long-term environmental effects of offshore oil and gas development. Elsevier Applied Science, NY, pp 343–410

Carr RS (1993) Sediment quality assessment survey of the Galveston Bay System. Galveston Bay National Estuary Program publication GBNEP-30.

Cherr GN, Shenker JM, Lundmark C, Turner KO (1987) Toxic effects of selected bleached kraft mill effluent constituents on the sea urchin sperm cell. Environ Toxicol Chem 6:561–569

Dinnel PA, Link JM, Stober QJ (1987) Improved methodology for sea urchin sperm cell bioassay in marine waters. Arch Environ Contam Toxicol 16:23–32

Gamble JC, Davies JM, Hay SJ, Dow FK (1987) Mesocosm experiments on the effects of produced water discharges from offshore oil platforms in the northern North Sea. Sarsia 72:383–386

Higashi R, Cherr G, Bergens C, Fan T, Crosby D (1992) Toxicant isolation from a produced water source in the Santa Barbara Channel. In: Ray JP, Englehardt FR (eds) Produced water: Technological/environmental issues. Plenum Publishing, NY, pp 223–233

Kobayashi N (1980) Comparative sensitivity of various developmental stages of sea urchins to some chemicals. Mar Biol 58:163–171

Krause PR (1994) Effects of an oil production effluent on gametogenesis and gamete performance in the purple sea urchin (*Strongylocentrotus purpuratus* Stimpson). Environ Toxicol and Chem 13(7):1153–1161

Krause PR, Osenberg CW, Schmitt RJ (1992) Effects of produced water on early life stages of a sea urchin: Stage specific responses and delayed expression. In: Ray JP Englehardt FR (eds) Produced water: Technological/environmental issues. Plenum Publishing, NY, pp 431–444

Lysyj I (1981) Chemical composition of produced water in selected offshore oil and gas extraction operations. Rockwell International, Newbury Park, CA, USEPA Report

Middleditch BS (1981) Hydrocarbons and sulfur. In: Middleditch BS (ed) Environmental effects of offshore oil production: The Buccaneer gas and oil field study. Plenum Press, NY

Middleditch BS (1984) Ecological effects of produced water discharges from offshore oil and gas production platforms. Final report on API project No. 248, American Petroleum Institute

Neff JM (1987) Biological effects of drilling fluids, drill cuttings, and produced waters. In: Boesch DF, Rabalais NN (eds) Long term environmental impacts of offshore oil and gas development. Elsevier Applied Science, NY, pp 469–538

Neff JM, Rabalais NN, Boesch DF (1987) Offshore oil and gas development activities potentially causing long-term environmental effects. In: Boesch DF, Rabalais NN (eds) Long term environmental impacts of offshore oil and gas development. Elsevier Applied Science, NY, pp 149–174

Neff JM, Sauer TC, Maciolek N (1992) Composition, fate and effects of produced water discharges to nearshore marine waters. In: Ray JP, Englehardt FR (eds) Produced water: Technological/environmental issues. Plenum Publishing, New York, pp 371–385

Osenberg CW, Schmitt RJ, Holbrook SJ, Canestro D (1992) Spatial scale of ecological effects associated with an open coast discharge of produced water. In: Ray JP, Englehardt FR (eds) Produced water: Technological/environmental issues. Plenum Publishing, NY, pp 387–402

Payne JR, Phillips CR, Hom W (1987) Transport and transformations: Water column processes. In: Boesch DF, Rabalais NN (eds) Long term environmental impacts of offshore oil and gas development. Elsevier Applied Science, NY, pp 175–232

Rabalais NN, McKee BA, Reed DJ, Means JC (1992) Fate and effects of produced water discharges in coastal Louisiana, Gulf of Mexico, USA. In: Ray JP, Englehardt FR (eds) Produced water: Technological/Environmental issues. Plenum Publishing, NY, pp 355–369

Raimondi PT, Schmitt RJ (1992) Effects of produced water on settlement of larvae: Field tests using red abalone. In: Ray JP, Englehardt FR (eds) Produced water: Technological/environmental issues. Plenum Publishing, NY, pp 415–430

Reilly WK, O'Farrell T, Rubin MR (1991) Development document for 1991 proposed effluent limitation guidelines and new source performance standards for the offshore subcategory of the oil and gas extraction point source category. USEPA, Washington, DC

Rose CD, Ward TJ (1981) Acute toxicity and aquatic hazard associated with discharged formation water. In: Middleditch BS (ed) Environmental effects of offshore oil production. The Buccaneer gas and oil field study. Plenum Press, NY

Runchal AK (1983) An evaluation of effluent dispersion and fate models for OCS platforms. Vol 1: Summary and recommendations. Proc. of the Minerals Management Service Workshop on Discharges Modeling, 7-10 February, 1983, Santa Barbara, CA. Prepared for MMS by MBC Applied Environmental Sciences and Analytical and Computational Research Inc

SAS Institute Inc. (1988) SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute Inc., Cary, NC

Shepherd MC, Shore FL, Mertens SK, Gibson JS (1992). Characterization of produced waters from natural gas production and storage operations: Regulatory analysis of a complex matrix. In: Ray JP Englehardt FR (eds) Produced water: Technological/environmental issues. Plenum Publishing, NY, pp 163–173

Spies RB (1987) The biological effects of petroleum hydrocarbons in the sea: Assessments from the field and microcosms. In: Boesch DF, Rabalais NN (eds) Long term environmental impacts of offshore oil and gas development. Elsevier Applied Science, NY

C. Osenberg, C.W., R.J. Schmitt, S.J. Holbrook and D. Canestro. 1992. Spatial scale of ecological effects associated with an open coast discharge of produced water. Pages 387-402 *in* J.P. Ray and F.R. Englehardt (eds.), *Produced water: technological / environmental issues and solutions*, Plenum Publishing Corp., New York.

SPATIAL SCALE OF ECOLOGICAL EFFECTS ASSOCIATED WITH AN OPEN COAST DISCHARGE OF PRODUCED WATER

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INTRODUCTION

The biological effects of produced water are poorly understood (Neff, 1987). Not only are laboratory studies of toxicity impeded because of the complex and variable chemical composition of produced water (Middleditch, 1984; Higashi et al., 1992), but field assessments are complicated by other types of production activities that confound effects of produced water (Carney, 1987; Spies, 1987) . For example, most field studies of biological effects of "produced water" have compared biological samples collected at various distances from produced water outfalls associated with production platforms. Spatial patterns that are detected in such studies are difficult to interpret, because biological responses can result not only from effects of produced water, but also from discharges of other substances from the platform, or from physical effects of the platform itself. Field studies that examine effects of only produced water discharge are relatively uncommon.

Studies of Trinity Bay (Armstrong et al., 1977), a shallow embayment within Galveston Bay, have provided some of the strongest evidence for effects of produced water on benthic fauna. Depressed infaunal densities were observed out to a distance of approximately one kilometer from the separator platform discharge. Despite other production activities in the area, this study convincingly implicated the discharge of produced water as the causative agent (Armstrong et al., 1977). However, the authors urged caution in extrapolating their results to other situations, because the study was conducted in very shallow (2 - 3 m), turbid water in a sheltered environment. In more exposed and/or deeper areas, dilution rates should be greater and biological effects could be much more localized (Armstrong et al., 1977, Middleditch, 1984). Because other studies of the effects of produced water have also been conducted in poorly mixed coastal or estuarine waters (e.g., Boesch and Rabalais, 1987) or have been confounded with other sources of impact (e.g., Bedinger et al., 1981; Spies, 1987), there exists little information regarding environmental effects of discharge of produced water to high energy coastal habitats.

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Although impacts from produced water are likely to occur in the benthos because of accumulation of contaminants in the sediments (e.g., Armstrong et al., 1977), such impacts may be difficult to detect due to natural spatial and temporal variability in population density of infaunal organisms. Examination of individual-based parameters (such as growth) might provide more sensitive indicators of environmental impacts than assessment of population density (Carney, 1987; Osenberg et al., 1993). This approach might reveal a different spatial extent of biological effects than documented by variation in population densities. However, biological effects other than those on densities, such as changes in growth or other measures of individual performance, rarely have been addressed in field studies of impacts of produced water. Even in the absence of natural variability, examination of individual-based parameters might lead to the detection of impacts that would not be identified by focusing only on population density. For example, changes in the production of a population are related not only to the density of the population, but also the amount of growth or reproduction attributed to each individual. Although density might be unaffected by discharge of produced water, production (and therefore the population's ability to persist in face of other sources of stress) might be affected adversely. This can be assessed by direct examination of, for example, individual growth or reproductive output, in addition to density.

Here we examine the spatial scale of biological impacts associated with discharge of produced water to a high energy coastal environmental in southern California. Discharge comes from an onshore facility and occurs through a single pipeline a few hundred meters offshore. Therefore, we were able to study effects of produced water discharge in the absence of localized effects from other production activities and structures (e.g., the nearest platform is at least several km from the outfall). The three principal goals in this study were to: (1) determine if produced water discharge had discernible biological effects in this high energy environment; (2) document the nature of observed effects; and (3) compare the spatial extent of impacts on infaunal densities with effects on growth and body condition of "indicator" species (outplanted mussels).

METHODS

Study Site

The study site is located near Carpinteria, CA, USA (34°23'N, 119°30'W), where the subtidal environment consists of a sand bottom with little or no physical relief. This area is a high energy, open coast environment, although it is somewhat sheltered from the swell due to the presence of the northern Channel Islands and the east-west orientation of the coast. The surf typically ranges from 1 - 2 m, but varies seasonally. The surf is greatest during the winter due to large amplitude, long period swells generated from Pacific storms; however the winter in which infauna were sampled (see below) was a relatively mild winter with few major storms. Current flow primarily is east-west (parallel to shore), with an offshore flow component probably due to tidal flux. Based on depth profiles from 30 sampling dates, currents flowed to the west, east, south (to offshore) and north, 42%, 27%, 25% and 6% of the time, respectively.

Discharge of produced water occurs approximately 200 - 300 m from shore along the last 25 m section of the pipe at bottom depths of 10 - 12 m. The effluent is discharged through 10 T-ports that are oriented perpendicular to the pipe about 0.75 m above the bottom, resulting in a calculated minimum initial dilution of 125:1. The volume discharged is extremely consistent from month to month, averaging 2.64 million liters/day (16,600 bbls/d) from February 1989 - October 1990. Produced water has been discharged from this diffuser array since 1978.

The effluent is comprised primarily of produced water, with minor amounts of wastewater added from natural gas processing vessels and the cleaning of equipment. An unspecified amount of storm water is discharged with the produced water. However, there was no storm run-off during the period of this study. Wastewaters are treated by gravity settling and induced gas flotation units. Chemical characteristics of the effluent are summarized in Higashi et al. (1992). The effluent is approximately 6° C warmer than the receiving waters and has a lower

salinity (~20 ppt). However, there is no discernible thermal gradient beyond 0.5 m of the discharge ports.

Infaunal Depth Distribution

In a preliminary study, the depth distribution of infauna was examined to determine the appropriate depth for our infaunal sampling survey. On 31 October 1989, divers sampled infauna at four sites that were within 1 km of the Carpinteria outfall. At each site, 10 cores (20.3 cm^2/core) were taken to a depth of 25 cm. Each core was sectioned into 5 cm depth intervals, and depth fractions from five cores (all from the same site) were pooled into a single sample, yielding two samples/site, each with five depth fractions. Buffered formalin was added to each sample to bring the total solution to approximately 10% formalin, preserved samples then were washed through a series of 2 mm, 1 mm and 0.5 mm sieves, and each fraction subsequently was stored in alcohol. Organisms were picked from the sediments in each fraction, counted and identified to broad taxonomic categories.

Benthic Survey

An intensive spatial survey of infauna was conducted along the 11 m isobath at the Carpinteria study site. A total of 20 sites was sampled along a gradient upcoast (west) and downcoast (east) of the diffusers. Scuba divers collected samples on 10 February 1991 from 2, 3, 5, 10, 25, 50, 100, 250, 500, and 1000 m west of the diffusers and on 11 February 1991 from 5, 7, 10, 15, 30, 55, 100, 250, 500, and 1000 m east of the diffusers. Infaunal densities were sampled by taking 8 coffee can cores (78 cm^2/core to a depth of 10 cm) at each site, and pooling four cores into a single sample. As before, buffered formalin was added to the samples to bring the total solution to approximately 10% formalin, washed through 2 mm, 1 mm and 0.5 mm sieves and stored in alcohol. Organisms were picked from the sediments, counted and identified to broad taxonomic categories. For each site and each taxonomic group a mean based on the two replicates was calculated, and this mean was used in all subsequent analyses.

At the same time that the biological cores were collected, 3 sediment cores also were collected at each site (20.3 cm^2/core, 5 cm deep) and frozen. One core was used to determine percent organic matter in the sediments, one core was used for grain size analysis, and the remaining core was archived. Percent organic matter (POM) was determined by thawing sediments, aspirating the overlying water, and drying the sediments for 48 hours at 45° C. Three subsamples (ca. 5 - 6 g) of sediments from each sample were then dried for 3 hours at 60° C, weighed, combusted for 4 hours at 450° C, and reweighed. POM was calculated as 100% X(ash free dry mass / total mass of subsample), and the mean of the three subsamples was determined for each site.

The percent of sediments comprised by silt and clay was determined by drying each sample at 60° C, breaking up the dried sample with a rubber stopper, and shaking the sample for 10 minutes through a series of standard ASTM soil sieves. The mass of sediments on each sieve was determined, and the percent silt-clay was estimated as the percent of the sample that passed through the 0.063 mm sieve. Surficial sediments also were collected for analyses of metals and organics, and results of their analyses will be reported elsewhere by collaborators; we refer briefly to their findings in the discussion.

Data were analyzed using three related techniques. For all analyses, infaunal densities were log(x+1) transformed and distance was log transformed to better satisfy assumptions of the analyses (e.g., linearity and homoscedasticity of residuals). In the first approach, we examined the correlation (Pearson's r) between infaunal densities and each of three environmental parameters - distance from outfall, POM, and percent silt-clay. Second, we used multiple regression analysis to examine the effects of each environmental variable (e.g., distance) after accounting for variation due to the other two variables (e.g., POM and percent silt-clay). Third, we simplified the infaunal data using Principal Components Analysis, PCA (Dillon and Goldstein, 1984). PCA provided a way to simultaneously examine the patterns for all infaunal

taxa by constructing a set of new (and fewer) dependent variables (i.e., PC1, PC2, etc.) from the original variables (i.e., densities of each infaunal group) (see Jolliffe, 1986). Principal components were determined using the SAS Proc Factor procedure, and axes were rotated using the quartimax technique (SAS, 1988). Interpretation of the principal components was achieved by examining the loadings (i.e., correlation) of each original variable (i.e., density of a particular group) on the principal components. Following preliminary analyses, we retained and rotated the first two principal components, as remaining axes yielded little aggregation of the infaunal data.

PCA defined groups of organisms that exhibited similar patterns of spatial variation in abundance: i.e., each of the new variables (e.g., PC1) provided a measure of the aggregated response of several infaunal taxa to environmental variation. We then explored how the principal component scores varied with distance from the outfall and with variation in sediment characteristics. In other words, PCA enabled us to compare the relationship between environmental factors and a much reduced number of biological variables (i.e., the principal component scores), rather than densities of 20 different taxa. In this way, we could more easily infer the spatial extent of impacts due to produced water discharge.

Outplanted Mussels

Mussels (*Mytilus californianus* and *M. edulis*) were transplanted to six of the study sites to determine if proximity to the outfall influenced performance attributes (e.g., growth). At each of the six study sites (1, 5, 10, 50, 100, and 1000 m West of the outfall), we installed buoy arrays, each consisting of a cement anchor, line, and a subsurface buoy. *M. californianus* were collected from the intertidal zone at Montana de Oro State Park, CA, and *M. edulis* were collected from subtidal buoy lines at a location 1.5 km offshore Gaviota, California.

Before being transplanted to the field, individual mussels were measured and the shell margin notched so that initial sizes could be estimated after subsequent growth. Forty mussels from a uniform size distribution (range 20 - 60 mm shell length) of one species were put into a bag of 1.25 mm oyster netting with a vexar mesh skeleton for support. One bag with *Mytilus californianus* and one with *M. edulis* were attached to each buoy line approximately 4.5 m above the sediments on 7 June 1990. A subsample of "control" mussels was frozen without being transplanted to the field to provide an estimate of the initial condition of the transplanted mussels. Transplanted mussels were retrieved on 4 October 1990 and frozen.

Survivorship of outplanted mussels was estimated as the number of marked individuals recovered in each bag divided by the number initially marked. Shell length and initial shell length (as estimated by location of the notch) were measured for every marked mussel of both species recovered alive from each site (distance). For each individual, tissue was removed, separated into gonadal and somatic tissue, dried at 60° C for 24 hours, and weighed. Like most organisms, mussel growth and tissue mass vary with mussel size. To simplify analyses (data were taken over a range of mussel sizes), we obtained size-independent measures of mussel performance using analyses of covariance (ANCOVA) for each parameter for each mussel species, with log(shell length) as the covariate. From these analyses, we obtained adjusted means for each parameter at each of the six sites, and these adjusted means are the relative measures of mussel performance we report. All ANCOVAs were performed on log-transformed data, and adjusted means were either left transformed or back-transformed to original units depending on the specific analysis. Analyses of shell growth (i.e., change in length) used log (initial shell length) as the covariate, while analyses of condition (i.e., gonadal or somatic mass) used log (final shell length) as the covariate.

Principal components analysis was used to summarize the mussel data, which consisted of six different estimates of mussel performance measured at each site (i.e., the log-transformed adjusted means for shell growth, gonadal mass, and somatic mass for each of the two mussel species). We extracted only one principal component because all mussel parameters strongly covaried and therefore the first principal component accounted for most of the variation in the mussel data set.

To estimate tissue production for each of the two mussel species we 1) dissected "control" mussels that were not outplanted, 2) estimated the allometric relationship between tissue mass and shell length for these "control" mussels (for each species the relationship explained over 94% of the variation in tissue mass), and 3) for each outplanted mussel, subtracted its initial tissue mass (estimated by using its initial shell length in the allometric regression derived from control mussels) from its final tissue mass. We used ANCOVA to obtain the adjusted means for tissue production for each site using log(initial shell length) as the covariate, and then examined the relationship between the adjusted means and distance from the outfall. Thus, we determined if mussels that began the transplant period at the same size varied in their production of new tissue as a function of distance from the produced water outfall.

