

# **Potential biogenic habitat alteration by OCS Activities: Adverse effects to early life-stages of giant kelp from chronic disturbance**

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**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**

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## FINAL TECHNICAL SUMMARY

**STUDY TITLE:** Potential biogenic habitat alteration by OCS Activities: Adverse effects to early life-stages of giant kelp from chronic disturbance

**REPORT TITLE:** Potential biogenic habitat alteration by OCS Activities: Adverse effects to early life-stages of giant kelp from chronic disturbance

**CONTRACT NUMBER:** 14-35-0001-30471

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**PROJECT MANAGER:** Russell J. Schmitt

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**KEY WORDS:** attachment, barium, behavior, Carpinteria, colonization potential, dispersal, giant kelp, ecological impact assessment, fertilization, immigration, *Macrocystis pyrifera*, nonpolar organic compounds, petroleum hydrocarbons, produced water, propagules, recruitment, salinity, settlement, southern California, swimming, toxins, zoospore

**BACKGROUND:** For both ecological and economic reasons, giant kelp (*Macrocystis pyrifera*) forests form the most important biogenic habitat in the nearshore coastal waters of the Pacific OCS region. Various OCS oil and gas activities, however, may adversely affect these prominent marine communities. In particular, chronic exposure to production discharges (e.g. produced water) and petroleum hydrocarbons may have persistent long-term effects on the dynamics of giant kelp populations. Although the adult macroscopic

sporophyte stage may be relatively invulnerable to acute exposure to spilled oil, there is growing evidence that the microscopic stages are susceptible to several forms of pollution at very low levels. Consequently, forests of giant kelp may be at risk to acute and chronic exposure to constituents in oil and produced water, which inhibit kelp recruitment

**OBJECTIVES:** Like all kelps, *Macrocystis* has a heteromorphic life-history in which motile spores settle on the bottom and germinate into free-living male and female gametophytes that produce eggs and sperm. A highly volatile pheromone produced by the egg causes the release and attraction of the sperm to the egg. The embryonic sporophytes developing from the fertilized egg ultimately become the macroscopic sporophyte with a characteristic kelp-like form. Low levels of refined oil have been shown to inhibit spore germination, gametogenesis, and early sporophyte development in the Atlantic kelp *laminaria saccharina*. Produced water effluents contain many heavy metals, some of which may later spore settlement and gametophyte reproduction if their concentrations are elevated above background levels in the water column. More importantly, the highest concentrations of pollutants are found on the bottom where metals and petroleum hydrocarbons accumulate in sediments near discharges. Such benthic accumulation of pollutants could severely alter patterns of kelp recruitment, especially since kelp beds growing on soft sediments are common near oil and gas development activities.

Our objective was to determine potential lethal and sublethal effects of discharges from oil and gas activities on growth and reproduction in the microscopic recruitment stages of giant kelp. Our goal was to predict the level at which effects would be noticeable in natural populations of giant kelp that are chronically exposed to produced water and petroleum hydrocarbons.

**DESCRIPTION:** Laboratory experiments were used to determine how water column exposure to produced water influences the colonization potential of giant kelp zoospores on the bottom. These experiments were designed to examine effects of produced water concentration and exposure duration on: (1) swimming duration of zoospores, (2) attachment ability of zoospores, and (3) germination of gametophytes from zoospores. Zoospores were maintained in suspension at relatively low densities in 18 L containers and exposed to one of five concentrations of produced water for varying amounts of time. Field experiments were used to determine the spatial scale of benthic effects of an active produced water outfall on various components of recruitment in the giant kelp. Field experiments were done in the shallow subtidal of a high energy open coast near Carpinteria CA, USA (34°23'N, 119°30'W). The outfall diffuser of produced water studied was located ≈ 200-300 m offshore in a sandy bottom at a depth of about 11 m that was approximately 200 m downcurrent from a giant kelp forest. The response variables measured in the field experiments were: zoospore production in transplanted sporophytes, survival, reproduction, and sporophyte production of outplanted gametophytes.

**STUDY RESULTS:** Zoospore swimming generally decreased with increasing produced water concentration and exposure duration; however, the specific pattern of decrease differed between experimental trials done on different dates. The effect of exposure duration on the ability of swimming zoospores to attach to plastic dishes placed on the bottom varied with produced water concentration. Zoospores placed in produced water concentrations of 1% and 10% showed a

steady decline in their ability to attach with increased exposure; lower concentrations of produced water had no such effects. The percentage of zoospores that germinated after attachment varied tremendously with exposure duration and date of experimental trial. Zoospores that settled during the first 12 h after release had very poor rates of germination indicative of a short precompetent period. Surprisingly, exposure of suspended zoospores to high concentrations of produced water during the first 12 h reduced this precompetent period and greatly improved germination success on the bottom. The magnitude of this enhancement, however, varied among dates.

Discernible effects on all parameters measured in the field were limited to areas very close to the outfall (i.e. < 50 m). Zoospore production in sporophytes transplanted to varying distances from the diffuser did not vary in a systematic way. Survival and successful reproduction (i.e. sporophyte production) of outplanted gametophytes varied significantly among experimental dates. Performance of these parameters was significantly reduced only at the site nearest the diffuser (i.e. 5 m away). Poor gametophyte survival near the outfall may have resulted from exploitative competition with *Beggiatoa* sp. (a fast growing filamentous marine bacteria that exploits areas high in hydrogen sulfide, an abundant constituent of the produced water effluent) rather than from toxicity of produced water. Laboratory assays indicated that gametophyte reproduction and subsequent sporophyte production were inhibited at levels likely to occur within the near vicinity of the diffuser. Nonetheless, field data indicate that the lack of sporophyte production observed near the diffuser probably resulted from factors affecting gametophyte survival.

**SIGNIFICANT CONCLUSIONS:** Our laboratory experiments indicate that the toxicity of produced water to giant kelp zoospores is generally low. Relatively high concentrations of produced water ( $\geq 1\%$ ) were needed to produce adverse effects on zoospore swimming, attachment, and germination. Moreover, with the exception of swimming, adverse effects of produced water were observed only after relatively long periods of exposure (i.e.  $\geq 24$  h). Interestingly, relatively short exposures to high concentrations of produced water actually promoted successful germination during what normally appeared to be a pre-competent period in all three experimental trials. How produced water could cause this unexpected pattern of early germination, however, remains unknown. It is possible that zoospore swimming is more sensitive to produced water than our results indicate. We may have underestimated the number of non-swimming zoospores in treatments containing produced water if toxins in the effluent inactivate flagella without causing the spore to change shape. We cannot be certain that all zoospores induced into a non-swimming mode by produced water changed from pear shaped to spherical. However, we never saw non-swimming zoospores that were not spherical in video recordings of zoospores placed in different concentrations of produced water. Results from our field experiments indicated that discernible effects on kelp recruitment are likely to be limited to areas very close to produced water outfalls; significant reductions in gametophyte survival and reproduction (measured as sporophyte production) were observed only as far as our site located 5 m from the outfall. However, data from this study alone are insufficient to implicate the discharge of produced water as the primary cause for these observed effects. Constraints on the design of our field experiments prevented us from completely isolating natural causes of spatial variability from that due to the discharge of produced water. There are compelling reasons to believe, however, that the discharge was the primary mechanism

causing reduced performance in giant kelp. First, the results were highly localized within a relatively large and uniform area. Second, the observed patterns were symmetrical about the diffuser in as much as the most distant locations on either side of the outfall (which were chosen to serve as controls) were similar and different from that observed near the diffuser. Lastly, companion studies done at Carpinteria using other organisms showed that previously observed effects dissipate during brief periods when the discharge is not operational.

**STUDY PRODUCTS:** The following peer-reviewed publications resulted from this work.

Reed, D.C. and R.J. Lewis. 1994. Effects of an oil and gas production effluent on the colonization potential of giant kelp (*Macrocystis pyrifera*) zoospores. *Marine Biology* **119**: 277-283.

Reed, D.C., R. J. Lewis, and M. Anghera. 1994. Effects of an open coast oil production outfall on patterns of giant kelp (*Macrocystis pyrifera*) recruitment. *Marine Biology* **120**: 26-31.

Raimondi, P.T. and D.C. Reed. 1995. Determining the spatial extent of ecological impacts caused by local anthropogenic disturbances in coastal marine habitats. Pages 179-198, *In* R.J. Schmitt and C.W. Osenberg (eds.). *Detecting Ecological Impacts: Concepts and Applications in Coastal Habitats*, Academic Press, San Diego.

## FINAL STUDY REPORT

Marine Biology (1994) 119: 277–283

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D. C. Reed · R. J. Lewis

**Effects of an oil and gas-production effluent on the colonization potential of giant kelp (*Macrocystis pyrifera*) zoospores**

Received: 13 January 1994 / Accepted: 19 January 1994

**Abstract** Point sources of pollution (e.g. industrial and municipal outfalls) may produce ecological impacts at distant locations if pollutants affect dispersive propagules. We used laboratory experiments to determine how water-column exposure to produced water (=the aqueous fraction of oil and gas production that is typically discharged into coastal waters) influences the colonization potential of giant kelp (*Macrocystis pyrifera*) zoospores on the bottom. Zoospores were maintained in suspension at relatively low densities in 18-liter containers and exposed to one of five concentrations of produced water for varying amounts of time. Zoospore swimming generally decreased with increasing produced-water concentration and exposure duration; however, the specific pattern of decrease differed between experimental trials done on different dates. The effect of exposure duration on the ability of swimming zoospores to attach to plastic dishes placed on the bottom varied with produced-water concentration. Zoospores placed in produced-water concentrations of 1 and 10% showed a steady decline in their ability to attach with increased exposure; lower concentrations of produced water had no such effects. The percentage of zoospores that germinated after attachment varied tremendously with exposure duration and date of experimental trial. Zoospores that settled during the first 12 h after release had very poor rates of germination, indicative of a short precompetent period. Surprisingly, exposure of suspended zoospores to high concentrations of produced water during the first 12 h reduced this precompetent period and greatly improved germination success on the bottom. The magnitude of this en-

hancement, however, varied among dates. The results suggest that adverse effects of discharging produced water on planktonic zoospores of giant kelp would most likely be limited to the immediate vicinity of the outfall.

**Introduction**

Numerous studies have documented localized effects of point sources of pollution (e.g. outfalls) on populations of benthic marine organisms (e.g. Littler and Murray 1975; Murray and Littler 1978; Rastetter and Cooke 1979; Moran and Grant 1989 a, b). Local impacts could potentially cause ecological impacts at distant locations if individuals move from one place to another. Such widespread movement is not necessarily restricted to highly mobile organisms. Many sessile or weakly mobile species produce planktonic propagules (e.g. gametes, spores and larvae) that can be dispersed over considerable distances (tens to hundreds of kilometers) by ocean currents. Moreover, these early developmental stages are among the most sensitive to a wide variety of toxicants (Moore and Dwyer 1974; Neff et al. 1976; Rossi and Anderson 1976; Rice et al. 1977; Kingsford and Gray 1994). Therefore, localized activities that alter rates by which propagules disperse from locally disturbed areas or immigrate to more distant non-disturbed sites may influence population dynamics and community structure over much larger spatial scales.

Effluents of aqueous wastes generated during oil and gas production (commonly called produced waters) have been identified as the single largest waste stream associated with the petroleum industry (Neff et al. 1987). For example, oilfield operations in the North Sea in 1989 generated nearly 130 billion liters of produced-water effluents (Jacobs et al. 1992), while those in the Gulf of Mexico generated over 135 billion liters of produced water in 1990 (Reilly et al. 1991). Produced waters contain a diverse array of potentially toxic substances including hydrocarbons, heavy metals, radionuclides, and chemical additives such as corrosion inhibitors and biocides (Stephenson 1992).

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Not surprisingly, the discharge of such large volumes of effluents containing these substances has raised concern among regulatory agencies and environmental organizations about the potential harmful effects of produced waters to marine organisms.

Until recently, most investigations of the ecological effects of produced-water effluents have focused on sessile life stages of benthic marine organisms (reviewed in Ray and Engelhardt 1992). In part this is because many of the harmful components of produced water quickly drop out of the water column and accumulate on the bottom, while water-soluble contaminants are thought to be diluted rapidly to levels well below those considered to produce harmful effects (Armstrong et al. 1979; Wheeler et al. 1980; Mitteditch 1981). Results from these studies generally have shown effects to be relatively localized and restricted to areas that are near the point of discharge. More recent studies, however, have demonstrated adverse water-column effects on gamete production, fertilization and larval viability that are directly attributable to very dilute concentrations of produced-water effluents at substantial distances from the point of discharge (Krause et al. 1992; Osenberg et al. 1992; Raimondi and Schmitt 1992). Thus, propagule dispersal may allow ecological impacts resulting from the discharge of produced water to occur over even greater spatial scales than has been previously documented.

*Macrocystis pyrifera*, whose vegetative canopy extends throughout the water column, forms the most prominent biogenic habitat in the nearshore waters of central and southern California (Foster and Schiel 1985), a region of considerable oil and gas production. Giant kelp is propagated solely by microscopic zoospores that are released into the water column and settle on the bottom following what is believed to be a relatively short period of planktonic dispersal (i.e., days; Reed et al. 1992). Because zoospores lack a protective outer cell wall, they may be more vulnerable to xenobiotic toxins than are other life stages. Here, we describe laboratory experiments designed to examine how exposure to a produced-water effluent affects the ability of planktonic zoospores to disperse to and colonize distantly located, non-polluted benthic habitats. Reed et al. (1994) present the complementary field studies that examine how the discharge of produced water into a high-energy, open-coast environment influences zoospore production in adult plants, and subsequent survival and reproduction of the benthic microscopic gametophyte stage that develops following zoospore settlement.

## Materials and methods

Laboratory experiments were used to determine how produced water influences the competency and subsequent dispersive potential of planktonic zoospores following their release from the parent plant. Experiments were designed to examine effects of produced-water concentration and exposure duration on (1) swimming duration of zoospores, (2) attachment ability of zoospores, and (3) germination of gametophytes from zoospores.

Zoospores were obtained from adult *Macrocystis pyrifera* plants using the methods of Reed et al. (1992). Source plants were chosen

from kelp beds near Santa Barbara, California (34°25' N; 119°57' W) that were located > 15 km from the nearest produced-water outfall. Upon release, zoospores were placed in suspension in white plastic buckets containing 18 liters of one of five mixtures of clean filtered (0.2 µm) seawater and produced water (0, 0.01, 0.1, 1 and 10% by volume produced water;  $n = 5$  buckets for each mixture). Experiments were done at 15 °C under an irradiance of 40 µE m<sup>-2</sup> s<sup>-1</sup> with a 14 h light : 10 h dark photoperiod, using seawater from the marine laboratory at the University of California, Santa Barbara. Zoospore density in all buckets was ≈ 1000 zoospores ml<sup>-1</sup> at the start of each experimental trial. This density was high enough for zoospores to be observed but low enough to ensure that spore motility was unlikely to be affected by contact with other zoospores (authors' personal observations).

Undiluted produced water used in experiments was obtained from an oil-processing facility in Carpinteria, California. This facility discharges its effluent ≈ 200 m up coast of a giant kelp forest. Samples were collected from an onshore test-faucet located on the discharge pipe just before it enters the ocean. Produced water was collected without head space in acid-washed plastic containers, transported to the laboratory on ice, and kept at 4 °C in the dark until the start of an experiment (within 18 h of collection). The salinity of the effluent was 16‰. We did not adjust the salinity of produced water to that of seawater in our experiments, because we were interested in examining all possible toxic components of the effluent including reduced salinity.

The percentage of zoospores still swimming and the density of zoospores remaining in suspension in different produced-water concentrations were determined from water samples withdrawn at 0.25, 6, 12, 24, 48 and 96 h following zoospore release. Samples were fixed immediately in 5% formalin seawater, and a known volume was concentrated onto a 0.4 µm Nuclepore filter and transferred to a glass microscope slide using the filter-transfer-freeze technique (Hewes and Holm-Hansen 1983). The density of zoospores that remained in suspension was estimated by counting the number of preserved zoospores in ten regularly arranged fields using a compound microscope equipped with phase-contrast optics. To estimate the number of zoospores that were still swimming at the end of each sample period, we recorded the shape of the first 50 zoospores encountered along uniform transects on each slide; a spore changes from pear shape to spherical when it stops swimming (Reed et al. 1992).

We examined zoospore settlement on plastic petri dishes placed at the bottom of each bucket to determine if the concentration and duration of exposure to produced water affected the ability of planktonic zoospores to attach to the bottom. Dishes were placed and retrieved at varying intervals of time after the start of an experiment (0 to 6 h, 6 to 12 h, 12 to 24 h, 24 to 48 h and 96 to 120 h) to vary the duration of exposure to produced water. Upon retrieval, dishes were rinsed with a steady stream of seawater to remove any loose or weakly attached germlings, and filled with nutrient-enriched seawater (Provasoli 1968). Attached zoospores (= germlings) were counted using an inverted microscope under phase contrast. The attachment ability ( $A$ ) of zoospores during a particular time interval was calculated as:

$$A = s/c_i t_i \quad (1)$$

where  $s$  = the density of zoospores that settled in a dish,  $c_i$  = the density of suspended zoospores at the beginning of time interval  $i$ , and  $t_i$  = duration (h) of time interval  $i$  allowed for settlement.

Dishes were returned to culture conditions after sampling, and the effects of exposure to produced water on the ability of zoospores to germinate after attachment was determined by calculating the percentage of attached zoospores that had begun forming germ tubes after 72 h of release.

Experiments were done on three different dates (31 July 1991, 12 March 1992 and 14 April 1992) using different batches of zoospores and produced water. Zoospore swimming and attachment ability were examined on only two of these dates (July and April). The effects of produced-water concentration, exposure duration, and date on zoospore swimming and attachment were determined by three-way mixed-model ANOVAs in which concentration and exposure

duration were considered fixed factors and date a random factor. In all statistical analyses, the data are presented as a percentage of the control (i.e., 0% produced water) and were transformed to  $\log(x + 0.1)$  to meet the assumption of homoscedasticity. Since data on germination could not meet this assumption, they are only presented graphically.

**Results**

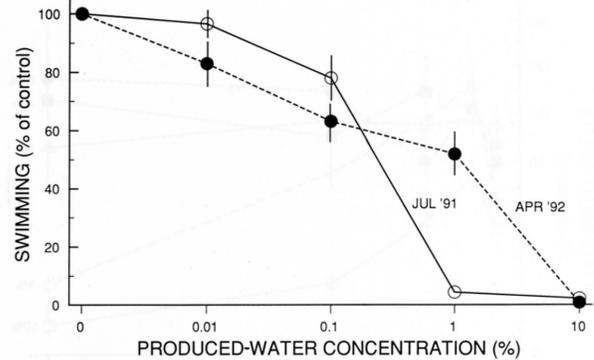
**Swimming**

*Macrocystis pyrifera* zoospores used in different experimental trials varied in their inherent swimming ability; the percentage of zoospores that were swimming at the start of each trial differed between dates (July 1991 = 54.9% and April 1992 = 40.6%;  $F_9 = 5.29$ ,  $P = 0.05$ ). Zoospore swimming generally decreased with increasing produced-water concentration (Fig. 1). The specific pattern of decrease, however, differed between experimental trials done on different dates (see significant date  $\times$  concentration interaction in Table 1). In July 1991, an abrupt and near total reduction in the percentage of zoospores that remained swimming was observed at produced-water concentrations of  $\geq 1\%$  (Fig. 1). In April 1992, zoospores had a somewhat higher tolerance to produced water; a near total reduction in zoospore swimming was observed only at a produced-water concentration of 10%. These drastic reductions in zoospore swimming at high concentrations of produced water were evident after only 15 min of exposure. This made the effects of produced-water concentration on zoospore swimming appear to be largely independent of exposure duration (see non-significant duration  $\times$  concentration interaction in Table 1). The fact that the concentrations which immediately caused zoospores to stop swimming varied between the two dates undoubtedly contributed to the significant three-way interaction of date  $\times$  duration  $\times$  concentration (Table 1).

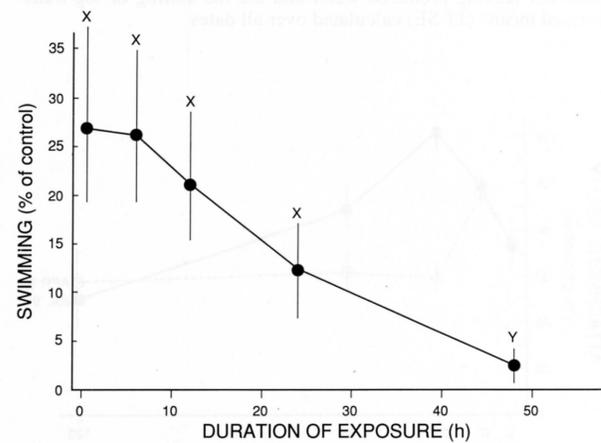
Swimming zoospores were never observed after 96 and 120 h, regardless of whether produced water was present or not. Prior to this time, increased exposure to produced water generally and gradually reduced the percentage of zoospores that remained swimming relative to zoospores that were not exposed to produced water (Fig. 2, Table 1).