RESULTS

Infaunal Depth Distribution

An average of 73% of all infauna collected from the top 25 cm of sediments occurred in the first 5 cm, with approximately 5 - 10% occurring in each of the deeper 5 cm depth fractions. A total of 82% of the infauna (range among the 4 sites: 80 - 86%) was collected in the top 10 cm. All taxonomic groups shared this general pattern, and similar results were obtained at nearby sites studied by Spies and Davis (1979). Based upon these findings, samples collected during our subsequent infaunal survey were taken to a depth of 10 cm.

Benthic Survey

Analyses revealed a number of strong associations between infaunal densities and the three environmental parameters: distance from outfall, POM and percent silt-clay (Table 1). The environmental parameters were not correlated with one another ($|r| < 0.22$; $P > 0.35$ for each comparison), leading to the similar results from correlation and multiple regression analyses. In general, densities were more strongly associated with distance from the outfall than with either of the sediment characteristics we measured. Among the 20 sites, POM ranged from 1.05 - 1.86 % ($\overline{X} = 1.29$), percent silt-clay ranged from 4.4 - 17.8 % ($\overline{X} = 10.2$), and there was no pattern in variation of either parameter with distance from the diffuser. In particular, sediments near the diffuser were not any finer or coarser (% silt-clay at 7 sites ≤ 10 m from outfall: $\overline{X} \pm$ 1 SE= 9.7 ± 0.8 %) than elsewhere along the gradient, and there was no evidence of elevated POM nearer to the discharge (% POM at 7 sites ≤ 10 m: $\overline{X} \pm$ 1 SE = 1.21 ± 0.06 %). The results of correlation analysis, multiple regression and PCA were similar. We categorized each taxon into one of four groups based on the way density covaried with the environmental variables (Table 1).

One group, consisting of Nematodes only, achieved greatest densities at sites near the outfall, and thus appeared to benefit from the discharge of produced water (Table 1). The second group, consisting of echinoderms, larval crustaceans, nemertean worms and several polychaete families, exhibited the opposite pattern and achieved greatest densities at sites farthest from the outfall. The third group included bivalves, two classes of crustacea (ostracods and cumaceans), and two polychaete families (Capitellidae and Spionidae) and exhibited a mixed pattern in which densities were generally positively associated with POM, and possibly more abundant near the outfall. The fourth group exhibited no discernible pattern with distance from the outfall or POM and included Glycerid polychaetes, gastropods, and all other crustaceans (e.g., gammarid amphipods, copepods).

The PCA produced two principal components that were associated with environmental features (Table 1). PC1 explained 25% of the variation in infaunal densities, and scores on PC1 were highly correlated with distance from the outfall ($r = 0.81$; $P < 0.0001$; $n = 20$). Importantly, the pattern of variation associated with PC1 was symmetrical to the east and west of the diffuser (Fig.1). However, the effect associated with distance was extremely non-linear, suggesting that impacts on the infauna were not present beyond a distance of approximately 50 - 100 m from

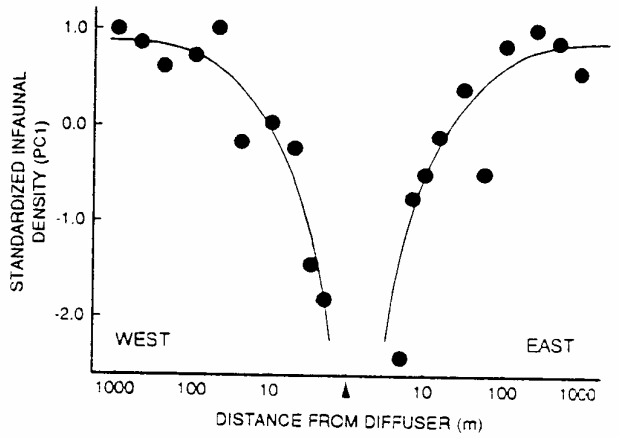

Figure 1. Standardized infaunal density as a function of distance from the produced water outfall. Standardized infaunal density (defined as PC1: Table 1) is an aggregate measure based on the densities of all infauna and is standardized to a mean of 0. The arrow indicates the location of the diffuser.

the outfall. Loadings on PC1 (Table 1) reveal that its variation was primarily associated with variation in the densities of organisms in Groups 1 and 2 (e.g., nematodes, nemerteans and polychaetes (Fig.2)

PC2 explained an additional 19% of the variation in infaunal densities and was primarily associated with organisms in Group 3 (e.g., ostracods, cumaceans and bivalves: Table 1). Scores on PC2 were significantly correlated with POM ($r = 0.48$; $P = 0.03$; $n = 20$). There was a weaker and non-significant relationship between PC2 and distance ($r = -0.36$; $P = 0.12$; $n = 20$). The pattern of variation in PC2 (Fig. 3) was not similar to the East and West of the diffusers. Therefore, it appears that variation in PC2 was driven by variation in POM and was not related to produced water discharge.

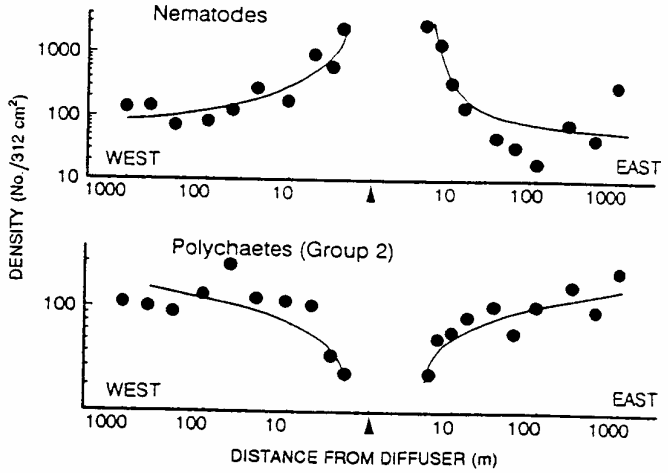

Figure 2. Densities of nematodes and Group 2 polychaetes (see Table 1), as a function of distance from the produced water outfall. Each data point gives the mean of two samples collected at each location. The arrow indicates the location of the diffuser.

Table 1. Summaries of three separate analyses of infaunal densities with environmental variables [log distance from the produced water outfall (DIST), percent organic matter in the sediments (POM), and percent of sediments in the silt-clay fraction (SC)]. Infaunal taxa were placed into one of four groups based upon the way their densities covaried with the environmental variables. Statistically significant ($P < 0.05$, n = 20) results from correlation and regression analyses are given and the sign indicates the direction of the effect. The signs of loadings from principal components analysis are indicated when they exceeded ± 0.50. PC1 and PC2 explained a total of 44% (25% and 19%, respectively) of the variation in infaunal densities. Because site score on PC1 was highly correlated with distance from the outfall (r=0.81, $P<0.0001$; Figure 1), a positive loading indicates greater densities were achieved far from the outfall. PC2 was positively correlated with POM (r = 0.48, $P = 0.03$) suggesting that taxa with positive loadings were more abundant at sites with greater POM.

		Multiple	Loadings on	
	Correlation	Regression	PC1	PC2
Group 1: (Greater density near outfall)				
Nematodes	-DIST	-DIST	—	
Group 2: (Greater density far from outfall)				
Polychaetes:				
Cirratulids			+	
Other Sedentariae	+DIST	+DIST	+	
Nephtyds			+	
Nereids	+DIST	+DIST	+	
Syllids	+DIST	+DIST	+	
Other Errantiae				
Nemerteans	+DIST	+DIST	+	
Larval crustaceans	+DIST	+DIST		
Echinoderms	+DIST	+DIST		
Group 3: (Greater density at sites with high POM and/or near outfall)				
Polychaetes:				
Spionids				+
Capitellids				+
Cumaceans	-DIST, +POM	-DIST, +POM		+
Ostracods	-DIST	-DIST, +POM		+
Bivalves				+
Group 4: (No apparent relationship to outfall or POM)				
Polychaetes:				
Glycerids	-SC	-SC		
Copepods		-SC		
Gammarids				
Other Crustaceans				
Gastropods				+

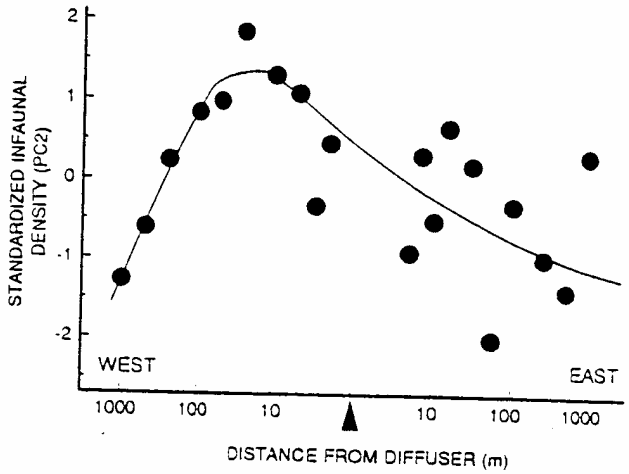

Figure 3. Standardized infaunal density, defined by PC2 (Table 1), as a function of distance from the produced water outfall. The arrow indicates the location of the diffuser. Compare with Figure 1.

Outplanted Mussels

Survivorship of mussels was relatively high (means ± 1 SE among sites: 0.71 ± 0.05 for *M. californianus*; 0.63 ± 0.5 for *M. edulis*), and showed no significant correlation with distance from the outfall ($|r| < 0.4$; $P > 0.4$ for each species). However, mussels did exhibit considerable variation in the intensity of sublethal effects, as measured by shell growth and condition (i.e., size-specific gonadal and somatic tissue masses). Both mussel species exhibited similar patterns of variation among sites in these sublethal components of performance; sites at which *M. californianus* performed well were the same sites at which *M. edulis* performed well (Figure 4).

Performance was highly correlated with distance from the outfall for all three measures of performance for *M. californianus*, and for two of three measures for *M. edulis* (Table 2). Due

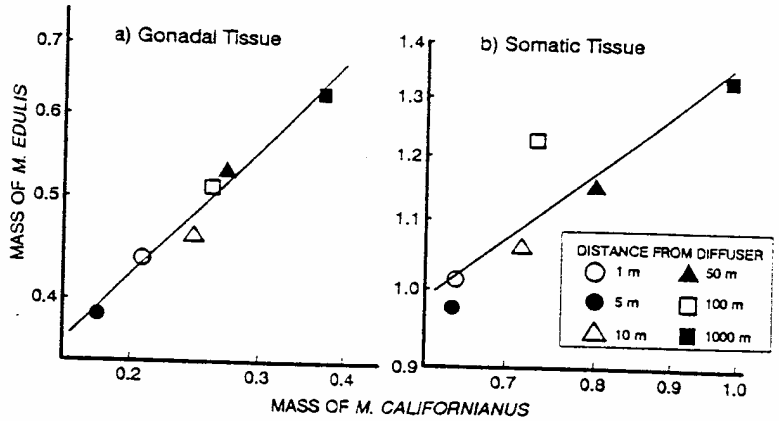

Figure 4. Pattern of covariation in condition (size-specific gonadal mass and somatic mass) of two mussel species (*M. californianus* and *M. edulis*). Each datum gives the response of the two species outplanted to the same site. In general sites close to the outfall yielded low values for each species, whereas sites far from the outfall yielded higher values. Condition of both species was highly correlated ($r = 0.99$, $P < 0.0001$ for gonadal tissue; $r = 0.90$, $P = 0.015$ for somatic tissue).

Table 2. Statistical summaries of analyses of mussel performance. Given are the correlations between adjusted means from analyses of covariance (based on log transformed data) and log (distance), as well as the correlations between the adjusted means and the first principal component (i.e., loadings). Correlations >0.81 are significant ($P<0.05$). PC1 explained 79% of the total variation in mussel performance and was highly correlated with distance from the outfall (r = 0.94, P = 0.005, n = 6).

Species	Parameter	Correlation with Distance	Loading on PC1
M. californianus			
	shell growth	+0.88	+0.92
	somatic mass	+0.92	+0.94
	gonadal mass	+0.90	+0.98
M. edulis			
	shell growth	+0.33	+0.36
	somatic mass	+0.95	+0.98
	gonadal mass	+0.89	+0.99

to the similarity in response of the three measures of performance for both species, the first principal component (PC1) provided a simple description of the aggregate response of mussels to environmental variation. PC1 explained 79% of the total variation in mussel performance, and was highly correlated with distance from the outfall (Fig. 5; Table 2), suggesting a major role of produced water discharge (relative to other factors) in determining variability in mussel performance among sites.

These results demonstrate that mussels near the produced water outfall tended to grow more slowly, and at any given size were in poorer condition (i.e., had less gonadal and somatic tissue) than mussels far from the outfall. These responses provide three separate measures of how produced water affected mussel production. To examine the aggregate effect, we estimated the per capita tissue production at each site. Tissue production was correlated with distance for

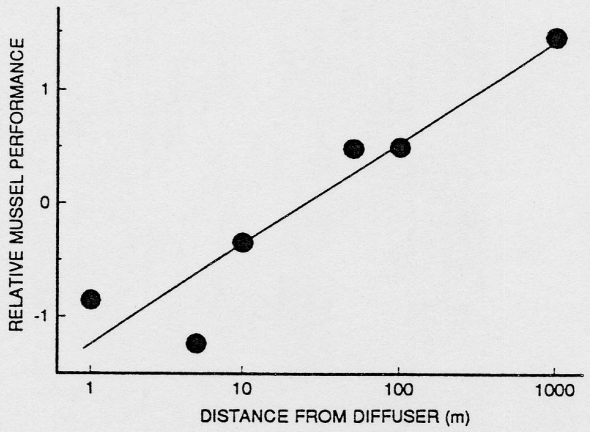

Figure 5. Relative mussel performance as a function of distance from the produced water outfall. Relative mussel performance is defined by the PC1 from analysis of mussel growth and condition (Table 2). Performance and log (distance) were highly correlated (r = 0.94, P = 0.005).

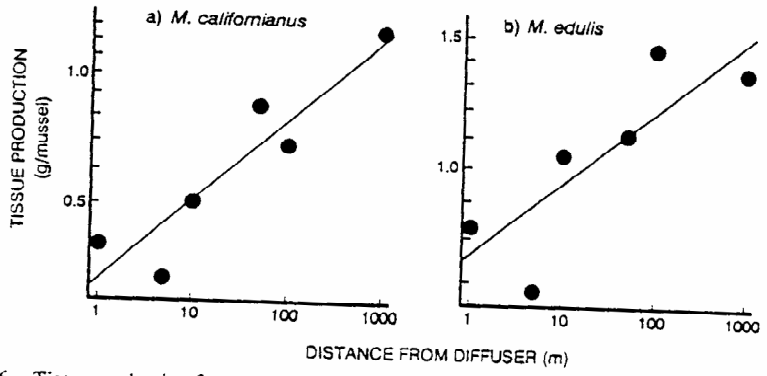

Figure 6. Tissue production for two species of mussel (*M. californianus* and *M. edulis*) as a function of distance from the diffuser. Tissue production and distance were positively correlated for each species ($r = 0.92$, $P = 0.009$ for *M. californianus*; $r = 0.82$, $P = 0.044$ for *M. edulis*).

both species (Fig. 6: based on log-transformed data, $r = 0.92$; $P = 0.009$ for *M. californianus* and $r = 0.82$; $P = 0.044$ for *M. edulis*). Sites farthest from the diffusers had production that was two to three times greater than sites near the outfall.

DISCUSSION

Interpretation of Spatial Patterns

Field studies of biological effects arising from point discharges such as produced water are hampered by a number of logistical and design problems. Principal among them are detecting effects amid natural variation and assigning causation for observed patterns (e.g., Carney, 1987). Ideally, this requires knowledge of environmental conditions at "impact" and "control" sites before and after the discharge occurs to determine whether (and by how much) attributes near the outfall change from those expected (e.g., Stewart-Oaten et al., 1986). While such an approach can provide powerful inference (e.g., Stewart-Oaten et al., 1986: Osenberg et al., 1992) , we know of no field assessment of environmental effects of produced water that has been able to collect pre-discharge information (but see ongoing study by Osenberg et al., 1993). Usually by necessity, the alternative approach is to seek spatial correlations between biological variables and environmental characteristics after a long period of produced water discharge. In these situations, natural spatial variability cannot be statistically isolated from the putative impact (Stewart-Oaten et al., 1986).

In addition to design constraints, many previous assessments of produced water discharge have been conducted where other oil and gas production activities occurred in the same immediate area, and/or where large, natural sources of disturbance existed (e.g., Bender et al., 1979; Bedinger et al., 1981; but see Armstrong et al., 1977). In these circumstances, it can be virtually impossible to isolate the contribution of produced water in shaping all but the most extreme environmental pattern (Carney, 1987). By contrast, the produced water outfall we examined is several kilometers from the nearest offshore platform, is the only discharge of any kind in the local vicinity, and, with the exception of small boat traffic, it is the only major industrial activity that occurs in the local area. Further, the closest potential source of a large natural disturbance is Carpinteria Salt Marsh, a small (93 ha) coastal wetland located ~ 3 km west of the outfall (Ferren, 1985). Thus, there are no obvious enterprises that covary in space with the discharge of produced water at Carpinteria that could account for observed patterns. It remains possible that the observed patterns resulted from natural spatial differences in environ-

mental attributes that were unrelated to, but were confounded in space with the discharge of produced water. Without pre-discharge information, it is difficult to reject this possibility. However, several lines of evidence suggest that the observed patterns resulted from the discharge of produced water.

The first supportive evidence for the causal involvement of produced water on mussel performance comes from the correspondence between the observed field effects and responses induced by exposure of mussels in controlled laboratory experiments. For example, based on *in vivo* imaging using nuclear magnetic resonance, Fan et al. (1992) showed that prolonged exposure to produced water from Carpinteria led to a severe reduction in the energetic status of mussels, *M. californianus*. This effect on the ability of mussels to maintain energy stores is a likely cause of our observed reductions in growth, condition, and tissue production.

The second line of evidence is the marked temporal consistency in effects that we have observed in four different outplants conducted after the one reported here (Osenberg et al., *unpublished data*). Although there was seasonal variation in the overall amount of mussel growth at each site, individual performance was consistently lowest at sites near the outfall and always greatest at the 1000 m site. Thus, the results of our first outplant (Figures 4 - 6) were not spurious and clearly represent consistent spatial variation in performance that is correlated with distance from the outfall.

Although suggestive, these results alone cannot isolate effects of the produced water from an unspecified factor that is correlated in space with the outfall. At a minimum, this would require additional evidence demonstrating that mussels actually encounter produced water at the sites with reduced performance. Because bivalves are known to incorporate a variety of trace elements into their shells (e.g., Carriker et al. 1982), and because barium is a marker of the Carpinteria produced water (Higashi et al. 1992), we recently determined the amount of barium in the shells of mussels that were outplanted to our six study sites during the same period as the mussels analyzed for performance (Osenberg, Fan and Collins, *unpublished data*). Barium concentration in shells of both *M. edulis* and *M. californianus* was significantly negatively correlated with distance ($r < -0.91$, $P \leq 0.01$, $n=6$ for each species based on log transformed data). Consequently, barium concentration was negatively correlated with mussel performance ($r < -0.92$, $P < 0.01$, $n = 6$); performance was greatest, and barium content lowest, at the 1000 m site. Thus, it appears that mussel performance varied inversely with relative exposure of mussels to the produced water plume.

The most conclusive evidence for evaluating if produced water exposure caused the observed pattern of mussel performance could be obtained by stopping the discharge of produced water and seeing if the spatial patterns of mussel performance and barium contamination disappear. If so, this would demonstrate the absence of an underlying cause for the patterns that was independent of produced water discharge. However, the mussel outplants require several months in the field, which makes it logistically infeasible for us to conduct such an experiment. Fortunately, operators of the Carpinteria plant plan to stop discharging produced water during the next year or two. At that time a conclusive test might be possible.

In contrast to the mussel outplants, that require several months to complete, shorter term field assessments have been possible. For example, a complementary investigation by Raimondi and Schmitt (1992) clearly demonstrated a cause-effect relationship between the discharge of produced water from the Carpinteria facility and the performance of abalone larvae. They documented marked reductions in survival, settlement and metamorphosis of abalone larvae with increased proximity to the diffuser when produced water was being discharged. The spatial pattern of effects on abalone was qualitatively identical to that we observed for mussels. Because their study relied on short-term exposures (≤ 4 days), they were able to show that effects were not present during a brief (10 day) period when produced water was not discharged.

Interpretation of the infaunal patterns is somewhat more difficult. Unlike parameters obtained from mussels, various infaunal groups did not show the same patterns of density as a

function of distance from the outfall: some groups were more abundant, some were less, and others showed no pattern with distance. The sedimentary environment is likely to be more heterogeneous than the water column where mussels were outplanted, and infaunal organisms respond to natural variation in such characteristics as grain size or particulate organic matter (e.g., Pearson and Rosenberg, 1978; Reish, 1979; Ferris and Ferris, 1979: Reish and Barnard, 1979. This heterogeneity can add considerable noise to patterns that arise from human disturbance. Despite this, interpretable relationships between density and proximity to the outfall were apparent, and the collective evidence strongly suggests an involvement of produced water discharge.

As revealed by principal components analysis, the major discernible source of variation in infaunal density was distance from the outfall (Fig. 1). The pattern of variation was symmetrical on both sides of the outfall, with the greatest alteration of densities closest to the diffuser. Symmetry of effects to the west and east of the diffuser would be crudely predicted by our water current data. Further, sediment samples collected concurrently with our infaunal cores revealed localized elevations of barium, a chemical marker of the effluent (Higashi et al., 1992), near to and on both sides of the diffuser (A. Flegal and K. Abu-Saba, *pers. com.*). In contrast to our analyses of barium in mussel shells, the elevation of barium in the sediments was much more localized, suggesting a more limited impact on the benthos (see below).

The particular organisms that varied systematically along the distance gradient also suggest that produced water was involved. Nematodes were more abundant closer to rather than further from the diffuser (Fig. 2a), and they are known to respond positively to organic enrichment of sediments from oil and sewage contamination (Chasse 1978; Spies et al., 1980; Sandulli and De Nicola 1991; Spies and DesMarais 1983) argued that enrichment by petroleum hydrocarbons at natural oil seeps led to higher densities of nematodes by stimulating the production of a food resource, the bacterium, *Beggiatoa sp.* We have observed *Beggiatoa* mats near the produced water outfall and coating buoy lines at our 1, 5, and 10 m sites.

In contrast to Spies' work at oil seeps, we found little evidence of trophic enhancement for infauna other than nematodes. Indeed, most carnivorous groups that we examined (e.g., nemerteans, and several polychaete families, including Nephtyds, Nereids and Syllids) varied inversely with the abundance of nematodes and were least abundant near the outfall. Pearson and Rosenberg (1978) suggested that carnivorous species might decline in abundance under moderate levels of trophic enrichment, and the pattern of variation we observed for this trophic group at Carpinteria was consistent with such a hypothesis.

An alternative explanation is that observed spatial variation in densities of the groups above resulted from current scour caused directly or indirectly by the outfall (e.g., Middleditch, 1981). Several lines of evidence argue against this hypothesis. First, sediment grain size and composition (i.e., percent silt-clay) were not coarser nearer to the pipeline than much further away, as would be expected by scouring. Second, mats of bacteria would not be expected to develop in even modestly scoured areas, yet they occurred on the sediment near the outfall. Third, the infaunal community near the diffuser was not that expected in a scoured area, but was characteristic of areas subject to organic enrichment from oil contamination and sewage discharges (e.g., Gray, 1979).

However, POM did not correlate with distance from the outfall, suggesting either that our estimates of POM were too crude to detect enrichment, or that particulate organic input provided only a minor portion of the source of trophic enrichment near the outfall. Our data did reveal that some groups of deposit and filter-feeding organisms (i.e., those in Group 3, Table 1, such as bivalves, cumaceans, ostracods and Spionid polychaetes) were more abundant in areas with greater POM.

Spatial Extent of Effects

Based on the foregoing discussion, we believe that the major effects of produced water on infaunal densities at Carpinteria are summarized by variation in Principal Component 1 (Figure

1). Effects on densities appeared limited to areas very close to the outfall (≤ 100 m). This spatial extent of effects on the benthos was more limited than reported previously for areas with shallower receiving waters (e.g., Armstrong et al. 1977; Boesch and Rabalais 1987; Neff et al. 1989), and perhaps for deeper sites with lower discharge rates (Middleditch 1981; Neff et al. 1989). Thus, our infaunal survey might lead to the conclusion that biological effects, in general, were extremely localized. However, results from our mussel outplants and from the related study by Raimondi and Schmitt (1992) suggested that important biological effects can occur over larger spatial scales at Carpinteria, despite the discharge to a high energy environment.

The production of mussel tissue was affected by produced water, and, by contrast with the limited spatial effect on infaunal density, mussel performance was reduced out to a distance of at least 100 m and perhaps beyond 1 km (Fig. 4). Unfortunately, our study provides no resolution between the 100 and 1000 m sites and we have no sites more than 1000 m from the outfall. In retrospect, we would be able to make much stronger statements about the spatial extent of these effects if we had also placed additional sites past 100 m. Previous suggestions that dilution was likely to reduce the spatial extent of effects in open coast environments (e.g., Middleditch, 1984; Neff et al., 1989) led us to focus on near-field effects. Since completing our first mussel outplant, we have added a site at 500 m and have completed two subsequent outplants. In each case, performance was greatest at the 1000 m site suggesting that effects on performance might extend past 500 m.

An area of effect of similar size also was observed at Carpinteria for performance of abalone larvae (Raimondi and Schmitt, 1992), and serves to illustrate how study of different biological parameters can reveal different spatial scales of environmental impact. The reasons for the different spatial extents of effects between densities of infauna and performance of mussels and abalone larvae are unknown, but they could be related to the two different types of parameters measured and/or the particular habitats (i.e., benthos vs. water column) sampled. Below, we discuss several possible explanations, which we offer as hypotheses for future field tests.

Mussels were suspended in the water column, and effects on their performance, like those on abalone larvae (Raimondi and Schmitt, 1992), may have arisen from dissolved and suspended fractions of the produced water plume. By contrast, it is possible that effects on infaunal organisms largely resulted from components of the effluent that settled to the benthos (e.g., Reynoldson, 1987). Our data suggest that water-borne contaminants may have caused effects over a much greater spatial scale than did the particulate fraction(s). This is consistent with the finding that the water-soluble fraction of the Carpinteria produced water was responsible for most biological effects observed in laboratory tests (Higashi et al., 1992). If true, this challenges conventional thinking regarding the relatively greater likelihood of produced water effects being mediated by toxicants accumulated in the sediments rather than those dispersed in the water column (e.g., Middleditch, 1984; Neff, 1987; Payne et al., 1987). As noted by Howarth (1989) and Raimondi and Schmitt (1992) this possibility suggests that more attention needs to be given to potential effects mediated through the water column, not only for planktonic organisms but also for benthic and demersal species that are potentially exposed to water soluble contaminants.

Differences in "habitats" notwithstanding, part of the explanation for the greater spatial effects on mussels than infauna probably relates to differences in the biological parameters examined. It has been suggested that individual-based parameters (e.g., growth and reproduction) are more sensitive indicators of pollutants than population-level characteristics (e.g., densities), especially with regard to detectability of actual effects (e.g., Carney, 1987; Osenberg et al., 1992, 1993). For example, the extent to which affected populations are depressed locally will depend on the size and nature of effects on local adults (e.g., survivorship, fecundity) and the supply of new individuals from all sources (e.g., Underwood and Peterson, 1987). Most marine organisms have a planktonic dispersive stage, which tends to decouple local production of propagules from subsequent recruitment into the local adult population. Thus, it is possible for a discharge to have no direct effect on local adult stocks (e.g., when recruitment and survival of subsequent age classes are independent of contaminant levels), but considerable effects on

the local production of larvae (e.g., reduced growth and reproductive output of adults). Mussels in the vicinity of the Carpinteria outfall, for instance, have about half the gonadal mass of adults further away, yet because of recruitment of larvae produced elsewhere, local population densities may not be depressed by local reduction in reproductive output. Furthermore, the expression (if any) of the reduced local output of mussel propagules would be spread over a much larger spatial scale, defined by the dispersal distance of the larvae, which would make effects on the "global" population virtually impossible to detect (see Raimondi and Schmitt, 1992; Nisbet et al., 1993).

Because of propagule dispersal, assessment of local population densities can lead to considerable underestimation of both near- and far-field biological impacts of a point source discharge. Conversely, the effect of effluent discharge might be to alter settlement behavior of propagules, resulting in depressed local populations, even in the absence of effects on local adult survivorship or per capita production. In this circumstance, it is possible that the local reduction in density overestimates the ecological impact if larvae that chose not to settle at the discharge site successfully recruited elsewhere. A similar argument can be made for measures of individual-based parameters. For these reasons, we contend that a primary focus of assessment studies should be on the dynamics and persistence of populations. In marine systems especially, this requires information not only on local density, but also production (e.g., assessed by density and changes in demographic rates or individual-based parameters), as well as the connection between local processes and larger scale dynamics. Given the limited information on metapopulation dynamics in marine systems (Roughgarden and Iwasa, 1986), it is unrealistic to expect such thoroughness in assessment studies. However, it is important that field studies begin addressing aspects of production in addition to static analysis of local density. Doing so will provide a more complete picture of the spatial extent of ecological impacts that arise from such local perturbations as the discharge of produced water.

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REFERENCES

Armstrong, H.W., Fucik, K., Anderson, J.W., and Neff, J.M., 1977, "Effects of Oilfield Brine Effluent on Benthic Organisms in Trinity Bay, Texas, API Publ. No. 4291," American Petroleum Institute, Washington, D.C.

Bedinger, C.A., Jr., Childers, R.E., Kimball, K.T., Cooper, J.W., and Kwok, A., 1981, Part 1 - Background, program organization and study plan, *in*: "Ecological Investigations of Petroleum Production Platforms in the Central Gulf of Mexico, Volume I. Pollutant Fate and Effects Studies," C.A. Bedinger, Jr., ed., Southwest Research Institute, San Antonio, Texas.

Bender, M.E., Reish, D.J., and Ward, C.H., 1979, Re-examination of the offshore ecology investigation, *Rice* Univ. Stud. 65:35-118.

Boesch, D.F., and Rabalais, N.N., eds., 1987, "Long-term Environmental Effects of Offshore Oil and Gas Development," Elsevier Applied Science, New York.

Carney, R.S., 1987, A review of study designs for the detection of long-term environmental effects of offshore petroleum activities, *in*: "Long-Term Environmental Effects of Offshore Oil and Gas Development," D.F. Boesch and N.N. Rabalais, eds., Elsevier Applied Science, New York.

Carriker, M.R., Swann, C.P., and Ewart, J.W., 1982, An exploratory study with the proton micropobe of the ontogenetic distribution of 16 elements in the shell of living oysters (*Crassostrea virginica*), Marine Biology 69:235-246.

Chasse, C., 1978, The ecological impact on the near shores by the *Amoco Cadiz* oil spill, Mar. Poll. Bull. 9(11):298-301.

Dillon, W.R., Goldstein, M., 1984, "Multivariate Analysis," John Wiley and Sons, New York.

Fan, T., Higashi, R., Cherr, G., and Pillai, M., 1992, Produced water perturbs reproduction in mussels as monitored *in vivo* by NMR spectroscopy and imaging, *in*: "Produced Water: Technological/ Environmental Issues and Solutions," J.P. Ray and F.R. Engelhardt, eds., Plenum Publishing Corp., New York, New York, USA.

Ferren, W.R., Jr., 1985, "Carpinteria salt marsh; environment, history, and botanical resources of a southern California estuary, University of California Santa Barbara Herbarium Publ. 4," University of California, Santa Barbara, California.

Ferris, V.R., and Ferris, J.M., 1979, Thread worms (Nematoda), *in*: "Pollution Ecology of Estuarine Invertebrates," C.W. Hart, Jr. and S.L.H. Fuller, eds., Academic Press, New York.

Gray, J.S., 1979, Pollution-induced changes in populations, Phil. Trans. R. Soc. Lond. B. 286:545-561.

Higashi, R., Cherr, G., Bergens, C., Fan, T., and Crosby, D, 1992, Toxicant isolation from a produced water source in the Santa Barbara Channel, *in*: "Produced Water: Technological/Environmental Issues and Solutions," J.P. Ray and F.R. Engelhardt, eds., Plenum Publishing Corp., New York, New York, USA.

Howarth, R.W., 1989, Determining the ecological effects of oil pollution in marine ecosystems, *in*: "Ecotoxicology: Problems and Approaches," S.A. Levin, M.A. Harwell, J.R. Kelly, K.D. Kimball, eds., Springer-Verlag, New York.

Jolliffe, I.T., 1986, "Principal Component Analysis," Springer-Verlag, New York.

Middleditch, B.S., ed., 1981, "Environmental Effects of Offshore Oil Production. The Buccaneer Gas and Oil Field Study," Plenum Press, New York.

Middleditch, B.S., 1984, "Ecological Effects of Produced Water Discharges from Offshore Oil and Gas Production Platforms. Final Report on API Project No. 248," American Petroleum Institute, Wash., D.C.

Neff, J.M., 1987, Biological effects of drilling fluids, drill cuttings and produced waters, *in*: "Long-Term Environmental Effects of Offshore Oil and Gas Development," D.F. Boesch and N.N. Rabalais, eds., Elsevier Applied Science, New York.

Neff, J.M., Sauer, T.C., and Maciolek, N., 1989, "Fate and Effects of Produced Water Discharges in Nearshore Marine Waters, API Publ. No. 4472," American Petroleum Institute, Washington, D.C.

Nisbet, R.M., Murdoch, W.W., and Stewart-Oaten, A., 1993, Consequences for Adult Fish Stocks of Human-Induced Mortality on Immatures, Ecological Applications, in review.

Osenberg, C.W., Holbrook, S.J., and Schmitt, R.J., 1992, Implications for the design of environmental assessment studies, *in*: "Perspectives on the Marine Environment," P.M. Grifman and S.E. Yoder, eds., USC Sea Grant, Los Angeles, California.

Osenberg, C.W., Schmitt, R.J., Holbrook, S.J., Abu-Saba, K.E., and Flegal, A.R., 1993, Detection of environmental impacts: power analysis and spatial inference, Ecological Applications, in review.

Payne, J.R., Phillips, C.R., and Hom, W., 1987, Transport and transformations: water column processes, *in*: "Long-Term Environmental Effects of Offshore Oil and Gas Development," D.F. Boesch and N.N. Rabalais, eds., Elsevier Applied Science, New York.

Pearson, T.H., and Rosenberg, R., 1978, Macrobenthic succession in relation to organic enrichment and pollution of the marine environment, Oceanogr. Mar. Biol. Ann. Rev. 16:229-311.

Raimondi, P.T., and Schmitt, R.J., 1992, Effects of produced water on settlement of larvae: field tests using red abalone, *in*: "Produced Water: Technological/Environmental Issues and Solutions," J.P. Ray and F.R. Engelhardt, eds., Plenum Publishing Corp., New York, New York, USA.

Reish, D.J., 1979, Bristle Worms (Annelida: Polychaeta), *in*: "Pollution Ecology of Estuarine Invertebrates," C.W. Hart, Jr. and S.L.H. Fuller, eds., Academic Press, New York.

Reish, D.J., and Barnard, J.L., 1979, Amphipods (Arthropoda: Crustacea: Amphipoda), *in*: "Pollution Ecology of Estuarine Invertebrates," C.W. Hart, Jr. and S.L.H. Fuller, eds., Academic Press, New York.

Reynoldson, T.B., 1987, Interactions between sediment contaminants and benthic organisms, *in*: "Ecological Effects of *In Situ* Sediment Contaminants," R. Thomas, R. Evans, A. Hamilton, M. Munawar, T. Reynoldson, and H. Sadar, eds., Dr W. Junk Publishers, Boston.

Roughgarden, J., and Iwasa, Y., 1986, Dynamics of a metapopulation with space-limited subpopulations, Theor. Pop. Biol. 29:235-261.

Sandulli, R., and De Nicola, M., 1991, Responses of meiobenthic communities along a gradient of sewage pollution, Mar. Poll. Bull. 22(9):463-467.