**Attachment**

The inherent attachment ability of zoospores varied between batches of zoospores used in different experimental trials; the attachment index of non-exposed zoospores (i.e., those placed in clean seawater lacking produced water) after the first 6 h differed between dates (July 1991 = 38.9 and April 1992 = 22.9;  $F_9 = 5.73$ ,  $p = 0.044$ ). The effect of produced-water concentration on the ability of suspended zoospores to settle and attach to plastic dishes placed on the bottom of each bucket varied with duration of exposure to the effluent (Fig. 3, see significant duration



**Fig. 1** *Macrocystis pyrifera*. Interactive effects of produced-water concentration and date of experimental trial on zoospore swimming. Data are standardized to that observed in seawater lacking produced water and are the antilog of log-transformed means ( $\pm 1$  SE) calculated over all exposure durations

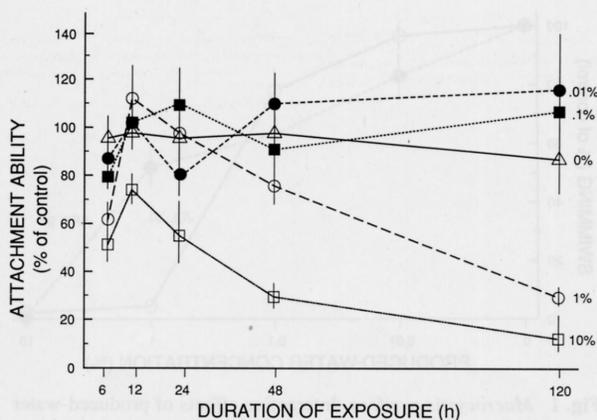


**Fig. 2** *Macrocystis pyrifera*. Main effect of duration of exposure to produced water on zoospore swimming. Data are standardized to that observed in seawater lacking produced water and are the antilog of log-transformed means ( $\pm 1$  SE) calculated over all dates and concentrations. Relatively low percentage (27%) of zoospores observed swimming after only 15 min reflects almost immediate response (cessation of swimming) by nearly all zoospores in high produced-water concentrations (see Fig. 1). Means with same letter (X, Y) are not significantly different from each other using REGWQ (Ryan-Einot-Gabriel-Welsch multiple-range test,  $p=0.05$ ; SAS Institute Inc. 1985)

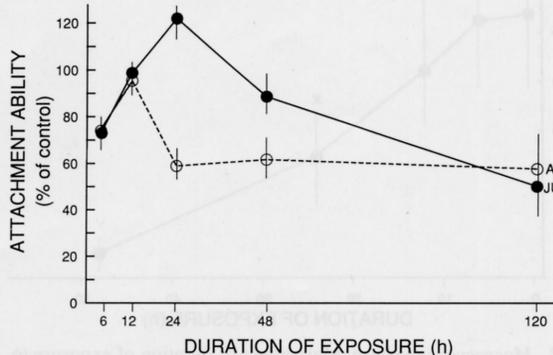
**Table 1** *Macrocystis pyrifera*. Results of analysis of variance on effects of date, exposure duration and produced-water concentration on zoospore swimming ability. (df degrees of freedom; MS mean square; F ratio of MS terms)

Source	df	MS	F	p
Date	1	0.0079	0.02	0.8752
Duration	4	9.3049	17.59	0.0084
Concentration	4	50.1907	13.25	0.0141
Date $\times$ duration	4	0.5289	1.66	0.1614
Date $\times$ concentration	4	3.7869	11.87	0.0001
Duration $\times$ concentration	16	1.0162	1.84	0.1158
Date $\times$ duration $\times$ concentration	16	0.5509	1.73	0.0442
Residual	200	0.3191		

280



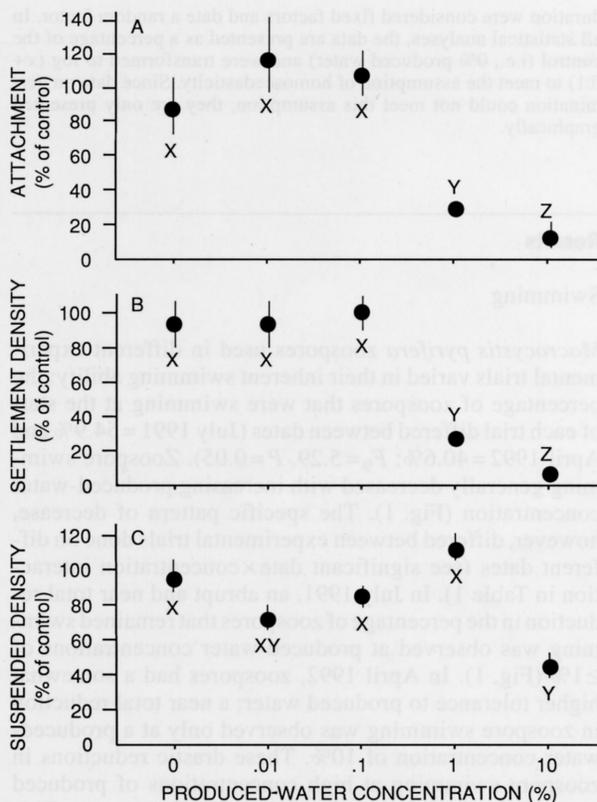
**Fig. 3** *Macrocyctis pyrifera*. Interactive effects of duration of exposure to produced water and produced-water concentration on spore attachment ability [=settlement density/(suspension density×time allowed for settlement)]. Data are standardized to that observed in seawater lacking produced water and are the antilog of log-transformed means ( $\pm 1$  SE) calculated over all dates



**Fig. 4** *Macrocyctis pyrifera*. Interactive effects of duration of exposure to produced water and date of experimental trial on zoospore attachment ability [=settlement density/(suspension density×time allowed for settlement)]. Data are standardized to that observed in seawater lacking produced water and are the antilog of log-transformed means ( $\pm 1$  SE) calculated over all concentrations

**Table 2** *Macrocyctis pyrifera*. Results of analysis of variance on effects of date, exposure duration, and produced-water concentration on rate of zoospore attachment [=settlement density/(suspension density×time allowed for settlement)]

Source	df	MS	F	p
Date	1	0.3961	7.47	0.0069
Duration	4	0.4829	1.66	0.3178
Concentration	4	1.5714	38.17	0.0019
Date×duration	4	0.2910	5.49	0.0003
Date×concentration	4	0.0412	0.78	0.5419
Duration×concentration	16	0.2620	5.56	0.0007
Date×duration×concentration	16	0.0471	0.89	0.5836
Residual	198	0.0530		



**Fig. 5** *Macrocyctis pyrifera*. Main effect of produced-water concentration on **A** attachment ability [=settlement density/(suspension density×time allowed for settlement)]; **B** zoospore settlement density; **C** zoospore suspension density during 96 to 120 h time interval. Data are standardized to that observed in seawater lacking produced water and are the antilog of log-transformed means ( $\pm 1$  SE) calculated over all dates. Means with same letter (X, Y, Z) are not significantly different from each other using REGWQ (Ryan–Einot–Gabriel–Welsch multiple-range test,  $p=0.05$ ; SAS Institute Inc. 1985)

×concentration interaction in Table 2). Zoospore attachment in produced-water concentrations of  $\leq 0.1\%$  was similar to that observed in seawater lacking produced water, regardless of exposure duration. In contrast, zoospores placed in produced-water concentrations of 1 and 10% showed a steady decline in their ability to attach with increased exposure time (following an initial depression during the first 6 h). The effects of duration of exposure to produced water on zoospore attachment, however, varied in an inexplicable way with date (Fig. 4, see date×duration interaction in Table 2).

The effects of produced-water concentration on the ability of zoospores to attach was greatest at longer exposure durations (Fig. 3). Further analyses revealed that the differences observed in zoospore attachment in the different concentrations of produced water were due largely to factors that influenced settlement rather than to factors that influenced the density of zoospores in suspension (recall that attachment ability is a function of the density of zoo-

spores in suspension, the density of zoospores that settle and the time allowed for settlement). The pattern of settlement during the 96 to 120 h interval was quite similar to that observed for attachment; settlement and attachment were both greatest at produced-water concentrations  $\leq 0.1\%$ , intermediate at  $1\%$  and lowest at  $10\%$  (Fig. 5 A, B). The density of suspended zoospores would have had to have been greatest at the higher concentrations of produced water for differences in suspension densities to have caused the observed patterns in attachment. This did not occur (Fig. 5 C).

### Germination

The effects of produced-water concentration on the percentage of attached zoospores that germinated varied tremendously with duration of exposure while in suspension and date of experimental trial. Zoospores that settled within 12 h after release generally had very poor rates of germination relative to those individuals that settled later (Fig. 6), suggesting that zoospores that settled shortly after release were not competent to germinate. The notable exception to this pattern were zoospores that were exposed to  $10\%$  produced water. Surprisingly, brief (up to 12 h) exposure to  $10\%$  produced water reduced the "precompetent period" and greatly improved germination success on the bottom. The magnitude of this enhancement in germination, however, varied among dates; germination after 6 and 12 h in  $10\%$  produced water was greatest in July, intermediate in March and lowest in April (Fig. 6). Longer exposures (i.e.,  $> 12$  h) to  $10\%$  produced water were extremely detrimental, and drastically reduced (even totally inhibited) zoospore germination. No consistent differences in the effects of produced water on germination were observed at produced-water concentrations  $\leq 1\%$ .