SAS Institute Inc., 1988, "SAS/STAT User's Guide, Release 6.03 Edition." Cary, NC. SAS Institute Inc., 1028 pp.

Spies, R.B., 1987, The biological effects of petroleum hydrocarbons in the sea: assessments from the field and microcosms, *in*: "Long-Term Environmental Effects of Offshore Oil and Gas Development," D.F. Boesch and N.N. Rabalais, eds., Elsevier Applied Science, New York.

Spies, R.B., and Davis, P.H., 1979, The infaunal benthos of a natural oil seep in the Santa Barbara Channel, *Mar. Biol.* 50:227-237.

Spies, R.B., Davis, P.H., and Stuermer, D.H., 1980, Ecology of a submarine petroleum seep off the California coast, *in*: "Marine Environmental Pollution, 1. Hydrocarbons," R.A. Geyer, ed., Elsevier, Amsterdam.

Spies, R.B., and DesMarais, D.J., 1983, Natural isotope study of trophic enrichment of marine benthic communities by petroleum seepage, Mar. Biol. 73:67-71.

Stewart-Oaten, A., Murdoch, W.W., and Parker, K., 1986, Environmental impact assessment: 'pseudoreplication' in time?, Ecology 67:929-940.

Underwood, A.J., and Peterson, C.H., 1987, Towards an ecological framework for investigating pollution, Mar. Ecol. Prog. Ser. 46:227-234.

V. STATISTICAL POWER OF BACIPS STUDIES: INFERENCES FROM BEFORE-AFTER STUDIES AND LONG-TERM BEFORE STUDIES.

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DETECTION OF ENVIRONMENTAL IMPACTS: NATURAL VARIABILITY, EFFECT SIZE, AND POWER ANALYSIS[1]

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Abstract. The power of any test of an environmental impact is simultaneously constrained by (1) the variability of the data, (2) the magnitude of the putative impact, and (3) the number of independent sampling events. In the context of the Before–After-Control–Impact design with Paired sampling (BACIP), the variability of interest is the temporal variation in the estimated differences in a parameter (e.g., population density) between two unperturbed sites. The challenges in designing a BACIP study are to choose appropriate parameters to measure and to determine the adequate number and timing of sampling events. Two types of studies that are commonly conducted can provide useful information in designing a BACIP study. These are (1) long-term studies that provide estimates of the natural temporal and spatial variability of environmental parameters and (2) spatial surveys around already-perturbed areas ("After-only" studies) that can suggest the magnitude of impacts.

Here we use data from a long-term study and an After-only study to illustrate their potential contributions to the design of BACIP studies. The long-term study of parameters sampled at two undisturbed sites yielded estimates of natural temporal variability. Between-site differences in chemical–physical parameters (e.g., elemental concentration) and in individual-based biological parameters (e.g., body size) were quite consistent through time, while differences in population-based parameters (e.g., density) were more variable. Serial correlation in the time series of differences was relatively small and did not appear to vary among the parameter groups. The After-only study yielded estimates of the magnitude of impacts through comparison of sites near and distant from a point-source discharge. The estimated magnitude of effects was greatest for population-based parameters and least for chemical–physical parameters, which tended to balance the statistical power associated with these two parameter groups. Individual-based parameters were intermediate in estimates of effect size. Thus, the ratio of effect size to variability was greatest for individual-based parameters and least for population and chemical–physical parameters.

The results suggest that relatively few of the population and chemical–physical parameters could provide adequate power given the time constraints of most studies. This indicates that greater emphasis on individual-based parameters is needed in field assessments of environmental impacts. It will be critical to develop and test predictive models that link these impacts with effects on populations.

Key words: Before–After-Control–Impact design; environmental impact; environmental monitoring; impact assessment; individual vs. population parameters; pollution: produced water; serial correlation; spatial variability; statistical power; temporal variability.

INTRODUCTION

A principal challenge posed in field assessments of environmental impacts is to isolate the effect of interest from noise introduced by natural spatial and temporal variability. If the size of an impact from a human disturbance is small relative to natural variability, it will be difficult to detect with any degree of confidence. Therefore, it is critical to consider statistical power in planning and interpreting environmental impact assessment studies (Green 1989, Fairweather 1991, Faith et al. 1991, Osenberg et al. 1992*a*, Mapstone, *in press*; see also Peterman 1990, Cooper and Barmuta 1993). Consideration of power can also guide the selection of environmental parameters and sampling intensity. These are important design criteria because time and

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financial constraints typically limit the number of parameters that can be measured and the number of samples that can be collected.

Calculation of statistical power, which is the probability of rejecting the null hypothesis of "no effect" when it is false, requires specification of the number of replicates as well as the ratio between the size of an effect and the variability among the replicates (Cohen 1977). Because there are many assessment designs, each of which makes different assumptions about the meaning of "effect," "variability," and "replicate" (Green 1979, Stewart-Oaten et al. 1986, Eberhardt and Thomas 1991, Underwood 1991, 1994, Osenberg et al. 1992*a*), the general assessment design must be specified before power can be discussed unambiguously. In assessing the environmental impacts of a particular anthropogenic activity, we typically require a design that explicitly deals with the lack of spatial replication and randomization (e.g., nuclear power plants are not replicated and placed at random sites along the United States coastline: Stewart-Oaten et al. 1986). The Before–After-Control–Impact design with Paired sampling (BACIP: Stewart-Oaten et al. 1986, Schroeter et al. 1993, Stewart-Oaten, *in press*; see also Campbell and Stanley 1966, Eberhardt 1976, Skalski and McKenzie 1982, Bernstein and Zalinksi 1983, Carpenter et al. 1989) meets this criterion, and is the focus of our analyses and discussion.

In its simplest formulation, BACIP requires simultaneous (Paired) sampling several times Before and After the perturbation at a Control and an Impact site. The measure of interest is the difference (hereafter referred to as "delta," Δ) in a parameter value (in its raw or transformed state) between the Control and Impact sites as assessed on each sampling date (e.g., $\Delta_{Pi} = \log(C_{Pi}) - \log(I_{Pi})$, where C_{Pi} and I_{Pi} are estimates of the parameter at the Control and Impact sites on the i^{th} date of the period P: i.e., Before or After). The average delta in the Before period is an estimate of the average spatial difference between the two sites, which provides an estimate of the expected delta that should exist in the After period in the absence of an environmental impact (i.e., the null hypothesis). The difference between the average Before and After deltas ($\Delta_{B\cdot} - \Delta_{A\cdot}$) provides a measure of the magnitude of the environmental impact. Confidence in this estimate is determined by the variation in deltas (among sampling dates within a period, S_Δ), as well as the number of sampling dates (i.e., replicates) in each of the Before and After periods ($n_B + n_A = n$). For the purposes of this study, we define

$$\text{Effect size} = \Delta_{B\cdot} - \Delta_{A\cdot}, \quad (1)$$

$$\text{Variability} = S_\Delta = [\Sigma (\Delta_{Pi} - \Delta_{P\cdot})^2]^{1/2}/(n_P - 1)^{1/2}, \quad (2)$$

$$\text{Standardized effect size} = |\Delta_{B\cdot} - \Delta_{A\cdot}|/(2 \times S_\Delta). \quad (3)$$

We assume for convenience that variability (S_Δ), as well as sample size (n_P), are equal in the Before and After periods (but see Stewart-Oaten et al. 1992). Note that the standardized effect size (Eq. 3), which consists of two components (defined by Eqs. 1 and 2) expresses the effect size in standard deviation units and enters directly into conventional calculations of power (Cohen 1977). We double the standard deviation of deltas (S_Δ) in the denominator of Eq. 3 based on the assumption that the resulting test will be two-tailed (Gill 1978).

Unlike other designs, the variability of interest, S_Δ, is not a simple measure of within-site sampling variability. Rather, it is a measure of the actual temporal variation in deltas, as well as within-site sampling error (which contributes to error in estimating the actual delta on any date). Fig. 1 illustrates how this variability of deltas can be altered without any change in the average temporal variability of a parameter (e.g., density), or in the amount of within-site sampling error. The critical feature in determining the variability among deltas is the extent to which estimates of parameters at the two sites track one another though time; Magnuson et al. (1990) refer to this as "temporal coherence."

To aid in the planning of a BACIP study, it would be helpful to find previous BACIP studies conducted in a comparable situation (e.g., similar perturbation in a similar environment) and review the results for variability and effect size. This would permit estimation of the number of sampling dates needed to achieve a given level of power (e.g., Bernstein and Zalinski 1983) or a given amount of confidence in estimates of the effect size (e.g., Bence et al., *in press*, Stewart-Oaten, *in press*). For example, parameters with large standardized effect size (i.e., relatively large effect size and small variability) will yield more powerful assessments with fewer sampling events than parameters with low standardized effect size. Obtaining an adequate number of sampling events in the Before period is crucial in a BACIP assessment, since once the perturbation begins it is no longer possible to obtain additional Before samples. Unfortunately, there are few existing BACIP studies that permit this type of analysis.

In the absence of this information, other data could be used to guide the design of BACIP studies. Two types of non-BACIP studies are more common and can offer insight. The first are long-term studies that document natural spatial and temporal variability, and therefore can provide estimates of S_Δ (Eq. 2). The second are "After-only" studies that assess impacts using a post-impact survey of sites that vary in proximity to the perturbation. After-only studies are a common type of field assessment approach, but they confound effects

TABLE 1. List of the types of parameters used to explore natural temporal variability in deltas—the differences in parameter values between the Control and Impact sites (from the long-term study)—and to obtain estimates of effect size from an existing perturbation (from the "After-only" study).

Parameter type*	Source: Long-term study (Variability)	Source: After-only study (Effect size)
Chemical–Physical		
Water temperature (no. depths)	2	2
Seston characteristics	3	0
Sediment quality	2	2
Sediment elements	11	9
Water column elements	12	8
Individual-based		
Field collections		
Urchin size and condition	5	0
Cumacean body size	2	0
Transplants		
Mussel performance	(10)†	12
Abalone performance	0	4
Population-based (no. of taxa)		
Band transects	6	0
Infaunal cores	11	10
Quadrats	1	0
Emergence traps	4	0
Re-entry traps	3	0

* For each parameter type, we give the number of parameters quantified at each site (e.g., for infaunal density, 11 taxonomic groups yielded sufficient data for analysis in the long-term study). Details on parameters are given in the Methods section.

† The 10 estimates of variability for mussel performance, in parentheses, were collected as part of the After-only study but analyzed in the same manner as data from the long-term study.

of the perturbation with natural spatial variability. Still, After-only studies can suggest the size of effects that might occur in response to a particular perturbation (Eq. 1).

In this paper we illustrate how information from long-term studies and After-only studies can be combined to help plan BACIP studies. We show how this information can be used to guide the selection of parameters and determine sampling schedules given constraints of time and funding. Our presentation consists of four analytical steps: (1) estimation of temporal variability of deltas using results from a long-term study; (2) estimation of the likely magnitude of impacts using results from an After-only study; (3) determination of the number of sampling dates required to detect the estimated impact given the background variability (at a specified level of power); and (4) exploration of serial correlation, using the long-term data set, to assess the time necessary to achieve the required number of independent sampling dates. We contrast results for chemical–physical (e.g., chemical concentrations, sediment characteristics), individual-based biological (e.g., body size, growth), and population-based biological (e.g., density) parameters, and conclude there is a critical need to increase the use of individual-based parameters in field studies of environmental impacts.

METHODS

Background

To help guide the planning of a BACIP study of a particular planned intervention, it would be best to examine results of several preexisting BACIP studies that examined impacts on many parameters in response to the same intervention in identical environments. Of course, such studies do not (and cannot) exist, but the congruence between this ideal and the realized match serves as a guide to the potential accuracy of the general guidelines that emerge.

The first step in this process is to define the intervention. To illustrate our approach, we focus on the nearshore discharge of an aqueous waste called "produced water." Produced water is a complex wastewater generated from the production of oil and contains a variety of petroleum hydrocarbons, heavy metals, and other potential pollutants (Middleditch 1984, Higashi et al. 1992). Although concerns have been raised about possible environmental effects of produced water in marine environments (Neff 1987, Neff et al. 1987, Osenberg et al. 1992*b*, Raimondi and Schmitt 1992), there have been no field assessments with sufficient Before data to allow separation of impacts from other sources of spatial and temporal variability (Carney 1987; also see Underwood 1991, Osenberg et al. 1992*a*).

We explore results from a long-term study of natural spatial and temporal variability and an "After-only" study to substitute for the absence of existing BACIP studies. The two studies were both conducted in nearshore habitats along the coast of Santa Barbara County in southern California. The benthic environments are both dominated by soft-bottom habitats, and the studies used many of the same methods and quantified many of the same parameters. In each study, parameters had been selected based upon their perceived relevance to the impacts of produced water (e.g., Boesch and Rabalais 1987). (Because the long-term study is actually part of the "Before" sampling of a BACIP study of produced-water impacts, even the parameters examined in this study were selected with respect to produced-water discharge.) However, these parameters, which include chemical, physical, and biological characteristics (Table 1), are commonly measured in field assessments of other impacts in marine environments. We next review the two studies, the methods that were used, and the parameters that were measured.

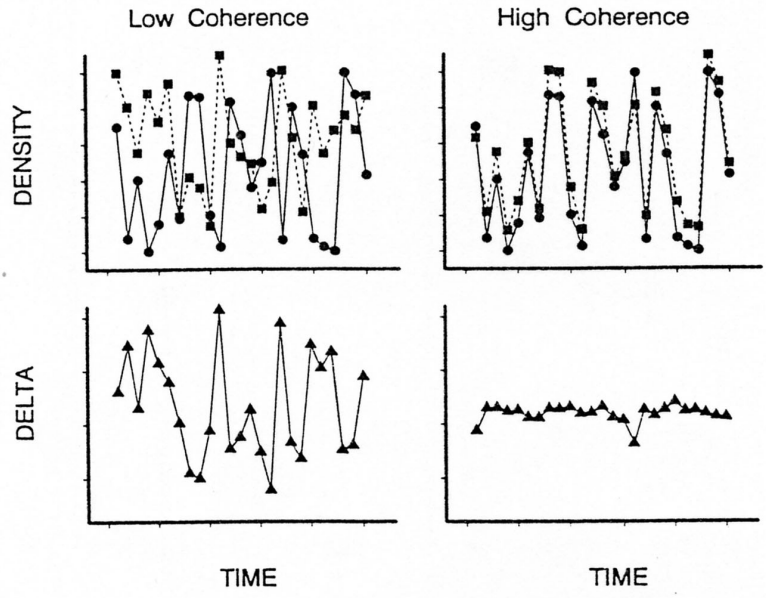

FIG. 1. Patterns of spatial and temporal variation in population densities that lead to high and low variation in deltas. (Δ = difference in parameter values between the Control and Impact sites.) Simulated data (top panels) are from two pairs of sites. In both panels temporal variation in density (at a site) and the average difference between the sites are similar. The panels differ in the degree to which the estimated densities at the paired sites track one another through time. On the left, poor tracking (i.e., low coherence: Magnuson et al. 1990) leads to a low correlation between densities at the two sites ($r = -0.25$), while on the right, good tracking (i.e., high coherence) leads to a stronger correlation in densities ($r = 0.98$). The bottom graphs show the resulting differences in density (deltas). Low temporal coherence in densities (or any other parameter of interest) leads to high variability in deltas, while high coherence leads to low variability in deltas.

Natural variability assessed from long-term study

The two sites that comprise the long-term study are located ≈1.6 km apart offshore of Gaviota, California (≈34°27′29″ N, 120°12′43″ W) at a water depth of ≈27 m. Various biological and chemical–physical parameters (Table 1) were sampled at the sites for periods ranging from 1.5 to just over 3 yr beginning in February 1988. For a given sampling date a single value was obtained for each parameter at each site, and a delta was calculated as the difference between the log-transformed values at the two sites for that i^{th} date:

$$\Delta_i = \log(X_{1i}) - \log(X_{2i}), \quad (4)$$

where X_{1i} and X_{2i} are the values of parameter X at each of the two sites (1 and 2) on the i^{th} date. Original parameter values were log-transformed to better satisfy assumptions of additivity required by BACIP (Stewart-Oaten et al. 1986) and to facilitate comparison of deltas for parameters measured in different units (the transformed deltas are unitless). For each parameter, variability was quantified as the standard deviation of the deltas (S_Δ) calculated over all available sampling dates (Eq. 2).

Population-based parameters.—Densities of infaunal organisms were estimated ≈8 times per year. On each sampling date 12 cores (each 78 cm² × 10 cm deep) were collected. Samples were preserved in a 10% buffered formalin solution and sieved through a 0.5-mm mesh sieve. Organisms were identified and counted from at least four of these cores per site per sampling date. Because this community is extremely speciose, with many species represented by only a few organisms or by zero counts on particular dates, and because zeros can cause difficulties in BACIP analyses (Stewart-Oaten et al. 1986), infaunal organisms were grouped into broad taxonomic units, such as families and classes (see discussions on aggregation in Herman and Heip [1988], Warwick [1988], and Frost et al. [1992]).

Numbers of infaunal organisms that migrated from the sediments into the overlying water (i.e., demersal zooplankton) were estimated using two emergence funnel traps (each covering a bottom area of 0.23 m²) and three reentry traps (each 0.05 m² in area), which were deployed at both sites ≈8 times per year (for more detail on trap designs and function, see Alldredge and King 1980, Stretch 1983). Traps were set out for a 24-h period. Following retrieval, contents were preserved, sieved through a 0.5-mm mesh sieve, and organisms

were identified and counted as with the infaunal cores (Table 1).

Densities of larger epifaunal and demersal organisms (e.g., fish, sea stars, tube anemones) were estimated visually along band transects by divers. Two band transects (each 40 m × 1 m) were established along the 27-m isobath at both sites on each sampling date, and all large organisms within the transect were counted. Most were identified to species, although we grouped many of them into larger taxonomic units for these analyses. Due to their greater maximum density, white sea urchins (*Lytechinus anamesus*) were counted in five non-permanent quadrats, each 1 m^2 in area, at both sites on all dates. Densities of urchins and other epifaunal and demersal organisms were estimated 8–12 times per year.

Individual-based parameters.—The size (length of metasome) of two cumacean species was measured from samples obtained from the emergence traps. Other individual-based parameters (Table 1), including average test diameter, gonad mass, somatic tissue mass, and gonadal/somatic index, were calculated from samples of the white sea urchin, *Lytechinus anamesus*. The average condition of individual urchins of a given size was estimated by calculating adjusted means for each site and date based on ANCOVA using each collection as a group, log(test diameter) as the covariate, and log(tissue mass) as the response parameter. Urchins were sampled for these analyses 11 times during the study. As part of the After-only study, we also obtained estimates of variability for several other individual-based parameters derived from study of the mussel *Mytilus californianus* (see below: *Combining results on effect size and natural variability*).

Chemical–physical parameters.—Chemical and physical parameters were examined that were thought to be indicative of the future plume's chemistry (e.g., elevated levels of certain heavy metals) or of the discharge's physical effects (e.g., altered sediment traits due to scouring of substrate or altered sedimentation rates and temperature due to local oceanographic effects) (Table 1). Seston flux was estimated by particulate accumulation in two sediment traps (5.1 cm in diameter) that were filled with a mixture of seawater, formalin, and salt; the dense preservative remained in the sediment traps during the deployment and had an initial salinity of ≈65 g/L and a formalin concentration of 5%. Sediment traps were deployed ≈3 m above the sediments and retrieved by divers after 3–7 d. Traps were deployed ≈8 times per year. Prior to analysis, large invertebrates were removed (aided by a dissecting microscope), following which the dry mass and ash free dry mass (AFDM) of the particles were determined. Sedimentation rate was calculated as the mass of material (on a dry-mass or AFDM basis) per square centimetre per day. The percentage of organic matter in the seston was estimated as the ratio of AFDM to dry mass.

Sediment grain size and percentage of organic matter were characterized from two sediment cores (20.3 cm^2/core, 5 cm deep) collected from both sites ≈8 times per year. Sediment organic matter (SOM) was estimated based on combustion (for 4 h at 450°C) of subsamples from one core. The fine sediment fraction (percentage) was estimated from the other core as the percentage (by dry mass) of the sample that passed through a 0.063-mm mesh sieve.

Water temperature was recorded approximately monthly at 3 m depth intervals. Here we use data for the 6 m and 21 m depths.

Surficial sediments (approximately the top 1 cm) were collected 4 times per year for analyses of trace and bulk elements. Three samples were collected at each site in acid-cleaned polyethylene containers by divers using trace metal clean-sampling techniques. Any overlying water was decanted and samples were frozen. Sediments were later thawed and extractions performed by leaching 2 g sediment in 20 mL of 0.5 mol/L HCl for 24 h. The leachate was then filtered through a 0.45-μm mesh teflon filter using procedures reported previously (Oakden et al. 1984). This extraction is considered to be relatively selective for the biologically available concentrations of many metals, such as Pb, Cu, and Ag (Luoma et al. 1991). Leachates were analyzed for bulk elements (Al, Ca, Fe, Mg, Mn, P) and trace elements (Ba and Zn) by inductively coupled plasma–atomic emission spectrometry (ICP–AES). Other trace element (Cr, Cd, and Pb) concentrations were determined by graphite furnace atomic absorption spectrometry (GFAAS). Environment Canada reference sediments (BCSS-1, MESS-1, PACS-1) were analyzed concurrently to quantify the extraction efficiency for each element. All analyses were normalized to sediment dry mass.

Unfiltered water samples were collected 2 times per year from each site at two depths (surface and 21 m). The samples were extracted using the ammonium 1-pyrrolidinedithiocarbamate/diethylammonium diethyldithiocarbamate (APDC/DDC) extraction method described by Bruland et al. (1985). Trace element concentrations (Ag, Cd, Co, Cu, Fe, Ni, Pb, Zn) were measured by GFAAS. Procedural blanks were measured in each sample set. Each set of samples was analyzed in duplicate after a series of intercalibrations with Environment Canada reference seawater (CASS-1). These analyses were conducted concurrently with analyses of sea water from San Francisco Bay, and details of the procedural blanks and intercalibrations are provided in a report on those data (Flegal et al. 1991).

Effect size estimated from an After-only study

The After-only study was conducted at a produced-water outfall located near Carpinteria, California (34°23′10″ N, 119°30′31″ W) that was the subject of recent investigations of potential environmental im-

pacts (Higashi et al. 1992, Krause et al. 1992, Osenberg et al. 1992*b*, Raimondi and Schmitt 1992). The Carpinteria sites are ≈50 km from the Gaviota sites. Although the two locations (Carpinteria and Gaviota) are both open-coast, soft-bottom environments in the Santa Barbara Channel and have many species in common, the bottom depths sampled differed between the Carpinteria (11 m) and Gaviota (27 m) sites.

An intensive spatial survey of infauna was conducted along the 11 m isobath at the Carpinteria study area in 1990, ≈12 yr after produced water was first discharged at this location (Osenberg et al. 1992*b*). In a single survey, 20 sites were sampled along a spatial gradient from 2 to 1000 m up coast (West) and down coast (East) of the diffusers. Infaunal densities were estimated at each site by collecting eight cores (78 cm^2 per core to a depth of 10 cm). These were processed as described for the long-term study, and a mean density was calculated for each taxon at each of the 20 Carpinteria sites.

All chemical–physical parameters examined as part of the long-term study at Gaviota were also estimated at the Carpinteria sites, except those related to seston quality and deposition and several elements. Methods were identical to those used at Gaviota (described above, see *Natural variability assessed . . .*).

Individual-based biological data were obtained by transplanting individuals of known size and/or age to several of the sites. Mussels (*Mytilus californianus* and *M. edulis*) were transplanted to six sites to determine if proximity to the outfall influenced their individual growth and condition (Osenberg et al. 1992*b*). Forty individuals from a uniform size distribution (range: 20–60 mm shell length) of a mussel species were put into a bag of 1.25-mm mesh oyster netting, and one bag of *M. californianus* and one of *M. edulis* were attached to buoy lines ≈3 m above the sediments. Mussels were retrieved and frozen after 3–4 mo in the field. Final shell length, initial shell length, dry gonadal tissue mass, and somatic tissue mass were then measured for each mussel. Site-specific estimates of average gonadal condition (gonad mass at a given size), somatic condition, total condition, and gonadal–somatic index were obtained by running analyses of covariance (ANCOVA) for each parameter for each mussel species using log(final shell length) as the covariate. Average shell growth and tissue production were estimated using log(initial shell length) as the covariate. Adjusted means were obtained for each parameter at each of the six sites.

Abalone larvae were raised in the laboratory and transplanted in small flow-through cages to 6–8 sites located 5–1000 m from the diffuser (Raimondi and Schmitt 1992). Three measures of per-capita settlement and metamorphosis were derived from transplants that lasted ≈4 d: (1) the proportion of late-stage larvae that successfully settled in the field, (2) the proportion of late-stage larvae that successfully metamorphosed in the field, and (3) the proportion of early-stage larvae that subsequently settled in the laboratory after addition of a chemical inducer (for details, see Raimondi and Schmitt [1992]). An additional measure of individual performance was obtained from a short-term transplant: the proportion of early-stage larvae still swimming after 6 h in the field.

To obtain estimates of the magnitude of impacts due to produced water we calculated means (e.g., of density or performance) for three distance categories: Near (sites <25 m of the diffuser), Far (25–200 m), and Control (>200 m). We then calculated near-field and far-field effect size as the difference between log(Mean Near or Mean Far) and log(Mean Control). This is equivalent to the impact size (expressed in log units) of a BACIP study (Eq. 1) assuming no natural spatial variation between the sites (i.e., $E(\Delta_B) = 0$). While this assumption cannot be tested without Before data, available evidence suggests that natural spatial gradients are small relative to the impacts of produced water (Osenberg et al. 1992*b*, Raimondi and Schmitt 1992).

Combining results on effect size and natural variability

For parameters that were common to both the After-only study and the long-term study, the standardized effect size was calculated as the ratio between the absolute value of the effect size, which was obtained from the long-term study, and twice the standard deviation of deltas, which was obtained from the After-only study (Eq. 3). In some cases, however, the same parameters were not measured in both studies, and other steps were required before proceeding with the power analyses.

For example, there were four chemical–physical parameters that provided estimates of effect size but not variability. All four parameters were elemental concentrations (i.e., Cu in sediments and Co, Ag, and Pb in the water column), so we used the average standard deviation for other elements (in either the sediments or water column) in the calculation of the standardized effect size.

Conversely, there were chemical–physical and population-based parameters that provided estimates of variability but not effect size (i.e., parameters estimated from sediment traps, band transects, emergence traps, reentry traps, and quadrats in addition to several elemental concentrations: Table 1). For these parameters we calculated standardized effect sizes using the average effect size for similar parameters that were measured as part of the After-only study.

Estimating standardized effect sizes for individual-based parameters posed a more difficult analytical problem because the individual-based data from the long-term study were derived from field collections of organisms, whereas the transplants conducted in the After-only study used organisms of known size, or cohorts of known number and age. Therefore, the trans-

plants removed several sources of potential variability present in estimates from the long-term study. Because mussels had been transplanted during four different periods (spread over a total of 14 mo), we were able to obtain estimates of variability for the mussel parameters. The standard deviation of differences between log-transformed parameters measured at the 1000-m and 100-m sites was calculated for ten of the mussel parameters over the four periods. Because the 100-m site is probably influenced slightly by the discharge of produced water (Osenberg et al. 1992*b*, Raimondi and Schmitt 1992), this approach will overestimate S_Δ if there is temporal variation in the effects of produced water.

Standardized effect size was then calculated as explained above using these new estimates of variability for all mussel parameters except tissue production (for which we had only one survey and therefore could not estimate S_Δ, the variation among sampling dates within a period). The mean standard deviation of deltas for the mussel parameters was used to estimate the standardized effect sizes for mussel tissue production and abalone performance parameters, which lacked estimates of S_Δ. The standardized effect sizes for the individual-based parameters derived from the long-term study were calculated using the mean effect sizes based on the mussel and abalone transplants.

For each parameter we estimated the sample size (total number of sampling dates in the Before and After periods) needed to have an 80% chance of detecting ($\alpha = .05$) an impact characterized by the parameter's standardized effect size. All power analyses were based on two-tailed t tests as provided in Gill (1978). The number of sampling dates in the Before and After periods was assumed to be equal.

Serial correlation

The power analyses yield the number of independent sampling events (i.e, dates) needed for a given level of power (e.g., 80%). The time scale over which those samples must be collected will depend on the amount of serial correlation in the time series of deltas for each parameter (Stewart-Oaten et al. 1986). Serial correlation can be directly incorporated into the analyses of BACIP data (Stewart-Oaten et al. 1992), but power is greatest when serial correlation is absent. Therefore, we tried to determine the most intensive sampling schedule that would avoid substantial amounts of serial correlation. By doing so, we could roughly translate the number of independent sampling events into an estimate of the minimum amount of time required by the BACIP study.

Because rigorous analyses of serial correlation require long time series of data, and because the approach we outline here is imprecise to begin with (i.e., extrapolating from two different studies to the design of a future one), we used a simpler approach to provide a general guide to sampling frequency. For each parameter sampled as part of the long-term study, we examined the correlation between the delta measured on one sampling date (Δ_i) and the delta measured on the next date on which sampling for that parameter was conducted (Δ_{i+1}). Only parameters with data from ≥ 8 dates were included in the analyses.

RESULTS

Natural variability assessed from a long-term study

Data from the long-term study revealed that the variation in deltas (i.e., in the difference in parameter values between sites) was lowest for chemical–physical parameters, intermediate for individual-based parameters, and greatest for population-based parameters (Fig. 2). Most (28 of 30) of the chemical–physical parameters exhibited less variation in deltas than did the least variable population-based parameter. Almost all of the population-based parameters (24 of 25) were more variable than the most variable of the 7 individual-based parameters. Within a parameter group, no systematic differences were apparent among data collected using different techniques (e.g., densities based on infaunal cores vs. band transects, or water column elements vs. sediment elements), and there were no apparent trends among the population-based parameters related to the level of taxonomic aggregation (see Frost et al. 1992). All else being equal, these data suggest that chemical–physical parameters will provide more reliable indicators of environmental impacts than population-based parameters due to their smaller variability.

Effect size estimated from After-only study

The After-only study provided estimates of effect sizes, which varied with proximity of the sampled sites to the produced-water diffuser. In general, sizes of effects were correlated ($r = 0.62$, $n = 47$) for sites near to and far from the diffuser (Fig. 3), and the magnitudes of effects consistently were greatest nearer the diffuser. This pattern suggests that impacts diminished with distance away from the disturbance.

Both positive and negative changes in parameter values with distance from the diffuser were observed, and the sign depended on the particular parameter or parameter group examined. For example, concentrations of water-column metals were higher nearer the diffuser, whereas most measures of individual performance were lower. Similarly, some taxa were more abundant closer to the diffuser, while others were less abundant. These two patterns in density probably reflect positive responses to organic enrichment (from oil constituents) and negative responses to toxicants present in pro-

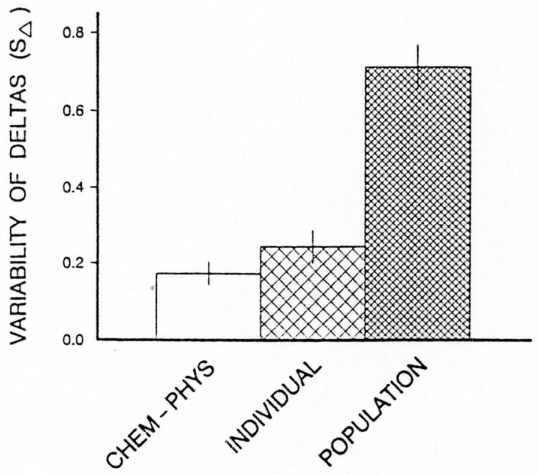

FIG. 2. Temporal variability in estimates of the deltas (S_Δ) for chemical–physical, individual-based, and population-based parameters. Data were derived from the long-term study. For each parameter on each sampling date, a delta was estimated based on the difference between the log-transformed means at two sites (e.g., Log(mean density at Site 1 on date *i*) − Log(mean density at Site 2 on date *i*)). Shown are the standard deviations of deltas (mean ± 1 SE) for parameters in each of the three groups. Means are based on 30, 7, and 25 different parameters for chemical–physical, individual, and population groups, respectively. Here all individual-based data are derived from field collections.

duced water (e.g., Spies and DesMarais 1983, Osenberg et al. 1992*b*; see also Pearson and Rosenberg 1978, Ferris and Ferris 1979).

In evaluating power the crucial factor is the absolute size of the change and not the sign (i.e., a positive or negative response). Although quite variable, the population-based parameters had absolute values of effect sizes that were about twice those for individual-based parameters, and four times larger than effect sizes for chemical–physical parameters (Fig. 4). This pattern was similar for both Near and Far sites ($r = 0.72$, $n = 47$), although the overall magnitude of effects was lower at the Far sites (Fig. 4). For simplicity, we focus on results from the Near sites in the following sections.

Combining results on effect size and natural variability

Estimates of natural variability in individual-based parameters were derived from field collections, whereas those for effect size were obtained from transplants. To make the estimates more comparable, we calculated variability of deltas for individual performance of mussels from four separate transplants in the After-only study. The results show that all 10 indices of mussel performance were relatively invariable over time (S_Δ mean ± 1 SE = 0.080 ± 0.20, range: 0.007–0.220). Indeed, most (70%) of these estimates of mussel performance were less variable than almost all (94%) of the parameters measured in the long-term study.

The results from the long-term study and the After-only study yielded the opposite conclusions about the power associated with different parameter groups. On one hand, the population-based (and individual-based) parameters should be the most powerful due to their larger average effect sizes (Fig. 4), while the chemical–physical (and individual-based) parameters should be more powerful due to their smaller average variability (Fig. 2). Ultimately, the more powerful parameters will be those with the greatest standardized effect size (i.e., signal to noise ratio: Eq. 3). Due to their relatively large effect sizes but low variability, individual-based parameters (particularly those derived from transplants) had larger standardized effect sizes than either the chemical–physical or population-based parameters (Fig. 5). With respect to the individual-based parameters, the transplants yielded standardized effect sizes that were >3 times larger than those derived from field collections.

The standardized effect sizes for both chemical–physical and population-based parameters were low and quite similar (Fig. 5), due to the lower variability associated with chemical–physical parameters (Fig. 2) and the greater effect sizes associated with population-based parameters (Fig. 4). The standardized effect sizes for these two groups of parameters were one-half and one-seventh the magnitude of those for individual-based parameters derived from field collections and transplants, respectively (Fig. 5).

These results indicate that power to detect changes from exposure to produced water should be greatest

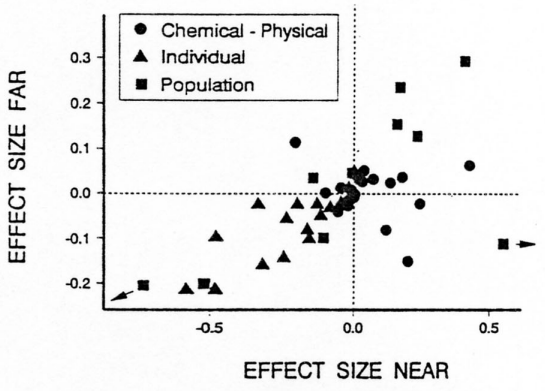

FIG. 3. Effect sizes estimated from sites near and far from an operating produced-water diffuser (an "After-only" study). Positive values indicate larger parameter values near (or far from) the diffuser relative to control sites, while negative values indicate the opposite. The two population-based parameters next to the arrows have effect sizes that are off the scale: (−0.92, −0.85) and (0.917, −0.13).