### Discussion

Our laboratory experiments indicate that the toxicity of produced water to giant kelp (*Macrocystis pyrifera*) zoospores is generally low. Relatively high concentrations of produced water ( $\geq 1\%$ ) were needed to produce adverse effects on zoospore swimming, attachment, and germination. Moreover, with the exception of swimming, adverse effects of produced water were observed only after relatively long periods of exposure (i.e.  $\geq 24$  h). Interestingly, relatively short exposures to high concentrations of produced water actually promoted successful germination during what normally appeared to be a precompetent period in all three experimental trials. How produced water could cause this unexpected pattern of early germination, however, remains unknown. It is possible that zoospore swimming is more sensitive to produced water than our results indicate. We may have underestimated the number of non-swimming zoospores in treatments containing produced water if toxins in the effluent inactivate flagella without causing

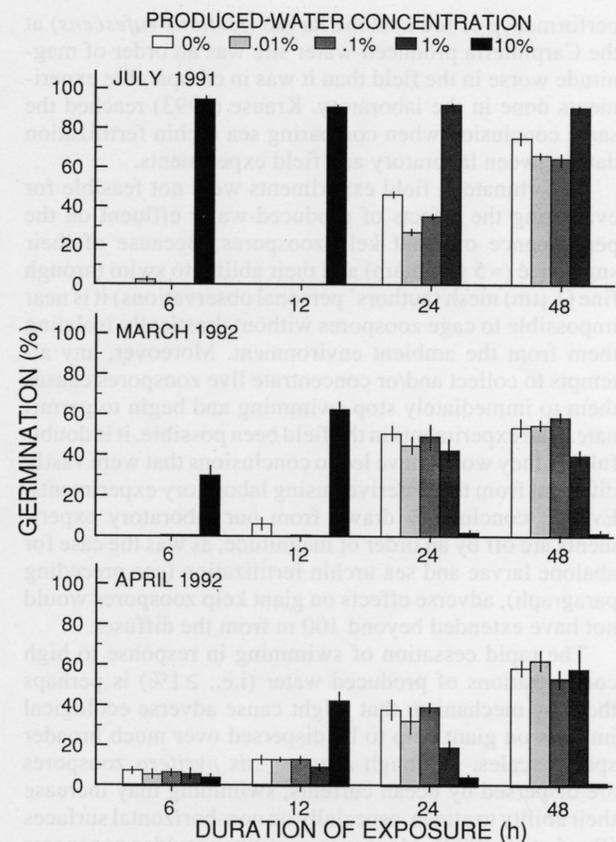


Fig. 6 *Macrocystis pyrifera*. Effects of produced-water concentration, exposure duration and date of experimental trial on percentage of attached zoospores that initiated germination after 72 h. Data are means ( $\pm 1$  SE)

the spore to change shape. We cannot be certain that all zoospores induced into a non-swimming mode by produced water changed from pear-shaped to spherical. However, we never saw non-swimming zoospores that were not spherical in video-recordings of zoospores placed in different concentrations of produced water.

Produced-water effluents are rapidly diluted in the near-shore environment along open coasts where kelp populations typically abound. Using salinity and elemental barium as tracers of produced water, Raimondi and Reed (1994) estimated the effluent concentration at the Carpinteria site to be  $\approx 0.1\%$  just 10 m from the diffuser. Thus, our laboratory results indicate that any adverse effects of discharging produced water on planktonic zoospores would be limited to the immediate vicinity of the outfall. However, conclusions drawn solely from laboratory studies are often inadequate for determining the spatial scale of ecological change that results from a local (i.e., point source) anthropogenic disturbance due to the inherent difficulties of mimicking field conditions in the laboratory. For example, Raimondi and Reed (1994) found that the

performance of red abalone larvae (*Haliotis rufescens*) at the Carpinteria produced-water site was an order of magnitude worse in the field than it was in comparable experiments done in the laboratory. Krause (1993) reached the same conclusion when comparing sea urchin fertilization data between laboratory and field experiments.

Unfortunately, field experiments were not feasible for evaluating the effects of produced-water effluent on the performance of giant kelp zoospores. Because of their small size ( $\approx 5 \mu\text{m}$ /diam) and their ability to swim through fine ( $1 \mu\text{m}$ ) mesh (authors' personal observations) it is near impossible to cage zoospores without drastically isolating them from the ambient environment. Moreover, any attempts to collect and/or concentrate live zoospores causes them to immediately stop swimming and begin to germinate. Had experiments in the field been possible, it is doubtful that they would have led to conclusions that were vastly different from those derived using laboratory experiments. Even if conclusions drawn from our laboratory experiments are off by an order of magnitude, as was the case for abalone larvae and sea urchin fertilization (see preceding paragraph), adverse effects on giant kelp zoospores would not have extended beyond 100 m from the diffuser.

The rapid cessation of swimming in response to high concentrations of produced water (i.e.,  $\geq 1\%$ ) is perhaps the only mechanism that might cause adverse ecological impacts on giant kelp to be dispersed over much broader spatial scales. Although *Macrocystis pyrifera* zoospores are dispersed by ocean currents, swimming may increase their ability to attach, especially on non-horizontal surfaces (Reed et al. 1992). Moreover, motility provides zoospores with the means to choose the microhabitat in which they settle. Once attached to the bottom, kelp zoospores orient chemotactically toward a variety of nutrients that are beneficial for growth and development (Amsler and Neushul 1989). Toxicants that inhibit zoospore swimming, therefore, should reduce a spores' chance for survival after settlement on the bottom. Since effects on swimming are immediate, zoospores passing through a small but concentrated effluent plume, even for a brief period of time, could be disoriented and prone to settle in an unsuitable environment even if subsequently dispersed far beyond the plume, so long as the effects on swimming were irreversible. It was not possible to determine if such effects on swimming were irreversible, however, because any handling effects of removing zoospores from produced water causes them to stop swimming. Similarly, Raimondi and Schmitt (1992) found that brief exposure to Carpinteria produced-water inhibited swimming in larvae of red abalone. In their study, however, which included both field and laboratory experiments, effects on swimming were partly reversible:  $\approx 50\%$  of the larvae resumed swimming after being placed into clean seawater. These authors and others (Krause et al. 1992; Garmen et al. 1994) suggest that produced-water inhibition of swimming and other activities (e.g. sperm function and nuclear migration) is caused by a disfunction of microtubules.

The effects of exposure to produced water on various aspects of zoospore performance generally varied with date

in a largely quantitative rather than qualitative way; the effluent concentration that caused a response varied among dates, but the general direction of the response did not. Such temporal differences could result from either variation in the toxicity of the produced water or in inherent differences in the quality of zoospores used on different dates. All available evidence suggests that quantitative differences among trials is better explained by temporal variation in zoospore quality. This is supported by the fact that swimming and attachment of zoospores placed in clean seawater (i.e., 0% produced water) differed significantly between dates. Similarly, Anderson et al. (1990) found that the toxic effects of copper on zoospore germination in *Macrocystis pyrifera* varied seasonally. In contrast, when assessing sea urchin fertilization, Krause (1993) found that the toxicity of produced water collected from the Carpinteria facility was extremely similar on three out of four dates. Moreover, concentrations of divalent cations, which have been identified as the most toxic constituents of Carpinteria produced-water (based on gamete toxicity assays using purple sea urchins and California mussels; Higashi et al. 1992), were quite similar among batches of produced water used in the different experimental trials of this study (T. Fan and R. Higashi unpublished data). As zoospore performance (i.e., swimming, germination, gametophyte reproduction) in giant kelp typically varies throughout the year (Reed 1989 and authors' unpublished observations), the impact of the effects of point sources of pollution on the dispersal potential of giant kelp will undoubtedly vary in concert.

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## Effects of an open-coast oil-production outfall on patterns of giant kelp (*Macrocystis pyrifera*) recruitment

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**Abstract** Field and laboratory experiments were used to investigate the spatial scale of benthic effects of an active nearshore produced-water (= aqueous wastes of oil and gas production) outfall on various components of recruitment in the giant kelp *Macrocystis pyrifera*. Results showed that discernible effects on all parameters measured were limited to areas very close to the outfall (<50 m). Zoospore production in sporophytes transplanted to varying distances from the diffusers did not vary in a systematic way. Survival and successful reproduction (i.e., sporophyte production) of outplanted gametophytes varied significantly among experimental dates. Performance of these parameters was significantly reduced only at the site nearest the diffusers (5 m away). Poor gametophyte survival near the outfall may have resulted from exploitative competition with *Beggiatoa* sp. (a fast-growing filamentous marine bacteria that exploits areas high in hydrogen sulfide, an abundant constituent of the produced-water effluent) rather than from toxicity of produced water. Laboratory assays indicated that gametophyte reproduction and subsequent sporophyte production were inhibited at levels likely to occur within the near vicinity of the diffusers. Nonetheless, field data indicate that the lack of sporophyte production near the diffusers probably resulted from factors affecting gametophyte survival.

### Introduction

Marine macroalgae are major primary producers in coastal marine waters, where net primary productivity can be as

high as anywhere on earth (Mann 1973). Recent work using carbon-isotope analyses has shown that macroalgal-derived carbon contributes significantly to the carbon assimilated by both primary and secondary consumers throughout the nearshore food web (Dunton and Schell 1987; Duggins et al. 1989). Thus, anthropogenic disturbances that threaten macroalgal populations are of major concern because of their potential for indirectly affecting a much broader group of marine organisms. One of the more common forms of human disturbance in coastal marine waters is the discharge of toxic substances via industrial and municipal outfalls. Until recently, macroalgae have been considered to be relatively insensitive to such environmental pollutants, as indicated by conclusions based on bioassays using vegetative growth as an endpoint (Jensen 1984). More recent studies, however, indicate that sexual reproduction and early development in seaweeds can be arrested by very small amounts of toxicants in the environment (Chung and Brinkhuis 1986; Thursby and Steele 1986; James et al. 1987; Anderson and Hunt 1988; Steele and Thursby 1988; Anderson et al. 1990; James et al. 1990), causing populations of macroalgae to be as much at risk from pollutants as populations of some of the more commonly assayed marine animals (Martin et al. 1981; Martin et al. 1989).

Giant kelp, *Macrocystis pyrifera*, the largest known alga, is one of the most important marine species in regions where it occurs. For both ecological and economic reasons, giant kelp forms the most prominent biogenic habitat in the nearshore coastal waters throughout its range (Foster and Schiel 1985). Long-term declines in the size of giant kelp populations at several locations in southern California have been attributed to reduced water quality caused by dredging and waste effluents from industrial and municipal outfalls (Wilson et al. 1978; Wilson and McPeak 1983; Murdoch et al. 1989). Although the adult macroscopic sporophyte stage may be relatively invulnerable to acute exposure to pollutants such as spilled oil (Foster et al. 1971), growth and reproduction of the microscopic gametophyte stages are sensitive to several forms of pollution at relatively low levels (James et al. 1987; Anderson

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and Hunt 1988; Anderson et al. 1990; James et al. 1990). Such stage-specific sensitivity may cause giant kelp forests to be particularly at risk to point sources of pollution, especially since results from studies (Murdoch et al. 1989) and predictions from population models (Nisbet and Bence 1989; Burgman and Gerard 1990) indicate that declines in the size of giant kelp populations caused by poor growing conditions result primarily from a reduction in recruitment rates rather than from an increase in the rate of adult mortality.