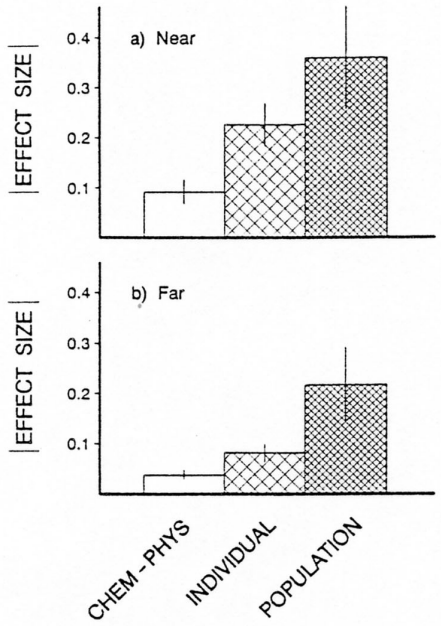

FIG. 4. Absolute effect sizes (mean ± 1 SE) for chemical–physical, individual-based, and population-based parameters based on sites (a) near and (b) far from the diffuser. Sample sizes (number of parameters) were 21, 16, and 10 for the chemical–physical, individual, and population groups, respectively.

for individual-based parameters derived from transplants, and next greatest for individual-based parameters obtained from field collections. For an equivalent number of estimates (i.e., sampling dates), power should be considerably lower for chemical–physical and for population-based parameters. For example, based upon average standardized effect sizes (Fig. 5) and a Type I error rate of .05, the numbers of independent sampling dates needed to achieve power of 80% are ≈4 for individual-based parameters from transplants, 24 for individual-based parameters from field collections, 90 for chemical–physical parameters, and 95 for population-based parameters.

Most individual-based parameters required <20 (and typically <10) sampling dates to achieve 80% power (Fig. 6). Over half of the chemical–physical and population-based parameters required 100 or more sampling dates to reach 80% power (Fig. 6). To provide an idea of how many parameters would have high power for a logistically reasonable number of surveys that would also permit model development and testing (Stewart-Oaten, *in press*), we determined the fraction of parameters in each group with a sufficiently large standardized effect size (>0.52) to yield power of at least 80% with 30 sampling dates ($n_B = n_A = 15$). Using this guideline, 81% (13/16) of individual-based parameters from transplants and 43% (3/7) of those from field collections had power that exceeded 80%. By contrast, only 18% (6/34) of the chemical–physical and 4% (1/26) of the population-based parameters achieved this level of power after 30 surveys.

The preceding analyses were based on effect sizes estimated from sites Near the produced-water diffuser. Repeating the analyses using data from the Far sites yielded similar patterns, although, as expected, the overall power was much lower or the number of sampling dates needed for a given level of power was much higher. For example, the smaller effect sizes (estimated from sites far from the diffuser) resulted in more than half of the parameters in each of the three groups requiring >100 sampling dates to achieve 80% power. Only 26% of the individual-based parameters (all from transplants) required <30 sampling dates, while none of the chemical–physical or population-based parameters achieved the same power with 30 dates.

Serial correlation

Our analyses suggested that impacts on individual-based parameters are the most likely to be detected with a limited number of sampling dates. The analyses assumed that each sampling date provided an independent estimate of the true deltas (i.e., the underlying difference in parameter values between the Control and Impact sites). We examined patterns of serial correlation from the long-term study to gain insight into the

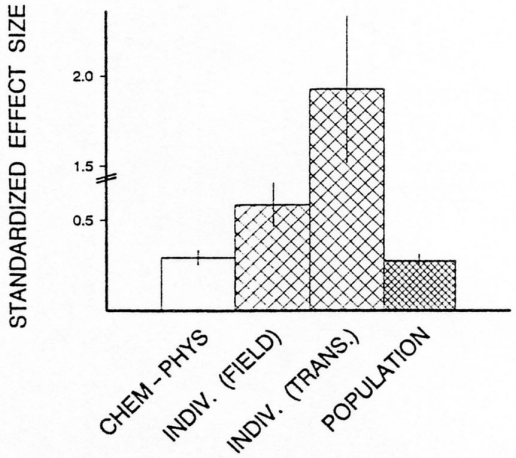

FIG. 5. Standardized effect size (|Effect size|/[2 × S_Δ]) for each parameter group; the measure is the ratio of effect size to twice the standard deviation of delta. Shown are means ± 1 SE, based on 34, 7, 16, and 26 parameters (from left to right). Individual-based parameters are divided into estimates derived from field collections and those derived from transplants of marked individuals or caged cohorts. Note the break in the vertical scale between 0.7 and 1.5.

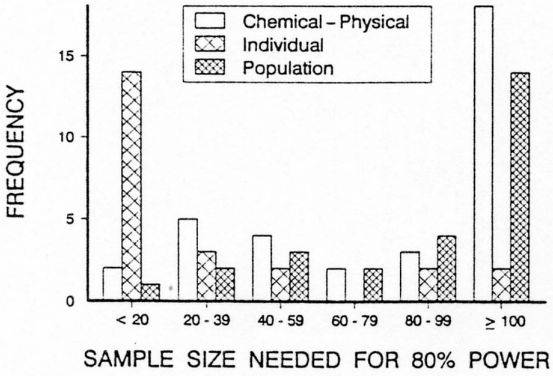

FIG. 6. Frequency distribution of the sample size (number of independent sampling dates) for parameters in each group that is required for 80% power. Power analyses are based on standardized effect sizes (Fig. 5).

frequency with which samples could be collected without grossly violating the assumption of temporal independence. This provided information on the time frame needed to collect series of independent samples.

There were no cases of significant ($P < .05$) negative serial correlation, and only 8% (4 of 50) of the parameters exhibited significant positive serial correlation (e.g., Fig. 7). Of the four parameters with positive serial correlation, two were chemical–physical parameters (seston sedimentation rate and seston percentage organic matter), and two were population-based parameters (densities of sea pens and sea urchins: Fig. 7c and d). None of the individual-based parameters exhibited significant serial correlation.

Serial correlation appeared to arise in the population-based parameters as a result of long-term trends in the deltas (Fig. 7c and d). For example, the white sea urchin (*Lytechinus anamesus*) exhibited strong seasonal migrations, and was present during the winter and spring but absent during the summer and fall. The relative density at the two sites appeared to be set when urchins reappeared in winter; the ranking of the two sites was consistent within a year, but varied greatly among years (Fig. 7c). This suggests that replicates should be collected only once per year, or a yearly average obtained from more frequent collections.

Density of sea pens (*Acanthoptilum* sp. and *Stylatula* sp.) exhibited an even longer term trend (Fig. 7d). One site tended to have a greater density than the other site prior to October 1989, but the reverse was true for all samples collected after this date (Fig. 7d). This could have arisen, for example, by a strong recruitment event in the fall of 1989 at only one of the sites.

Despite these two examples, serial correlation was not a general problem for the various parameters estimated in our long-term study (e.g., Fig. 7a and b). On average, the serial correlation for each of the three parameter groups was only 0.1–0.2 (Fig. 8). Simulations suggest that serial correlation of this order intro-

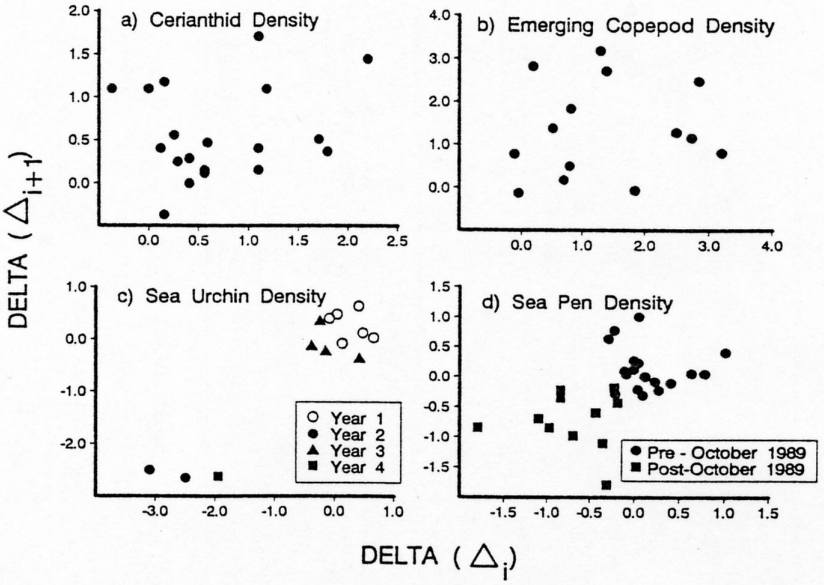

FIG. 7. Patterns of serial correlation in deltas for four population-based parameters. These are the difference in density of: (a) cerianthid (burrowing) anemones (from band-transect estimates); (b) copepods (from emergence traps); (c) white sea urchins (*Lytechinus anamesus*) (from quadrat samples); and (d) sea pen density (from band transects). There is significant serial correlation in (c) and (d), and data are separated into temporal groups to help distinguish the long-term patterns.

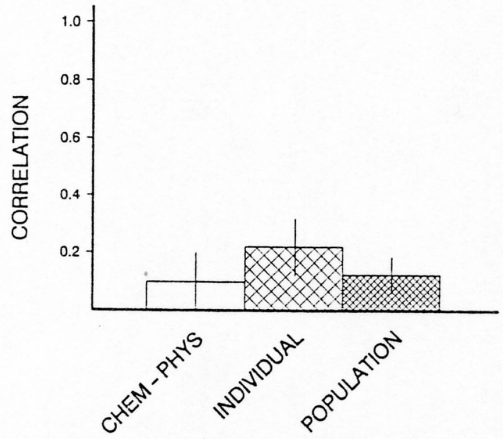

FIG. 8. Degree of serial correlation in deltas for each parameter group. Shown are means ± 1 SE, based on 18, 7, and 25 parameters for chemical–physical, individual, and population groups respectively.

duce only small error into tests of impacts (Carpenter et al. 1989, Stewart-Oaten et al. 1992).

Based on these results, we assumed that sampling could occur every 60 d without yielding substantial amounts of serial correlation. Assuming that six samples are collected per year and the Before and After periods are of equal duration, the estimates of sample size (number of independent sampling events) can be translated into the number of years the assessment study must be conducted. Achieving 80% power would require 16 yr for population-based parameters, 15 yr for chemical–physical parameters, 4 yr for individual-based parameters from field collections, and 1 yr for individual-based parameters from transplants. To achieve 80% power for only a quarter of the parameters in each group, the required study duration is reduced to 11 yr for population-based parameters, 7 yr for chemical–physical parameters, 3 yr for individual-based parameters from field collections, and <1 yr for individual-based parameters from transplants.

DISCUSSION

Because relatively few well-designed studies of planned perturbations have been completed, there is a sparse empirical base to guide the design of future assessment programs (e.g., Carney 1987, Spies 1987, Underwood 1991, Stewart-Oaten, *in press*). Recent discussions have highlighted general design considerations that should be incorporated in Before–After-Control–Impact approaches (e.g., Stewart-Oaten et al. 1986, 1992, Underwood 1994, Stewart-Oaten, *in press*), but these say little about specific considerations regarding sampling frequency and parameter selection. Often a study must be planned in the absence of sufficient preliminary data to properly guide sampling decisions (Stewart-Oaten, *in press*). It is crucial to obtain good estimates of sampling variability and the size of impacts that might arise (or that are deemed ecologically important: Underwood and Peterson 1987, Yoccoz 1991), but this information typically is lacking. In the absence of a BACIP (or analogous) study conducted previously on a similar perturbation in a similar habitat, it is vital that other existing data be used to guide specific design considerations.

Given limitations on time and funding, the selection of parameters and frequency of sampling are especially crucial features of the design process. One of the most acute constraints is the time available to collect data prior to the perturbation. In many situations the Before period probably will be rather abbreviated for a variety of reasons beyond scientific control. Therefore, parameter selection and sampling design should take into account the low numbers of temporal replicates that likely can be collected prior to the commencement of the disturbance (see Stewart-Oaten [*in press*] for discussion of model development based on these data). Key considerations in this regard are the likely variability in the parameter estimate (e.g., delta) and the probable magnitude of response to the disturbance, both of which influence statistical power to detect an effect. Constraints on the number of temporal replicates in the Before period are most likely to hamper detection of impacts on population density and chemical–physical characteristics, and least likely to affect detection of effects on individual performance. Unfortunately these results suggest that many field monitoring programs might be compromised because individual-based parameters rarely are examined (e.g., Carney 1987).

There are, however, compelling reasons to examine population and chemical–physical parameters despite the expected low power. First, chemical and physical properties describe the direct effect of many perturbations, and in many cases impacts could be ameliorated by subsequent intervention (e.g., source reduction, reduced discharge limits). Second, population attributes, such as density, reflect the ecological consequences of the disturbance, and are features of fundamental concern to resource managers and regulatory agencies. In addition, some species receive special regulatory consideration. Another reason is that, while the average power for population or chemical–physical parameters is low, some species or chemical–physical parameters will have greater power than others. The approach described here is equally useful in identifying promising candidates within a parameter group as it is in guiding allocation of effort among groups. Finally, the actual impacts of the new disturbance, of course, cannot be known a priori, and effects on populations and chemical–physical parameters certainly can be much larger (or variation much smaller) than anticipated based on extrapolations from other data sets.

It is useful to consider why the population-based and chemical–physical parameters had low and similar power, because low power arose for different reasons. Population parameters were highly responsive to produced water (i.e., larger impact), but exhibited much greater natural variability. In contrast, the chemical–physical parameters had much lower variability in deltas, but were not greatly altered by the discharge of produced water. It appears that these results generally will hold for other types of point-source disturbances in the marine environment. Many chemical–physical parameters probably are influenced largely by large-scale oceanographic processes that similarly affect nearby sites. For example, certain chemical–physical attributes (e.g., sedimentation rate, water temperature, nutrient flux) are strongly associated with upwelling conditions, which is a region-wide phenomenon (e.g., Landry and Hickey 1989). In these situations, differences in these parameter values between Control and Impact sites (i.e., the deltas) will be similar through time (see also a related discussion in Magnuson et al. [1990], which discussed temporal coherence of chemical–physical and biological parameters in freshwater lakes).

The relatively small response that we observed of chemical–physical parameters to the discharge of produced water is also consistent with recent analyses of the general effect of waste discharges on the distribution of trace elements in coastal waters. For example, massive discharges (10^9 L/d) of wastewaters in the Southern California Bight have had a negligible (<1%) impact on concentration of cadmium in those waters (Sañudo-Wilhelmy and Flegal 1991). Similarly, Schmidt and Reimers (1991) found that, in the Santa Barbara Basin, the fraction of certain metals (Cd, Cu, Ni, Pb) from human sources that is deposited in sediments near municipal outfalls is quite small (<1%) compared to the amount released. In both cases, natural inputs and physical mixing processes appeared to have reduced the contribution from human inputs to a small fraction of the background level. So for chemical–physical parameters the large spatial scale of events that drive natural variation can lead to low variability in deltas, while other natural processes can greatly diminish the signal provided by anthropogenic perturbations.

Population density, by comparison, is known to be highly responsive to local conditions, and can exhibit considerably different temporal patterns among neighboring sites (e.g., Holbrook et al. 1990, Magnuson et al. 1990, Schmitt and Holbrook 1990). The high sensitivity to local conditions potentially can translate into strong local responses to natural phenomena (thus increasing S_λ) as well as anthropogenic perturbations such as wastewater discharges (thus increasing effect size). Within-site sampling error also can contribute to the high variability as benthic populations are notoriously difficult to sample (Vezina 1988, Thrush et al. 1994).

It is important to note that the variability reported here (e.g., Fig. 2) is a measure of the variability (over time) in *estimates* of the *differences* between sites. This variability includes both the true temporal variation in deltas and variation due to sampling error within a site (which adds error to the estimation of delta on any date). The contribution of sampling error will be a function of spatial variability within a site and sampling intensity, and therefore will vary with the within-site sampling design. This suggests that the variation in deltas (S_λ) for population-based parameters could be reduced by more intensive sampling on each date, rather than increasing the number of dates. However, partitioning of observed variation for the long-term data set revealed that the deltas for population-based parameters were more variable due both to sampling error (i.e., high within-site spatial variation) and site-specific temporal variability (i.e., high variation in the actual deltas through time) (C. W. Osenberg, *personal observation*); increasing the sampling intensity within a date would reduce the observed variation (S_λ) by only $\approx$50%. Therefore, even if sampling error were removed (e.g., through more exhaustive sampling), population-based parameters still would be more variable than the chemical–physical or individual-based parameters (see Fig. 2).

Our estimates of S_λ probably are typical because the within-site sampling design of our long-term study is similar to that used in many assessment studies (see Thrush et al. 1994). The costs and benefits of adjusting within-site sampling intensity to achieve greater power can be analyzed (e.g., the importance of within-site accuracy vs. more sampling dates), although with limited resources greater precision ultimately would be accomplished at the cost of fewer sampling dates (which is the unit of replication in a BACIP design).

Difficulty in sampling populations or other parameters within sites not only can affect the variance of the estimate, it also might lead to overestimation of effect sizes from After-only studies (Figs. 3 and 4). This would be true especially for a parameter that is not affected by the perturbation, and thus should have an effect size of zero. Our approach would overestimate this effect by confounding sampling error and any underlying spatial gradient as an effect of the perturbation. If so, the calculated number of surveys (sample size) needed for a given level of power would be underestimated. While this bias will exist for any parameter, our data suggest that, on average, it will be most acute for population-based parameters. Hence, limitations on detecting impacts at the population level may be even more difficult than our analyses suggest.

In contrast to population and chemical–physical parameters, individual-based parameters had relatively high power owing to relatively low levels of variability (Fig. 2) and intermediate effect sizes (Fig. 4). Although 80% power could be achieved for many of the param-

eters we examined with fewer than 10 sampling dates, it is unwise to reduce the sampling intensity below a level at which model development and testing can be performed (Stewart-Oaten, *in press*). Our data also indicate that variability in the deltas for individual-based parameters can be reduced by use of transplants (Fig. 4), which results in increased power (Fig. 5). This presumably occurs because, compared with estimates from field collections, transplants remove noise introduced by individual variation as well as variability between sites over time. For example, size-specific growth rates can be assessed accurately using marked individuals of known size; because size can influence growth and size distributions can vary among sites (e.g., Osenberg et al. 1988), an analysis based on marked individuals is likely to be more powerful than one based on field collections.

It should be noted that several of the transplant-derived parameters for which we had relatively high power are closely related to population-based parameters, which had much lower power. For example, transplants of abalone larvae provided estimates of per capita settlement rates. In the field, natural rates of per capita settlement can be estimated from observed settlement rates and/or larval supply, both of which require estimation of density (e.g., Olson 1985, Keough 1986, Victor 1986, Raimondi 1990). Therefore, these field estimates would have considerable error for the same reasons that population parameters were highly variable. The use of transplants surmounted much of this problem by using cohorts of known size, thereby eliminating much of the variability that plagues the population parameters.