In terms of volume, effluents of aqueous wastes generated during oil and gas production (commonly called produced waters) are among the largest industrial waste streams in the world (Jacobs et al. 1992; Stephenson 1992). Not surprisingly, the discharge of such large volumes of produced water has raised significant environmental concerns over their fate and effects in the marine environment (see Boesch and Rabalais 1987 and Ray and Engelhardt 1992 for reviews). Produced waters contain a diverse array of potentially toxic substance including hydrocarbons, heavy metals, radionuclides, and chemical additives such as corrosion inhibitors and biocides (Middleditch 1984; Stephenson 1992; Tibbets et al. 1992). Since these effluent constituents tend to concentrate on the bottom (Armstrong et al. 1979), they have the potential to alter recruitment rates of sessile organisms such as giant kelp that are common near areas of oil and gas production activities. The spatial extent to which recruitment rates of *Macrocystis pyrifera* are impacted by the chronic discharge of produced waters into the nearshore marine environment is entirely unknown.

Here we describe field and laboratory experiments which examined the effects of an active nearshore produced-water outfall on various developmental stages of giant kelp that are necessary for successful recruitment: zoospore production in adult sporophytes and survival and successful reproduction of microscopic gametophytes on the bottom. Water-column effects of produced water on the colonization potential of planktonic zoospores are described elsewhere (Reed and Lewis 1994). The principal goals of this study were to: (1) define the spatial scale of discernable benthic effects on giant kelp recruitment associated with the discharge on produced water into a high-energy coastal environment, and (2) determine the toxicity of produced water on specific events occurring during gametophyte reproduction.

## Materials and methods

### Study site

Our study site was located in the shallow subtidal of a high-energy open coast near Carpinteria California USA (34°23'N; 119°30'W). The diffusers of the produced water outfall under study are located = 200 to 300 m offshore in a sandy bottom at a depth of ~ 11 m. Populations of *Macrocystis pyrifera* are located on hard substrate = 200 m east (up-current) and 2000 m west (down-current) of the diffusers. The salinity of the effluent during the study was 16‰. Daily records show that discharge of effluent was very constant during the

study period, averaging  $2.64 \times 10^6$  liters  $d^{-1} \pm 5\%$  (unpublished data of California State Water Resource Control Board). More detailed descriptions of the study site and outfall design can be found in Osenberg et al. (1992) and Raimondi and Schmitt (1992) and chemical characteristics of the effluent discharged at the site have been summarized by Higashi et al. (1992).

### Zoospore production

The effects of distance from the outfall on zoospore production in *Macrocystis pyrifera* were examined in similar-sized adult sporophytes collected from a nearby rocky reef and transplanted to five different locations at 5, 50, 100 and 1000 m to the west (down-current) and at 1000 m to the east (up-current) of the diffusers on 10 July 1991 (*M. pyrifera* produces zoospores all the year round; McPeak 1981). Seven plants were transplanted to each location by fastening the holdfast of each plant to a sand anchor using nylon line; all sporophylls on each plant were removed at this time. Zoospore production in transplanted individuals was evaluated 75 d later (23 September 1991) by measuring the area of sporogenous tissue produced on each plant since the start of the experiment. Sporogenous tissue in *M. pyrifera* is identified by its coloration, which reflects the presence of mature sporangia containing zoospores. Sorus area is a good indicator of zoospore standing stock because the density of sporangia is relatively uniform in non-senescent sori (mean sporangia density of *M. pyrifera* sori =  $10420 \text{ mm}^{-2} \pm 29\%$ ,  $n = 75$  plants; Reed unpublished data). Sorus area ( $A$ ) was calculated as:

$$A = slw/f \quad (1)$$

where  $s$  = the number of fertile sori,  $l$  = estimated mean length of sori on a plant,  $w$  = estimated mean width of sori on a plant, and  $f$  = the number of fronds on a given plant at the start of the experiment. Sorus area was standardized to frond number because experimental plants initially varied slightly in size (see Reed 1987). The effect of distance from the diffusers on sorus production was determined by ANOVA.

### Gametophyte survival and sporophyte production in the field

Gametophyte survival and successful reproduction (i.e., the production of embryonic sporophytes) were examined in situ by outplanting newly settled zoospores to the bottom at the above five locations along the same distance gradient from the outfall diffusers. Zoospores were seeded onto clear vinyl microscope slides and outplanted every two weeks between 26 February 1992 and 20 May 1992 (a total of seven dates). On three dates (26 February, 13 March and 20 May), slides were seeded on-site on board a small boat and gametophytes were = 2 h old when outplanted to the bottom; on the other four dates, slides were seeded in the laboratory = 18 h prior to being outplanted. No consistent differences in gametophyte survival or reproduction were observed as a result of the two different methods of seeding (see "Results - Gametophyte survival and sporophyte production in the field"). Slides were fastened in an upright position 0.5 m off the bottom on concrete blocks designed to mimic a low-relief rocky reef. Twenty-four slides were outplanted at every location on each date. Eight of the 24 slides were retrieved at 1, 3, and 6 wk after outplanting and brought back to the laboratory, where the density of female gametophytes and sporophytes on them was determined using a compound microscope. Only females were counted because they provide a more reliable estimate of survival in the field (Reed et al. 1988). Gametophyte survival was estimated for each location and date combination from the ratio: mean female gametophyte density at time of retrieval (1, 3, or 6 wk): mean settlement density of females when outplanted to the sea. Sporophyte density was used as an indicator of successful reproduction. The effects of date of outplant and distance from the diffusers (=location) were analyzed in two-way mixed-model ANOVAs with site considered a fixed factor and date a random factor. Data were transformed to  $\log(x + 0.1)$  to meet the assumption of homoscedasticity.

### Laboratory bioassays

Causation of effects of produced water on sporophyte production observed in the field was investigated in the laboratory. Produced wa-

ter used in all laboratory experiments was obtained from a spigot on the discharge pipe at the Carpinteria oil-processing facility just before it enters the ocean. The water was collected without head space in acid-washed plastic containers, transported to the laboratory on ice, and frozen at  $-70^{\circ}\text{C}$  until use. A bioassay was done to determine whether exposure to produced water affected sporophyte production in *Macrocystis pyrifera*. Zoospores were settled in plastic dishes at a density of  $\approx 30\text{ mm}^{-2}$  and cultured in clean, sterile-filtered, nutrient-enriched seawater (Provasoli 1968) at  $15^{\circ}\text{C}$  under an irradiance of  $40\ \mu\text{E m}^{-2}\text{ s}^{-1}$  with a 14 h light: 10 h dark photoperiod. These conditions are within the optimum range for gametophyte growth and reproduction in *M. pyrifera* (Lüning and Neushul 1978, Deysher and Dean 1984). The reproductive status of developing gametophytes was examined daily, and at the first sign of sexual maturity (i.e., egg extrusion by  $<5\%$  of the females on Day 11), media were replaced and varying amounts of produced water were added to the dishes to generate five different concentrations of produced water (0, 0.01, 0.1, 1, and 10%;  $n = 5$  dishes for each concentration). Four days later, the density of females, the percentage of females that had extruded eggs, and the mean number of embryonic sporophytes produced per female were estimated for each dish.

Additional laboratory experiments were made to elucidate the mechanisms by which produced water can potentially influence sporophyte production in *Macrocystis pyrifera*. Fertilization in kelps relies on male recognition of a pheromone that is released by the egg when it is extruded from the oogonium. The pheromone induces the release of sperm from the male and attracts sperm to the egg. Previous studies on algae have shown that petroleum hydrocarbons can interfere with the sperm's ability to respond to pheromones (Derenbach and Gereck 1980). We tested the null hypothesis that produced water does not alter the chemical communication system between male and female gametes in giant kelp. A clonal male gametophyte of *M. pyrifera* was fragmented in a tissue grinder and fragments were uniformly distributed among the wells of two 24-well tissue culture plates and cultured under the conditions detailed above. When males were sexually mature (7 d later), 0.45 ml of sterile filtered seawater containing varying concentrations of produced-water was added to each well to generate four different produced water treatments (0, 0.1, 1, and 10%;  $n = 12$  wells for each treatment). Female pheromone was introduced into each well by adding 50  $\mu\text{l}$  of media taken from cultures containing females with extruded eggs. After 5 min, the contents of each well were transferred to a microcentrifuge tube containing 25  $\mu\text{l}$  of formalin, and the concentration of sperm in each tube was estimated using a hemocytometer.

Reduced rates of fertilization could result if toxicants in produced water impair the swimming ability of sperm. To test this hypothesis, we collected sperm from male cultures by inducing their release with media from female cultures and added similar amounts of sperm to each of five petri dishes containing different concentrations of produced water (0, 0.01, 0.1, 1, and 10%). The percentage of sperm swimming at each concentration of produced water was assessed from replicate aliquots taken from each petri dish over a 40 min period. Sperm swimming was examined using a video camera attached to a compound microscope with differential interference contrast.

The effects of produced-water concentration on egg extrusion, sporophyte production and sperm release were determined by single-factor ANOVAs and differences among mean concentrations were resolved using the Ryan–Einot–Gabriel–Welsch multiple-range test (SAS Institute Inc. 1985). The effect of produced-water concentration on the percentage of sperm swimming was determined by ANCOVA, with time as the covariate.

## Results

### Zoospore production

We found no evidence that the discharge of produced water adversely affected zoospore production in *Macrocystis*

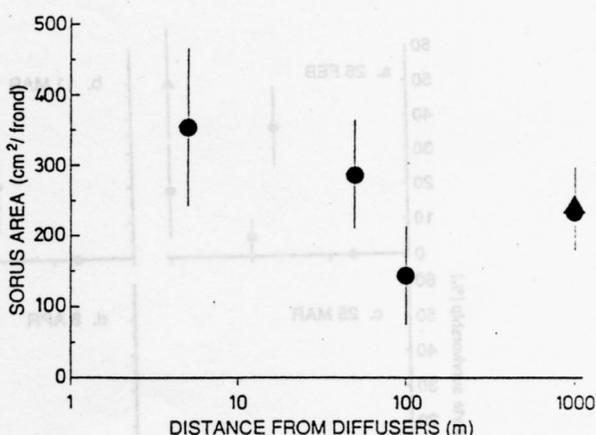


Fig. 1 *Macrocystis pyrifera*. Mean sorus production ( $\pm 1$  SE) of adult sporophytes at varying distances from diffusers (● west of diffusers; ▲ east of diffusers)

*pyrifera* at the Carpinteria site. The production of new sporogenous tissue was highly variable, and distance from the diffusers had no significant (nor systematic) effect on the amount of sorus area produced in transplanted adult sporophytes ( $F_{4,33} = 1.01$ ,  $P = 0.417$ ; Fig. 1). Strong flow from the diffuser ports was noticeable only at the 5 m location, where the basal portions of transplanted individuals (the region where zoospores are produced) were physically scoured by sand and often covered with a slimy film of the marine bacteria *Beggiatoa* sp. (a marine microbe that thrives in habitats high in hydrogen sulfide, a common constituent of the effluent discharged at the Carpinteria site). Nonetheless, the highest levels of sorus production were recorded at the 5 m location.

### Gametophyte survival and sporophyte production in the field

The effects of proximity to the diffusers on gametophyte survivorship after 1 wk varied among dates on concrete blocks used to mimic low-relief reefs ( $F_{24,279} = 3.2$ ,  $P < 0.0001$  for location  $\times$  date interaction). On all dates but one (6 May), survivorship was zero or nearly zero at 5 m from the diffusers and generally higher at locations farther away (Fig. 2). No consistent differences among the more distant locations (i.e., 50, 100, 1000 m west and 1000 m east) were observed, indicating that adverse effects of the outfall on gametophyte survivorship did not extend out to 50 m.

Survivorship of female gametophytes for 3 wk was generally low (always  $<5\%$  and usually  $<1\%$ ) at all locations on all outplant dates. Nonetheless, substantial sporophyte production was observed on slides retrieved 3 wk after being outplanted on 6 May, indicating that in the field female gametophytes die and disappear shortly after extruding

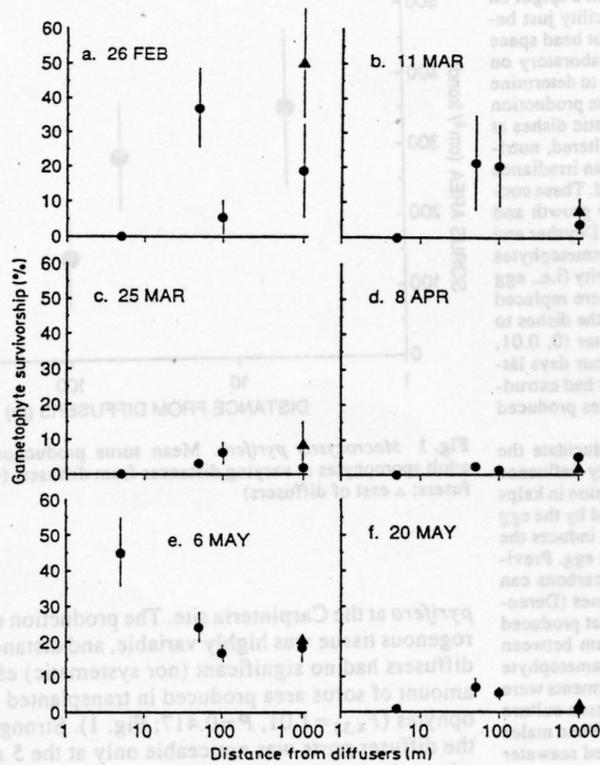


Fig. 2 *Macrocyctis pyrifera*. One-week survival of female gametophytes outplanted along a distance gradient from diffusers on six different dates; data are means  $\pm$  1 SE;  $n=8$  slides (● west of diffusers; ▲ east of diffusers)

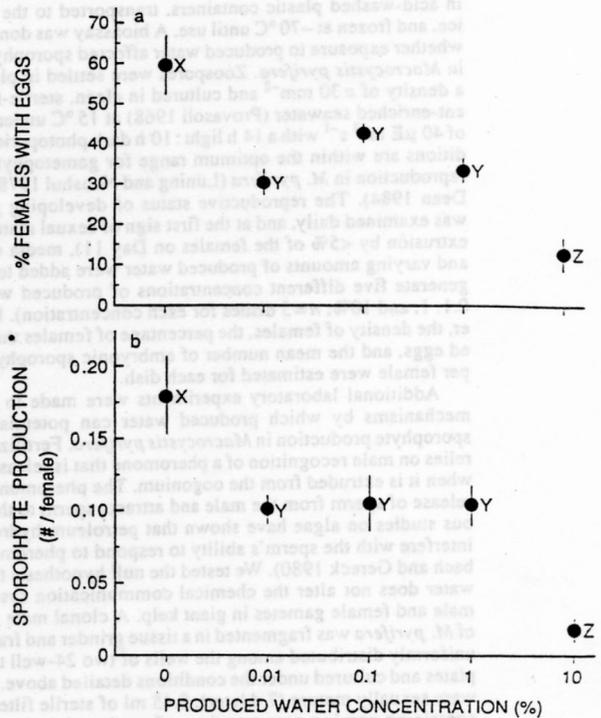


Fig. 4 *Macrocyctis pyrifera*. Effects of produced-water concentration on: a) percentage of females that became reproductively mature (i.e., extruded eggs from their oogonium), and b) number of sporophytes produced per female; data are means  $\pm$  1 SE,  $n=5$  dishes. Means with same letter (X, Y, Z) are not significantly different from each other using REGWQ (Ryan-Einot-Gabriel-Welsch multiple-range test,  $P=0.05$ ; SAS Institute Inc. 1985)

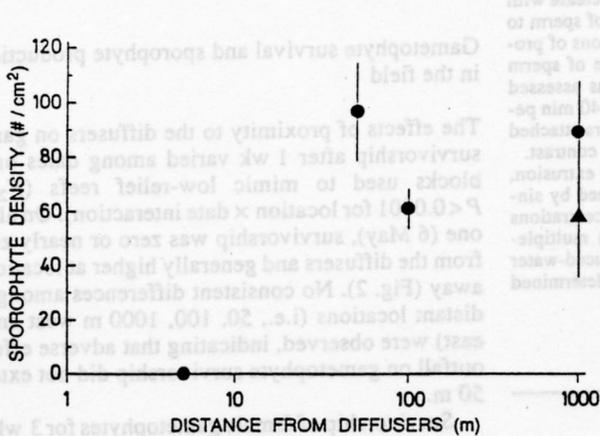


Fig. 3 *Macrocyctis pyrifera*. Recruitment density of embryonic sporophytes at varying distances from diffusers. Newly settled zoospores were outplanted on 6 May 1992 and sporophyte density was sampled 3 wk later; data are means  $\pm$  1 SE,  $n=8$  slides (● west of diffusers; ▲ east of diffusers)

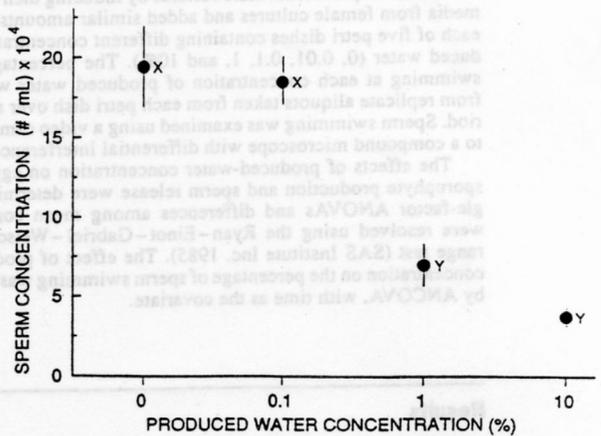


Fig. 5 *Macrocyctis pyrifera*. Effects of produced-water concentration on amount of sperm released by male gametophytes; data are means  $\pm$  1 SE,  $n=12$  wells. Means with same letter (X, Y) are not significantly different from each other using REGWQ (Ryan-Einot-Gabriel-Welsch multiple-range test,  $P=0.05$ ; SAS Institute Inc. 1985)

eggs. Relatively large numbers of embryonic sporophytes were observed at all locations except 5 m on this date (Fig. 3). Sporophytes were not produced from any of the outplant experiments done on other dates.

#### Laboratory bioassays

Results from laboratory bioassays indicate that produced-water toxicity can cause significant adverse effects to gametophyte reproduction in *Macrocystis pyrifera* at relatively low levels ( $F_{4,24} = 11.15$ ,  $P < 0.0001$ ). A two-fold reduction (relative to control) in the percentages of females extruding eggs (Fig. 4a) and females producing sporophytes (Fig. 4b) was observed at produced-water concentrations as low as 0.01%, the lowest dilution examined. Significantly greater declines in these two parameters were observed in 10% produced water.

The addition of produced water appeared to alter chemical recognition between male and female gametes, which caused a significant reduction in the amount of sperm available for fertilization ( $F_{3,47} = 26.8$ ,  $P < 0.001$ ). Significantly fewer sperm were released by male gametophytes exposed to 1 and 10% produced water relative to a 0.01% concentration of produced water or clean seawater (Fig. 5). In contrast, the addition of even high concentrations of produced water had no effect on the percentage of sperm swimming ( $F_{3,21} = 0.12$ ,  $P = 0.945$ ; Fig. 6).

#### Discussion

Discernible effects on recruitment of *Macrocystis pyrifera* appeared limited to areas very close to the outfall; significant reductions in gametophyte survival and reproduction (measured as sporophyte production) were observed only at the 5 m site. However, data from this study alone are insufficient to implicate the discharge of produced water as the primary cause for these observed effects. Constraints on the design of our field experiments prevented us from completely isolating natural causes of spatial variability from that due to the discharge of produced water (Stewart-Oaten et al. 1987). There are compelling reasons to believe, however, that the discharge was the primary mechanism causing reduced performance in giant kelp. First, the effects were highly localized within a relatively large and uniform area. Second, the observed patterns were symmetrical about the diffusers in as much as the most distant locations on either side of the outfall were similar to each other, and were different from that observed near the diffusers. Lastly, companion studies done at Carpinteria using other organisms showed that previously observed effects dissipated during brief periods when the discharge was not operational (Raimondi and Schmitt 1992; Krause 1993).