The observation that individual-based parameters may yield more powerful assessments is troubling given the rarity with which they are measured in field assessments. Care must be taken to guard against only considering parameters that yield low probabilities of demonstrable results (e.g., chemical–physical and population attributes), and inclusion of individual-based parameters could greatly increase the sensitivity of assessment studies (Carney 1987, Osenberg et al. 1992*a*; see also Jones et al. 1991). However, the need to investigate individual-based parameters goes far beyond power considerations; it is the individual-based (and demographic) parameters that provide the mechanisms that underlie changes at the population (and therefore community) level. Furthermore, these individual-based parameters provide an explicit connection with detailed laboratory studies that focus on individuals and mechanisms of toxicity. What is needed are more realistic studies of individual-based effects under field conditions combined with both mechanistic laboratory studies and field assessments of population-level consequences. Recent advances with individual-based models (DeAngelis and Gross 1992) provide an explicit framework for making these fundamental linkages among environmental chemistry, physiology, and population ecology (e.g., Hallam et al. 1990). Such models provide a powerful, mechanistic approach to assessing impacts on natural populations and complement the traditional approach of monitoring environmental impacts.

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LITERATURE CITED

Alldredge, A. L., and J. M. King. 1980. Effects of moonlight on the temporal migration patterns of demersal zooplankton. Journal of Experimental Marine Biology Ecology **44**: 133–156.

Bence, J. R., A. Stewart-Oaten, and S. C. Schroeter. *In press.* Estimating the size of an effect from a Before-After-Control-Impact-Pairs design: the predictive approach applied to a power plant study. *In* R. J. Schmitt and C. W. Osenberg, editors. Ecological impact assessment: conceptual issues and application in coastal marine habitats. University of California Press, Berkeley, California, USA.

Bernstein, B. B., and J. Zalinski. 1983. An optimum sampling design and power tests for environmental biologists. Journal of Environmental Management **16**:35–43.

Boesch, D. F., and N. N. Rabalais, editors. 1987. Long-term environmental effects of offshore oil and gas development. Elsevier Applied Science, New York, New York, USA.

Bruland, K. W., K. H. Coale, and L. Mart. 1985. Analysis of seawater for dissolved cadmium, copper and lead: an intercomparison of voltametric and atomic absorption methods. Marine Chemistry **17**:285–300.

Campbell, D. T., and J. C. Stanley. 1966. Experimental and quasi-experimental designs for research. Rand McNally, Chicago, Illinois, USA.

Carney, R. S. 1987. A review of study designs for the detection of long-term environmental effects of offshore petroleum activities. Pages 651–696 *in* D. F. Boesch and N. N. Rabalais, editors. Long-term effects of offshore oil and gas development. Elsevier Applied Science, New York, New York, USA.

Carpenter, S. R., T. M. Frost, D. Heisey, and T. K. Kratz. 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. Ecology **70**: 1142–1152.

Cohen, J. 1977. Statistical power analysis for the behavioral sciences. Academic Press, New York, New York, USA.

Cooper, S. D., and L. A. Barmuta. 1993. Field experiments in biomonitoring. Pages 399–441 *in* D. M. Rosenberg and V. H. Resh, editors. Freshwater biomonitoring and benthic

macroinvertebrates. Chapman and Hall, New York, New York, USA.

DeAngelis, D. L., and L. J. Gross, editors. 1992. Individual-based models and approaches in ecology: populations, communities, and ecosystems. Chapman and Hall, New York, New York, USA.

Eberhardt, L. L. 1976. Quantitative ecology and impact assessment. Journal of Environmental Management **4**:27–70.

Eberhardt, L. L., and J. M. Thomas. 1991. Designing environmental field studies. Ecological Monographs **61**:53–73.

Fairweather, P. G. 1991. Statistical power and design requirements for environmental monitoring. Australian Journal of Marine and Freshwater Research **42**:555–567.

Faith, D. P., C. L. Humphrey, and P. L. Dostine. 1991. Statistical power and BACI designs in biological monitoring: comparative evaluation of measures of community dissimilarity based on benthic macroinvertebrate communities in Rockhole Mine Creek, Northern Territory, Australia. Australian Journal of Marine and Freshwater Research **42**: 589–602.

Ferris, V. R., and J. M. Ferris. 1979. Thread worms (Nematoda). Pages 1–33 *in* C. W. Hart, Jr., and S. L. H. Fuller, editors. Pollution ecology of estuarine invertebrates. Academic Press, New York, New York, USA.

Flegal, A. R., G. J. Smith, G. A. Gill, S. Sañudo-Wilhelmy, and L. C. D. Anderson. 1991. Dissolved trace element cycles in the San Francisco Bay estuary. Marine Chemistry **36**:329–363.

Frost, T. M., S. R. Carpenter, and T. K. Kratz. 1992. Choosing ecological indicators: effects of taxonomic aggregation on sensitivity to stress and natural variability. Pages 215–227 *in* D. H. McKenzie, D. E. Hyatt, and V. J. McDonald, editors. Ecological indicators. Volume 1. Elsevier Applied Science, New York, New York, USA.

Gill, J. L. 1978. Design and analysis of experiments in the animal and medical sciences. Volumes 1–3. Iowa State University Press, Ames, Iowa, USA.

Green, R. H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley & Sons, New York, New York, USA.

———. 1989. Power analysis and practical strategies for environmental monitoring. Environmental Research **50**: 195–205.

Hallam, T. G., R. R. Lassiter, J. Li, and W. McKinney. 1990. Toxicant-induced mortality in models of *Daphnia* populations. Environmental Toxicology and Chemistry **9**:597–621.

Herman, P. M. J., and C. Heip. 1988. On the use of meiofauna in ecological monitoring: who needs taxonomy? Marine Pollution Bulletin **19**:665–668.

Higashi, R., G. Cherr, C. Bergens, T. Fan, and D. Crosby. 1992. Toxicant isolation from a produced water source in the Santa Barbara Channel. Pages 223–233 *in* J. P. Ray and F. R. Englehardt, editors. Produced water: technological/environmental issues and solutions. Plenum, New York, New York, USA.

Holbrook, S. J., R. J. Schmitt, and R. F. Ambrose. 1990. Biogenic habitat structure and characteristics of temperate reef fish assemblages. Australian Journal of Ecology **15**: 489–503.

Jones, M., C. Folt, and S. Guarda. 1991. Characterizing individual, population and community effects of sublethal levels of aquatic toxicants: an experimental case study using *Daphnia*. Freshwater Biology **26**:35–44.

Keough, M. J. 1986. The distribution of a bryozoan on seagrass blades: settlement, growth, and mortality. Ecology **67**:846–857.

Krause, P. R., C. W. Osenberg, and R. J. Schmitt. 1992. Effects of produced water on early life stages of a sea urchin: stage-specific responses and delayed expression. Pages 431–444 *in* J. P. Ray and F. R. Englehardt, editors. Produced water: technological/environmental issues and solutions. Plenum, New York, New York, USA.

Landry, M. R., and B. M. Hickey, editors. 1989. Coastal oceanography of Washington and Oregon. Elsevier Science, Amsterdam, The Netherlands.

Luoma, S. N., D. J. Cain, C. Brown, and E. V. Axtman. 1991. Trace metals in clams (*Macoma balthica*) and sediments at the Palo Alto mudflat in South San Francisco Bay: April, 1990–April, 1991. United States Geological Survey Report **91-460**.

Magnuson, J. J., B. J. Benson, and T. K. Kratz. 1990. Temporal coherence in the limnology of a suite of lakes in Wisconsin, U.S.A. Freshwater Biology **23**:145–159.

Mapstone, B. D. *In press.* Scalable decision criteria for environmental impact assessment: Type I and Type II errors. *In* R. J. Schmitt and C. W. Osenberg, editors. Ecological impact assessment: conceptual issues and application in coastal marine habitats. University of California Press, Berkeley, California, USA.

Middleditch, B. S. 1984. Ecological effects of produced water effluents from offshore oil and gas production platforms. Ocean Management **9**:191–316.

Neff, J. M. 1987. Biological effects of drilling fluids, drill cuttings and produced waters. Pages 469–538 *in* D. F. Boesch and N. N. Rabalais, editors. Long-term environmental effects of offshore oil and gas development. Elsevier Applied Science, New York, New York, USA.

Neff, J. M., N. N. Rabalais, and D. F. Boesch. 1987. Offshore oil and gas development activities potentially causing long-term environmental effects. Pages 149–173 *in* D. F. Boesch and N. N. Rabalais, editors. Long-term environmental effects of offshore oil and gas development. Elsevier Applied Science, New York, New York, USA.

Oakden, J. M., J. S. Oliver, and A. R. Flegal. 1984. Behavioral responses of a phoxocephalid amphipod to organic enrichment and trace metals in sediments. Marine Ecology Progress Series **14**:253–257.

Olson, R. 1985. The consequences of short-distance larval dispersal in a sessile marine invertebrate. Ecology **66**:30–39.

Osenberg, C. W., S. J. Holbrook, and R. J. Schmitt. 1992*a*. Implications for the design of environmental assessment studies. Pages 75–90 *in* P. M. Grifman and S. E. Yoder, editors. Perspectives on the marine environment. Sea Grant Institutional Program, Hancock Institute for Marine Studies, University of Southern California, Los Angeles, California, USA.

Osenberg, C. W., R. J. Schmitt, S. J. Holbrook, and D. Canestro. 1992*b*. Spatial scale of ecological effects associated with an open coast discharge of produced water. Pages 387–402 *in* J. P. Ray and F. R. Englehardt, editors. Produced water: technological/environmental issues and solutions. Plenum, New York, New York, USA.

Osenberg, C. W., E. E. Werner, G. G. Mittelbach, and D. J. Hall. 1988. Growth patterns in bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish: environmental variation and the importance of ontogenetic niche shifts. Canadian Journal of Fisheries and Aquatic Sciences **45**:17–26.

Pearson, T. H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review **16**:229–311.

Peterman, R. M. 1990. Statistical power analysis can im-

prove fisheries research and management. Canadian Journal of Fisheries and Aquatic Science **47**:2–15.

Raimondi, P. T. 1990. Patterns, mechanisms, consequences of variability in settlement and recruitment of an intertidal barnacle. Ecological Monographs **60**:283–309.

Raimondi, P. T., and R. J. Schmitt. 1992. Effects of produced water on settlement of larvae: field tests using red abalone. Pages 415–430 *in* J. P. Ray and F. R. Englehardt, editors. Produced water: technological/environmental issues and solutions. Plenum, New York, New York, USA.

Sañudo-Wilhemy, S., and A. R. Flegal. 1991. Trace element distributions in coastal waters along the US-Mexican boundary: relative contributions of natural processes vs. anthropogenic inputs. Marine Chemistry **33**:371–392.

Schmidt, H., and C. E. Reimers. 1991. The recent history of trace metal accumulation in the Santa Barbara basin, Southern California Borderland. Estuarine, Coastal and Shelf Science **33**:485–500.

Schmitt, R. J., and S. J. Holbrook. 1990. Contrasting effects of giant kelp on dynamics of surfperch populations. Oecologia **84**:419–429.

Schroeter, S. C., J. D. Dixon, J. Kastendiek, R. O. Smith, and J. R. Bence. 1993. Detecting the ecological effects of environmental impacts: a case study of kelp forest invertebrates. Ecological Applications **3**:331–350.

Skalski, J. R., and D. H. McKenzie. 1982. A design for aquatic monitoring programs. Journal of Environmental Management **14**:237–251.

Spies, R. B. 1987. The biological effects of petroleum hydrocarbons in the sea: assessments from the field and microcosms. Pages 411–467 *in* D. F. Boesch and N. N. Rabalais, editors. Long-term environmental effects of offshore oil and gas development. Elsevier Applied Science, New York, New York, USA.

Spies, R. B., and D. J. DesMarais. 1983. Natural isotope study of trophic enrichment of marine benthic communities by petroleum seepage. Marine Biology **73**:67–71.

Stewart-Oaten, A. *In press.* Problems in the analysis of environmental monitoring data. *In* R. J. Schmitt and C. W. Osenberg, editors. Ecological impact assessment: conceptual issues and application in coastal marine habitats. University of California Press, Berkley, California, USA.

Stewart-Oaten, A., J. R. Bence, and C. W. Osenberg. 1992. Assessing effects of unreplicated perturbations: no simple solutions. Ecology **73**:1396–1404.

Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental impact assessment: "pseudoreplication" in time? Ecology **67**:929–940.

Stretch, J. J. 1983. Habitat selection and vertical migration of sand-dwelling demersal gammarid amphipods. Dissertation. University of California, Santa Barbara, California, USA.

Thrush, S. F., R. D. Pridmore, and J. E. Hewitt. 1994. Impacts on soft-sediment macrofauna: the effects of variation on temporal trends. Ecological Applications **4**:31–41.

Underwood, A. J. 1991. Beyond BACI: experimental designs for detecting human environmental impacts on temporal variations in natural populations. Australian Journal of Marine and Freshwater Research **42**:569–587.

———. 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. Ecological Applications **4**:3–15.

Underwood, A. J., and C. H. Peterson. 1987. Towards an ecological framework for investigating pollution. Marine Ecology Progress Series **46**:227–234.

Vezina, A. F. 1988. Sampling variance and the design of quantitative surveys of the marine benthos. Marine Biology **97**:151–155.

Victor, B. C. 1986. Larval settlement and juvenile mortality in a recruitment-limited coral reef fish population. Ecological Monographs **56**:145–160.

Warwick, R. M. 1988. The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. Marine Pollution Bulletin **19**:259–268.

Yoccoz, N. G. 1991. Use, overuse and misuse of significance tests in evolution and ecology. Bulletin of the Ecological Society of America **72**:106–111.

VI. RECOVERY OF INFAUNA AND MUSSELS FOLLOWING CESSATION OF PRODUCED WATER DISCHARGE: APPLICATION OF THE BEFORE-AFTER-CONTROL-IMPACT PAIRED SERIES (BACIPS) DESIGN

A. Introduction

Our previous field research documented spatial variation in ecological parameters that correlated with distance from the produced water outfall. These patterns suggested that there were effects of produced water, but could not equivocally isolate effects of produced water from other factors that covaried in space with produced water. To provide stronger inferences we applied the BACIPS assessment design to data collected during two periods: 1) during discharge (or while the plant was "in operation"), and 2) after discharge stopped (or while the plant was "shut down"). Krause (1995) and Raimondi and Schmitt (1992) both took similar approaches, but had data from only a single "shut down" period. Ideally, a time series of samples should be taken both during operation and after the plant has shut down. Here, we report data from several surveys conducted during operation and several conducted after the separation facility stopped discharging produced water. The results provide the best documentation to date of the ecological effects of produced water.

B. Mussel Production and Shell Barium Content

During each survey taken when the plant was in operation, we consistently found a negative relationship between mussel growth, condition and production and distance from the diffuser. These spatial patterns disappeared as soon as the plant stopped discharging produced water (Figure VI-1), as anticipated if the toxicants were water-borne. Based on 7 (and 4) surveys obtained during operation and 6 (and 2) after shut down for *M. californianus* (and *M. edulis*), produced water reduced tissue production by 20 - 30% at sites within 10 m of the outfall (Figure VI-1). As distance increased, the effects diminished. Because our most distant sites were at 1 km, we were unable to discern effects beyond this point.

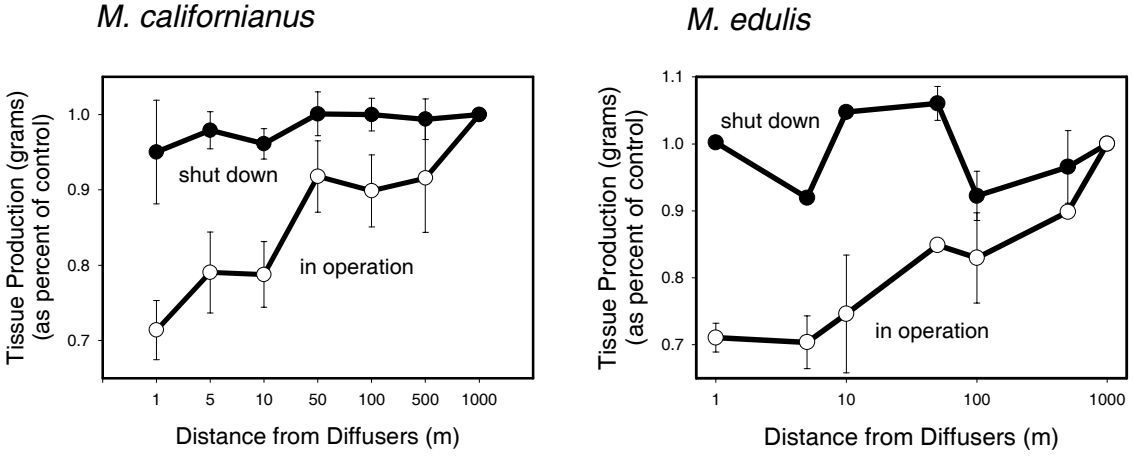

Figure VI-1. Tissue production of *M. californianus* and *M. edulis*. The difference between the two periods provides an estimate of the effect of produced water on tissue production.

Shell barium content was a useful marker of exposure to produced water. During operation, mussels placed close to the diffuser accumulated the highest levels of barium (e.g., Osenberg *et al*. 1992b), but after discharge ceased, these spatial patterns disappeared (Figure VI-2). When the plant was in operation, we consistently found a negative relationship between barium concentration in shells and distance from the diffuser. Barium content typically reached background levels around 100 m (range: 50-500 m) from the diffuser. As expected, *r* barium content and mussel performance were negatively correlated (e.g., for the first survey: = -0.96, n = 6, P<0.01), suggesting that the observed variation in mussel performance was

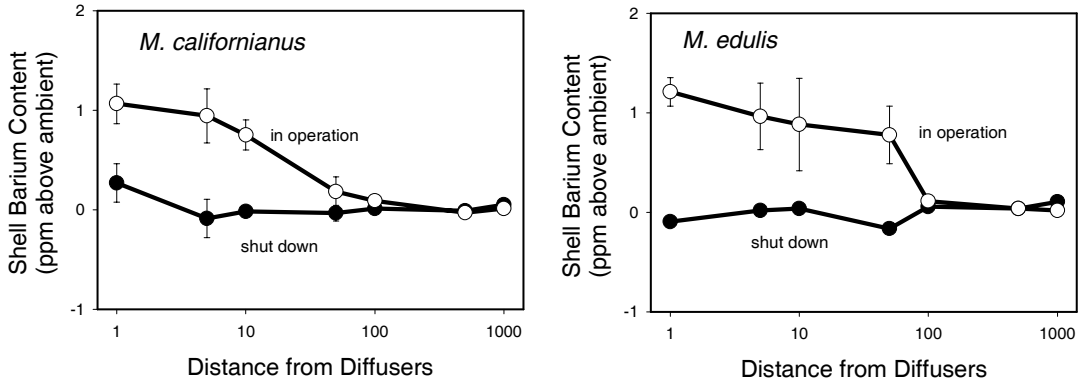

Figure VI-2. Shell barium content in *M. californianus* and *M. edulis*. The difference between the two periods provides an estimate of the effect of produced water on shell barium content.