Daily rates of effluent discharge were quite constant during our study (unpublished data California State Water

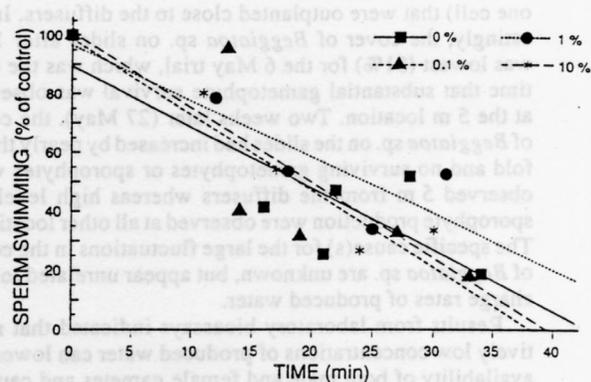


Fig. 6 *Macrocystis pyrifera*. Percentage of sperm swimming over time at different concentrations of produced water. Data are standardized to that observed in clean seawater at time = 0 h.

Resource Control Board), yet gametophyte survival and sporophyte production were highly variable in time and space. In this study, gametophytes survived to produce sporophytes on only 1 of 7 outplant dates at all locations except 5 m from the diffusers, where sporophyte production was never observed. Such poor survival rates are not surprising, since environmental conditions favorable for recruitment in *Macrocystis pyrifera* are known to occur infrequently in southern California (Deysler and Dean 1986; Reed et al. 1988; Reed 1990). The high rates of gametophyte survival observed at all locations during the week following the 6 May outplant most probably reflect an infrequent "recruitment window" (sensu Deysler and Dean 1986) of favorable environmental conditions for gametophyte survival and reproduction. The fact that high survival was observed 5 m from the outfall during this time suggests that some factor(s) associated with these favorable conditions somehow lessened the adverse effects of the outfall on the adjacent benthic habitat.

Controlled laboratory experiments testing the toxic effects of produced water on gametophyte survival through reproduction were not done because of difficulties in maintaining levels of toxicity over prolonged periods in culture. Without such information it is difficult to reliably attribute poor survival near the diffusers to toxic components of produced water. Toxicity, however, need not be invoked to explain reduced survival near the diffusers. Reed (1990) found that a cover of filamentous brown algae inhibited sporophyte production from *Macrocystis pyrifera* gametophytes outplanted to an offshore rocky reef. In the present study, dense mats of the filamentous marine bacteria *Beggiatoa* sp. covered much of the bottom near the diffusers and were quick to colonize microscope slides outplanted to the 5 m location. Cover of *Beggiatoa* sp. often exceeded 90% on outplanted slides after just 1 wk in the field. *Beggiatoa* sp. was not observed on slides placed at any of the other more distant locations. This extremely fast-growing microbe may have simply overgrown the smaller kelp gametophytes (female gametophytes rarely grew larger than

one cell) that were outplanted close to the diffusers. Interestingly, the cover of *Beggiatoa* sp. on slides after 1 wk was lowest (21%) for the 6 May trial, which was the only time that substantial gametophyte survival was observed at the 5 m location. Two weeks later (27 May), the cover of *Beggiatoa* sp. on the slides had increased by nearly three-fold and no surviving gametophytes or sporophytes were observed 5 m from the diffusers whereas high levels of sporophyte production were observed at all other locations. The specific cause(s) for the large fluctuations in the cover of *Beggiatoa* sp. are unknown, but appear unrelated to discharge rates of produced water.

Results from laboratory bioassays indicated that relatively low concentrations of produced water can lower the availability of both male and female gametes and cause a reduction in sporophyte production. Rates of egg extrusion and sperm release were significantly reduced by exposure to produced water. Egg extrusion appeared more sensitive to produced water than sperm release and was of sufficient magnitude to independently account for the reduction observed in sporophyte production. Because the lowest concentration of produced water examined (0.01%) reduced egg extrusion and sporophyte production by >40%, significant effects may be discernible at even lower concentrations. This contrasts with the relatively high tolerance to produced-water toxicity observed for *Macrocystis pyrifera* zoospores. Reed and Lewis (1994) found that adverse effects on zoospore swimming, attachment and germination were generally limited to effluent concentrations >1%. Differences in the sensitivities observed between gametophytes and zoospores cannot be attributed to variation in produced-water toxicity, since the batch of produced water used in the present study (collected 30 July 1991) was the same as one of those used to examine zoospores. Thus, based on sensitivities of different life stages, the area of effect surrounding a produced-water discharge is likely to be less for zoospores in the water column than for gametophytes on the bottom (irrespective of patterns of dilution or accumulation of toxicants in the two habitats).

The theoretical initial dilution achieved by the diffusers at 5 m from the outfall is  $\approx 1\%$  (Raimondi and Schmitt 1992). Toxic plume models of the Carpinteria outfall indicate that a produced-water concentration of  $\geq 0.01\%$  extends out  $\approx 20$  m from the diffusers (Krause 1993, Raimondi and Reed 1994). These models are based on comparisons of biological performance recorded in the field at different distances from the diffusers with that observed in the laboratory under known concentrations of produced water. Thus, the concentration of produced water at the 5 m location was probably high enough to account for at least some of the reduction in sporophyte production observed there. Nonetheless, the results from the field experiments suggest that the lack of sporophyte production from the 6 May outplant near the diffusers was not entirely caused by a toxicant-mediated inhibition of gametophyte reproduction, but rather resulted primarily from conditions affecting gametophyte survival. This is supported by the fact that neither gametophytes nor sporophytes were observed at the 5 m location 3 wk after the 6 May outplant, a time

when the overgrowth of *Beggiatoa* sp. was relatively high (see third paragraph of "Discussion").

Companion studies at Carpinteria have found the area of effect to be considerably larger ( $\geq 100$  m) for various reproductive traits and developmental processes of marine invertebrates, including gonad mass and gamete performance in sea urchins (Krause 1993), settlement and metamorphosis of red abalone larvae (Raimondi and Schmitt 1992), and growth and gonad mass in mussels (Osenberg et al. 1992). The reason(s) for the smaller response in giant kelp is unclear. Data from field and laboratory experiments on sea urchins and abalone larvae strongly suggest that reduced performance of these animals in the field was directly attributable to the toxicity of produced water. These conclusions might indicate that the reproductive traits and developmental processes examined in *Macrocystis pyrifera* are much less sensitive to produced water than those examined in sea urchins and abalone. Results from laboratory experiments indicate that this is not true. Raimondi and Schmitt found only a  $\approx 15\%$  reduction (relative to control) in settlement of abalone larvae at 0.01% produced water (the lowest concentration tested), while Krause observed a  $\approx 20\%$  reduction in sea urchin fertilization in seawater containing 0.01% produced water. In contrast, we found that sporophyte production in *M. pyrifera* was reduced by >40% in 0.01% produced water. The reasons for the inconsistencies between the results of our study and those on marine invertebrates is unclear, but may be due in part to the difficulty of detecting significant adverse impacts on rates of early survival in organisms such as kelp that typically have frequently low and highly variable rates of recruitment.

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## CHAPTER 10

# DETERMINING THE SPATIAL EXTENT OF ECOLOGICAL IMPACTS CAUSED BY LOCAL ANTHROPOGENIC DISTURBANCES IN COASTAL MARINE HABITATS

**Peter T. Raimondi and Daniel C. Reed**

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Determining the spatial scale of ecological change that results from a local (i.e., point source) anthropogenic disturbance is the focus of many ecological impact assessment studies. Frequently, measures of physical parameters are used to evaluate the potential maximum spatial extent of ecological change resulting from an impact (Spellerberg 1991). Such an approach assumes that changes in the physical environment (e.g., changes in seawater temperature, salinity, or chemistry) are comparable to, or provide indirect measurements of, impacts on ecological parameters (e.g., population density and community structure). This is unlikely to be the case unless the spatial extent of the physical and ecological impacts are the same.

There are compelling reasons to expect that the spatial extents of physical and ecological impacts are not always the same in coastal marine habitats. Many species typically display a nonlinear or threshold response to changes in the physical environment. This is because various physiological mechanisms allow many organisms to tolerate some degree of changing environmental conditions associated with a disturbance (e.g., increased levels of toxicants) without undergoing any appreciable change in their demographic rates. Moreover, because marine organisms vastly differ in physiology, resource requirements, and life-history characteristics, their tolerances to a given perturbation (and the corresponding spatial extent of the ecological impact that results from it) are likely to vary among species as well as among different ecological parameters within a species. This type of phenomenon can cause the spatial extent of the ecological impact to be partially or even completely unrelated to alterations in the physical environment caused by a perturbation. Unfortunately there are few published data sets that provide information on the spatial relationships among different

physical and ecological variables affected by a local impact. The scarcity of such information further complicates the task of determining the spatial scale of ecological change that results from a local disturbance. In this chapter we examine spatial relationships among various physical/chemical and biological variables used to measure the spatial extent of impacts caused by an industrial outfall in a coastal marine habitat.

Of equal concern in ecological impact assessment studies is the potential for ecological impacts to spread to distant locations far from the area that is physically disturbed. Such spatial separation between physical and ecological impacts is possible in nearshore habitats because many marine species reproduce via planktonic propagules (e.g., gametes, spores, and larvae) that are capable of dispersing substantial distances (tens to hundreds of kilometers). This causes renewal rates in local populations of many species to be largely dependent on the transport and arrival of propagules produced elsewhere. Thus, a local perturbation that alters demographic rates (e.g., birth, death, immigration, emigration) of nearby populations can potentially affect the structure and dynamics of distant populations.

If the physical and ecological effects caused by human activities covary in space and time, then assessment of ecological change, while often laborious, should not be overly complex. However, if physical and ecological effects of a disturbance are separated, ecological assessment can become very difficult. This is because when ecological effects of a local impact are dispersed to distant sites they usually become diluted making it more difficult to detect ecological change at any particular site. Furthermore, natural variation in population parameters coupled with the high costs of increased sampling effort serves to reduce the statistical power of detecting small changes caused by a distant perturbation. For these reasons, efforts to monitor ecological impacts that are spatially separated from physical impacts are almost surely destined to fail if typical protocols (including the BACIPS design, Chapters 6–8) are used. Nevertheless, local impacts can in theory substantially influence the dynamics and structure of more distantly located populations (Nisbet et al., Chapter 13).

Designing effective and logistically-tractable ecological assessment studies hinges critically on understanding the possible relationships between the spatial scale of physical and ecological impacts. The most fundamental cause of spatial uncoupling between physical and ecological impacts is the ability of individuals to move from one location to another. If movement of individuals is severely limited, then ecological impacts are likely to be restricted to the area of physical impact, or some subset of that area. In contrast, if organisms are highly mobile the effects of a local perturbation can spread to distant areas, far removed from the site of local activities causing physical and ecological impacts to be either partially or fully separated in space.