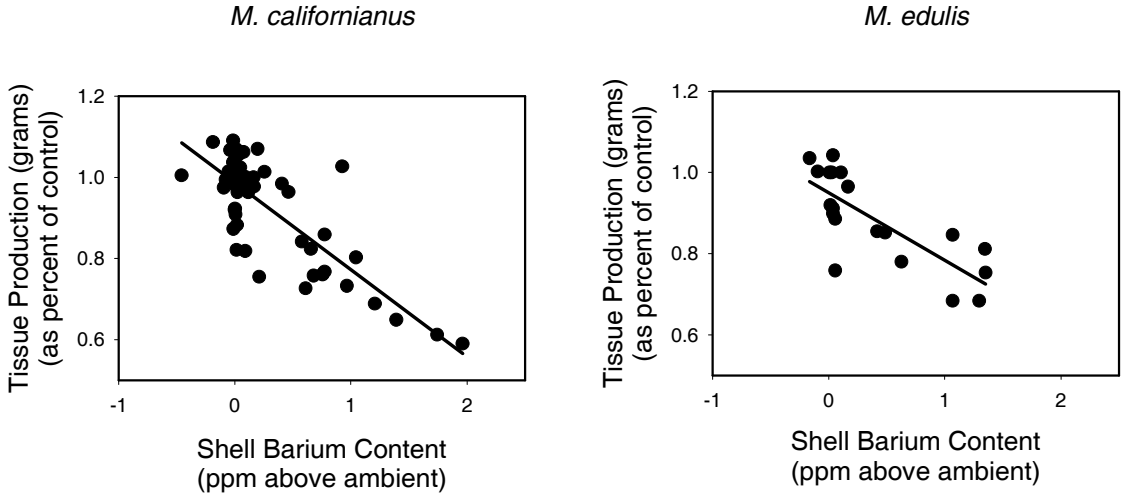

Figure VI-3. Reductions in tissue production in relationship to increased shell barium content in *M. californianus* and *M. edulis*. Tissue production was standardized as the percent of the distant sites (1000 m), whereas shell barium content was standardized as the absolute deviation from background. As a result, "no effect" would be indicated by standardized values of 1 (for tissue production) and 0 (for shell barium content).

directly related to exposure to produced water. In contrast to the data from the discharge period, we found no consistent variation in barium content during the post-shutdown period. Barium content was relatively uniform among sites and did not covary with distance from the diffuser, although it exhibited considerable temporal variation. Comparison of the "in operation" and "shut down" periods suggests that barium remained elevated in the water column only out to 50 – 100 m from the diffuser. Yet, effects on mussels seemed to persist out to 500 m (Figure VI-1). Overall, however there was a strong negative correlation between the degree to which barium was elevated above ambient and the extent to which mussel production was depressed (Figure VI-3).

In addition to its role as a marker of produced water, barium may also be an important source of toxicity in the produced water. For example, research by Cherr and Higashi and their co-workers have shown that the majority of toxicity is removed by Chelex (Higashi *et al*. 1992), and that this component contains high concentrations of Ba and Sr, but not other more "classical" toxic elements (e.g., Cu, As, and Cr). Subsequent work by Spangenberg and Cherr (1996) has demonstrated that barium can produce similar patterns of toxicity as observed in the Carpinteria produced water.

C. Infaunal Density

As also observed for mussels, there were spatial patterns of infaunal density during the period when produced water was discharged (Osenberg *et al*. 1992b). Following shut down of the facility, these patterns largely disappeared, demonstrating that previously documented patterns had resulted from the effects of produced water on infaunal density. These results, however, were less dramatic and more variable than observed for mussels and occurred on a more limited spatial scale. For example, nematodes were stimulated by the discharge of produced water, showing higher densities near the diffuser and lower densities at more distant sites. Within the first year following cessation of discharge, this signal was reduced considerably and by the second year, there was no noticeable association between nematode abundance and the diffuser (Figure VI-4a). Stimulatory effects of organic enrichment on nematodes have been observed at wastewater outfalls and natural oil seeps (Davis and Spies 1980, Motagna *et al*. 1989, Steichen *et al*. 1996). Most other taxa, showed depressed densities near the diffuser (Osenberg *et al*. 1992b), and many of these patterns dissipated after cessation of discharge (Figure VI-4b).

Several taxa showed more complex spatial patterns, possibly due to negative ("toxic") effects combined with stimulatory, enrichment effects (Osenberg *et al*. 1992, Steichen *et al*. 1996): Figure VI-5a. For example, bivalves showed a pattern during discharge that suggested a humped-shaped relationship with distance: maximum abundances occurred at intermediate distances (e.g., 10 – 100 m). Shortly after cessation of produced water discharge, bivalve abundances peaked near the diffuse and declined as distance increased (Figure VI-5b), suggesting a continuing response to enrichment but the absence of any lingering deleterious effects. After additional time (on the order of six months), this enrichment effect was reduced by about 50%, and after a year, there was little remaining effect (Figure VI-5b). Overall, however, these effects on population density occurred over very limited spatial scales compared to effects on individual-based and demographic parameters (Osenberg et al. 1992b): e.g., compare Figure VI-1 with Figures VI-4 and VI-5.

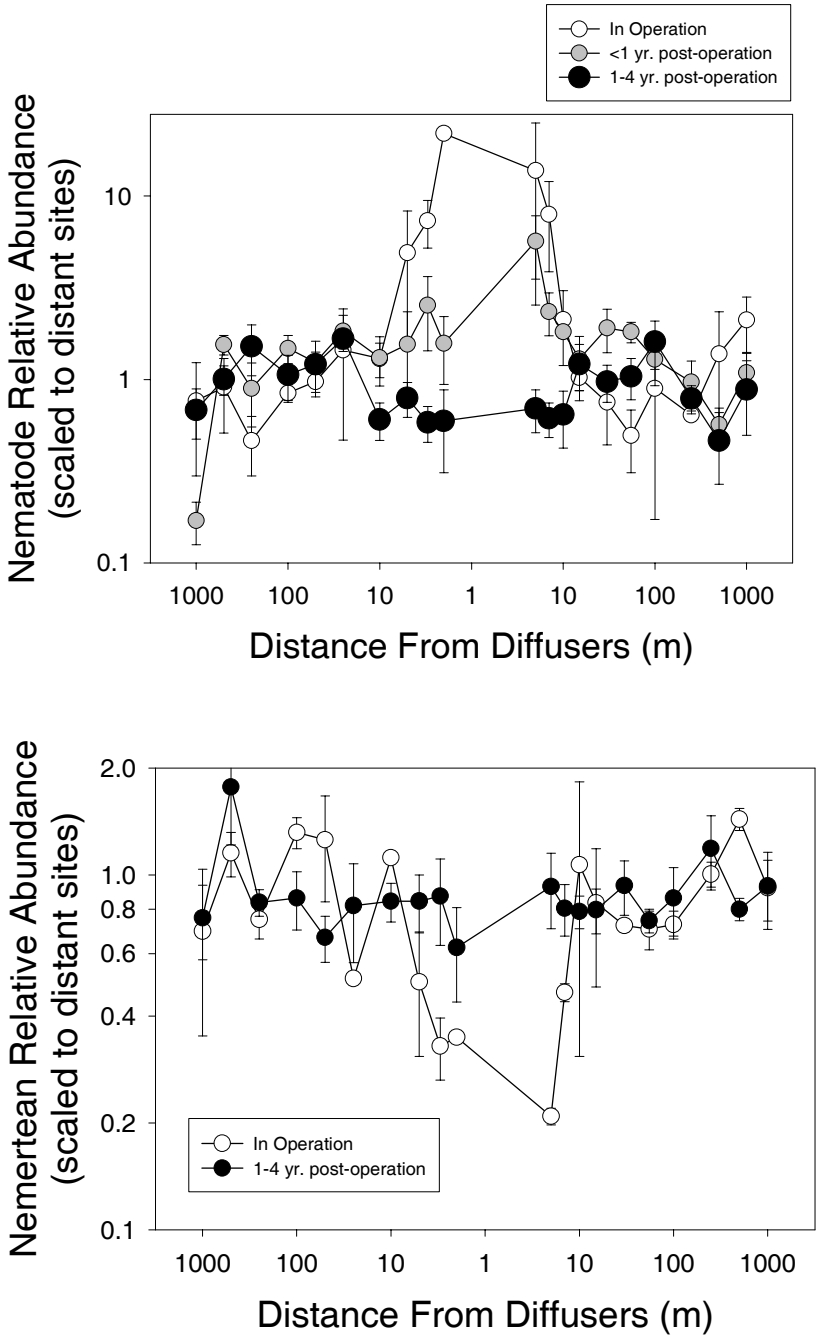

Figure VI-4. Density of nematodes (a, top) and nermerteans (b, bottom) as functions of the distance from the diffuser during three different sampling periods: 1) when produced water was discharged (open symbols); 2) within one year after discharged ceased (gray); and 3) from 1 – 4 years after cessation of produced water discharge (closed circles). The intermediate sampling period is omitted in the bottom panel for clarify. Densities were rescaled relative to the density at the most distant sites. As a result, the absence of spatial variation would be indicated by uniform relative abundances of 1.0. Produced water effects are indicated by differences between the "In operation" and "Post-operation" lines. Please note that the diffuser is located a the midpoint of the transect.

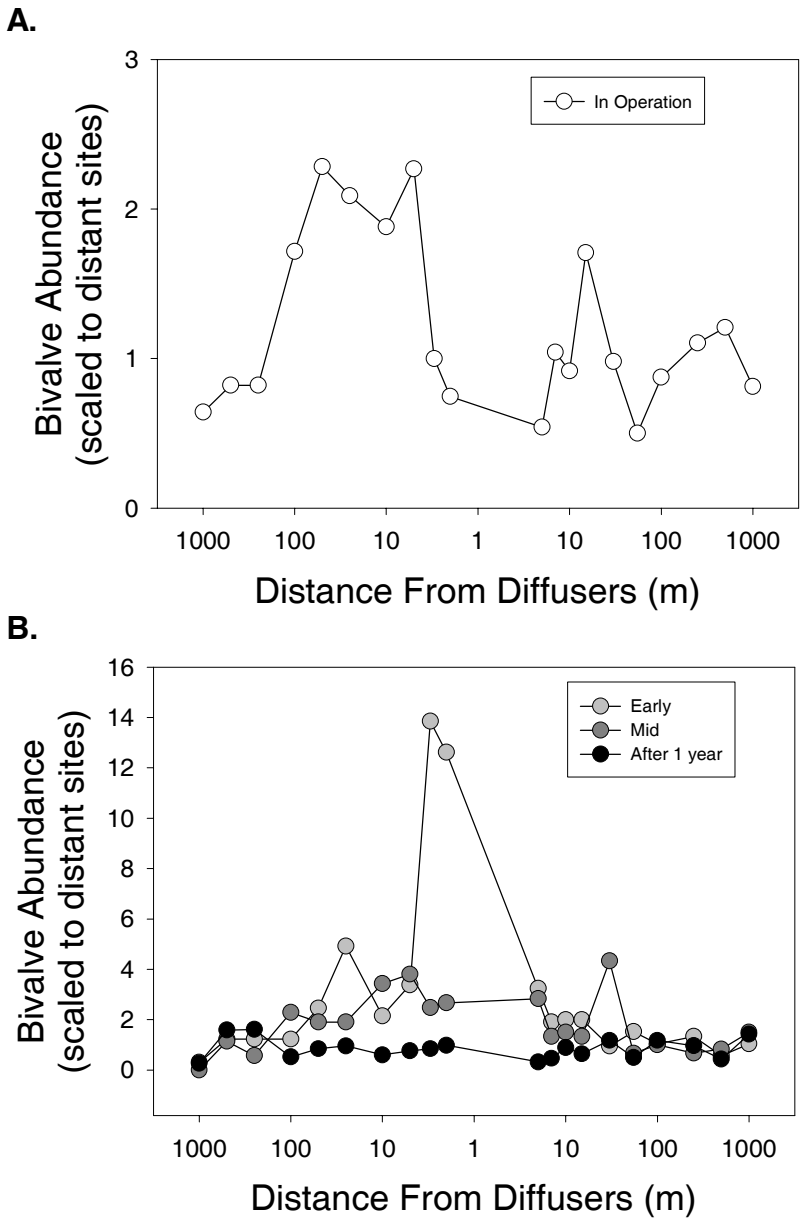

Figure VI-5. Density of bivalves as a function of distance from the diffuser during four different sampling periods: a (top) when produced water was discharged (open symbols); and b (bottom) during three periods following cessation of produced water discharge. Densities were rescaled relative to the density at the most distant sites. As a result, the absence of spatial variation would be indicated by uniform relative abundances of 1.0. Produced water effects are indicated by differences between the "In operation" and "Post-operation" lines. The diffuser is located at the midpoint of the transect.

REFERENCES

(see also the Literature Cited sections in the reprints in sections III, IV and V)

Carney, R.S. 1987. A review of study designs for the detection of long-term environmental effects of offshore petroleum activities. Pages 651-696 *in* D.F. Boesch and N.N. Rabalais, editors. *Long-term effects of offshore oil and gas development*. Elsevier Applied Science, New York.

Davis, P. H., Spies, R. B. 1980. Infaunal benthos of a natural petroleum seep: study of community structure. *Mar. Biol*. **59**:31-41.

Higashi, R., G. Cherr, C. Bergens, T. Fan, and D. Crosby. 1992. Toxicant isolation from a produced water source in the Santa Barbara Channel. Pages 223-233 *in* J.P. Ray and F.R. Englehardt, editors. *Produced water: technological/environmental issues and solutions*. Plenum Press., New York.

Krause, P.R. 1994. Effects of an oil production effluent on gametogenesis and gamete performance in the purple sea urchin (*Strongylocentrotus purpuratus* Stimpson). *Env. Tox. and Chem.* **13**:1153-1161.

Krause, P.R. 1995. Spatial and temporal variability in receiving water toxicity near an oil effluent discharge site. *Archives of Environmental Contamination and Toxicology* **29**:523-529.

Krause, P.R., C.W. Osenberg, R.J. Schmitt. 1992. Effects of produced water on early life stages of a sea urchin: gender-specific responses and delayed expression. Pages 431-444 *in* J.P. Ray and F.R. Englehardt (eds.), *Produced water: technological/environmental issues and solutions*, Plenum Publishing Corp., New York.

Montagna, P. A., Bauer, J. E., Hardin, D. Spies, R. B. 1989. Vertical distribution of microbial and meiofaunal populations in sediments of a natural coastal hydrocarbon seep. *J. Mar. Res*. **47**:657-680

Osenberg, C.W., S.J. Holbrook and R.J. Schmitt. 1992a. Implications for the design of environmental assessment studies. Pages 75-90 *in* P.M. Grifman and S.E. Yoder (eds.) *Perspectives on the Marine Environment*. USC Sea Grant, Los Angeles, California.

Osenberg, C.W., R.J. Schmitt, S.J. Holbrook and D. Canestro. 1992b. Spatial scale of ecological effects associated with an open coast discharge of produced water. Pages 387-402 *in* J.P. Ray and F.R. Englehardt (eds.), *Produced water: technological/environmental issues and solutions*, Plenum Publishing Corp., New York.

Osenberg, C.W. and R.J. Schmitt. 1994. Detecting human impacts in marine habitats. *Ecological Applications* **4**:1-2.

Osenberg, C.W., R.J. Schmitt, S.J. Holbrook, K. E. Abu-Saba, and A. R. Flegal. 1994. Detection of environmental impacts: natural variability, effect size, and power analysis. *Ecological Applications* **4**:16-30.

Osenberg, C.W. and R.J. Schmitt. 1996. Detecting ecological impacts caused by human activities. Pages 3-16 *in* R.J. Schmitt and C.W. Osenberg (eds.) *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press, San Diego.

Piltz, F.M. 1996. Organizational constraints on environmental impact assessment research. Pages 317-328 *in* R.J. Schmitt and C.W. Osenberg (eds.) *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press, San Diego.

Raimondi, P.T., and R.J. Schmitt. 1992. Effects of produced water on settlement of larvae: field tests using red abalone. Pages 415-430 in J.P. Ray and F.R. Englehardt, editors. *Produced water: technological/environmental issues and solutions*. Plenum Press., New York.

Schmitt, R.J. and C.W. Osenberg (editors and contributing authors). 1996. *Detecting ecological impacts: Concepts and applications in coastal habitats*. Academic Press, San Diego.

Spangenberg, J.V. and G.N. Cherr. 1994. Developmental effects of barium exposure in a marine bivalve (*Mytilus californianus*). *Environmental Toxicology and Chemistry* **15**:1769-1774.

Spies, R.B. 1987. The biological effects of petroleum hydrocarbons in the sea: assessments from the field and microcosms. Pages 411-467 in D.F. Boesch and N.N. Rabalais, (editors), *Long-term environmental effects of offshore oil and gas development*. Elsevier Applied Science, New York.

Steichen, D.J., Jr., S.J. Holbrook, and C.W. Osenberg. 1996. Distribution and abundance of benthic and demersal macrofauna within a natural hydrocarbon seep. *Marine Ecology Progress Series* **138**:71-82.

Stewart-Oaten, A., W.W. Murdoch, and K.R. Parker. 1986. Environmental impact assessment: "pseudoreplication" in time? *Ecology* **67**:929-940.

Stewart-Oaten, A., J.R. Bence, and C.W. Osenberg. 1992. Assessing effects of unreplicated perturbations: no simple solutions. *Ecology* **73**:1396-1404.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.