While organismal mobility seems to be a necessary condition for the extension of ecological effects beyond the area of physical impact, other attributes may make such separation between physical and ecological effects more or less

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likely to occur. Thus, in addition to examining spatial relationships between physical and biological variables we also discuss how various life-history attributes of marine organisms may cause ecological impacts to become spatially separated from physical impacts.

### **Spatial Relationships among Physical and Ecological Variables Following a Local Disturbance**

Detecting the spatial extent of ecological impacts resulting from local physical disturbances is often difficult. A major complication arises when physical measures of impact, which are often used to estimate biological impacts, do not correlate well with ecological ones. This may happen when: (i) the physical variables measured are not those that cause ecological effects (or are not correlated with the causal agents), or (ii) physical variables are not adequately measured (e.g., instrumentation and/or sampling design are inadequate to detect changes caused by disturbance). That individual physical measures of impact may not correlate with other physical measures and individual ecological measures of impact may not correlate with other ecological measures further complicates the task of assessing the spatial extent of local impacts. If physical and ecological measures of impact are poorly correlated, then the spatial extent of an impact can be meaningful only relative to the measured parameter. This would reduce our ability to predict and determine the extent and type of ecological effects that result from localized physical disturbances.

To provide insight into the degree of correlation among different physical and ecological measures of impact we examined results from a group of companion studies designed to assess the impact of a produced water (a byproduct of oil and gas development) discharge on the nearshore marine environment. The outfall is located off the coast of Carpinteria, California, USA (34°23'N, 119°30'W) on a flat sandy bottom at a depth of 11 m. This area has been characterized as an open coast high energy environment (Osenberg et al. 1992). The effluent is discharged ~ 300 m offshore through a series of small diameter (4–5 cm) diffuser ports. The average discharge of effluent during the course of the investigations (1989–1992) was 2.64 million liters/day (Osenberg et al. 1992). A discussion of the chemical nature of the effluent can be found in Higashi et al. (1992).

Surveys and experiments were done to estimate the spatial extent of the physical and biological/ecological impacts resulting from the discharge of produced water. Data were collected at a series of locations, along an 11 m isobath, arranged in a gradient away from the diffusers. Physical variables most likely to be affected by the outfall were chosen and used to estimate physical extent of the impact (Table 10.1). Water clarity (estimated from secchi disk measurements) and sediment grain size were measured because of the belief that the rapid discharge of effluent to a sand-bottom habitat would stir up sediments and

**Table 10.1. Estimates of Spatial Extent of Impact at Carpinteria for Various Physical/Chemical and Biological Variables**

	Distance (meters) from outfall where measured variables = background or control levels	Source*
<i>Physical/chemical variables</i>		
Barium in the shell of <i>Mytilus edulis</i>	500–1000	a
Barium in the shell of <i>Mytilus californianus</i>	500–1000	a
Barium in sediments	10–20	b
Salinity	5–10	c
Water clarity	0	d
Sediment grain size	0	a
% Organic matter	0	a
<i>Biological variables</i>		
Nematode density	50–100	e
Polychaete density	50–100	e
<i>Mytilus edulis</i>		
Tissue production	500–1000	e
<i>Mytilus californianus</i>		
Tissue production	500–1000	e
<i>Strongylocentrotus</i>		
Male gonadal mass	50–100	c
Female gonadal mass	50–100	c
Fertilization	50–100	c
Sperm attack rate	50–100	c
<i>Haliotis rufescens</i> - precompetent larvae		
Settlement	100–1000	f
Survivorship	100–1000	f
<i>Haliotis rufescens</i> - competent larvae		
Settlement	100–500	f
Survivorship	0	f
Metamorphosis	50–100	f
Swimming	100–500	f
<i>Macrocystis pyrifera</i>		
Fecundity (spore production)	0–5	g
Sporophyte recruitment	5–50	g

\*Sources: a = Osenberg and Fan (personal communication); b = Higashi and Fan (personal communication); c = Krause (1993); d = Reed (unpublished data); e = Osenberg et al. (1992); f = Raimondi and Schmitt (1992); g = Reed et al. (1994).

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increase turbidity near the diffusers. Produced water discharged at Carpinteria is hyposaline (about 17 ppt) and contains numerous hydrocarbons and heavy metals, the most conspicuous being barium which has an elevated concentration of 6–19 ppm (compared to ambient control levels of <0.002 ppm). Therefore, water-column salinity, and barium and organic matter in the sediments were measured to map the extent of the outfall plume. In addition, barium was measured in the shells of the mussels *Mytilus californianus* and *M. edulis*. Measuring constituents of the effluent in sediments and mussels can be useful in determining the spatial extent of impacts because sediments and mussels potentially integrate the temporally variable supply of effluent over time. To measure the biological/ecological response to the produced water discharge, 16 variables from 8 species were examined (Table 10.1).

Less than 50% (3/7) of the physical/chemical variables measured at the Carpinteria site were significantly correlated with proximity to the diffusers, an event we consider to be evidence for an impact (Table 10.2). By contrast, a greater percentage, 75% (12/16), of the biological variables examined were correlated with distance from diffusers. A concern about the validity of comparing correlations involving physical/chemical variables to those from biological variables is that the results might be affected by selection of variables. We agree, but note that all physical/chemical variables which as a group were less correlated with distance from diffusers, were studied because of specific predictions about the physical results of the impact (e.g., likely increase in salinity and decrease in barium concentration as a function of distance from the diffusers; as above).

The spatial extent to which each variable was affected by the outfall was estimated by determining the distance from the diffusers where levels of each variable were no different from background or control levels (Table 10.1). In general, the distances over which the biological variables were affected were ~ an order of magnitude greater than those over which the physical/chemical variables were affected with the exception of barium in the shells of the mussels, *M. californianus* and *M. edulis*.

We investigated the relative value of different biological and physical/chemical variables in predicting the spatial extent of ecological impacts by examining the degree to which: (i) biological measures of impact were correlated with each other and, (ii) physical/chemical measures of impact were correlated with biological measures. Pairwise correlations were done on all combinations of variables by arranging variable values by distance from the diffusers. The significance levels for all correlations are given in Table 10.2 and the ratios of statistically significant to nonsignificant correlations involving each variable are summarized in Table 10.3 along with whether the variables themselves were significantly correlated with distance from the diffusers.

Results indicate that certain variables were better than others at predicting the spatial pattern of the ecological impact (here defined as the area over which any biological variable was affected: Table 10.3). For example, polychaete number was not significantly correlated with any of the other 15 biological variables. By



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Table 10.3. Relationships among Variables

	P/B	B/B	Correlated with distance from diffusers?
<i>Physical/chemical variables</i>			
Barium in the shell of <i>Mytilus edulis</i>	9/16		Yes
Barium in the shell of <i>Mytilus californianus</i>	9/16		Yes
Barium in sediments	1/16		No
Salinity	1/16		Yes
Water clarity	0/16		No
Sediment grain size	0/16		No
% organic matter	0/16		No
<i>Biological variables</i>			
Nematode density		1/15	Yes
Polychaete density		0/15	No
<i>Mytilus edulis</i>			
Tissue production		7/15	Yes
<i>Mytilus californianus</i>			
Tissue production		4/15	Yes
<i>Strongylocentrotus purpuratus</i>			
Male gonadal mass		7/15	Yes
Female gonadal mass		7/15	Yes
Fertilization		8/15	Yes
Sperm attack rate		9/15	Yes
<i>Haliotis rufescens</i> - precompetent larvae			
Settlement		7/15	Yes
Survivorship		5/15	Yes
<i>Haliotis rufescens</i> - competent larvae			
Settlement		7/15	Yes
Survivorship		0/15	No
Metamorphosis		8/15	Yes
Swimming		9/15	Yes
<i>Macrocystis pyrifera</i>			
Fecundity (spore production)		1/15	No
Sporophyte recruitment		0/15	No

Note: Ratios are the number of significant correlations ( $P \leq .05$ ) over the total number correlations (significant and nonsignificant). P/B = Physical/chemical vs biological variables; B/B = Biological vs other biological variables. See Table 10.2 for description of data.

contrast, sea urchin fertilization was significantly correlated with 9 of 15 biological variables. Interestingly, the same variable measured in different ways yielded different results. Concentration of barium in the shells of mussels (a physical/chemical variable) was as good as any biological variable in predicting the spatial extent of ecological impacts, while barium in the sediments was correlated with only one biological variable. Like barium in the sediments, other physical/chemical variables were generally poor predictors of ecological impacts. There were large differences in ability to predict the response of other variables even among those biological and physical/chemical variables affected by the impact (i.e., those significantly correlated with distance from the diffusers). The range in number of significant correlations for such variables was from 1 to 9 out of 15. The magnitude of this range should act as a precautionary warning about the importance of selecting indicator variables (species) that are truly representative of other ecologically important variables (see also Chapter 3).

Finally, one piece of information that is often missing from studies of local impacts in the marine environment is an assessment of the spatial distribution of the causative agent. This information is potentially important for at least two reasons. First, laboratory assays cannot be used to predict performance in the field without knowledge of the concentration gradient of the causative agent in the environment. Second, the relationship between causative agent and biological performance can be obtained by comparing the spatial distributions of performance and causative agent(s). Obviously, the best method for assessing the spatial distribution of a causative agent is to directly measure it in the vicinity of the impact. However, this is often complicated by uncertainty about the identity of the causative agent, or concern that multiple agents could be producing the effect. For example, a large amount of information (Table 10.1) suggests that the discharge of produced water is toxic to a wide variety of marine organisms, yet there is no consensus as to the identity of the specific toxin (Higashi et al. 1992). Furthermore, even if the causative agent is known, environmental processes can cause it to be extremely variable in its occurrence, making it difficult to detect unless variables that integrate over time are used.

An alternative to measuring the spatial distribution of causative agents in the field is to predict it from performance under known concentrations in the laboratory and at known distances from the effluent source in the field. We did this for the *Carpinteria* study using data on abalone settlement. Abalone settlement was chosen because it was as sensitive (as measured by spatial extent of effect) as any other biological variable examined at *Carpinteria* (Table 10.1) and because it was a variable that could easily be examined in laboratory and field. We estimated the concentration of produced water as a function of distance from the diffusers from the observed settlement of abalone larvae at known concentrations of produced water in the laboratory (Raimondi, *personal observation*) and from data on settlement in the field.

The estimated concentration of produced water near the diffusers was greater than 100% (at the 5 m Site it is >1000%) and gradually declined to ~0.0001% at

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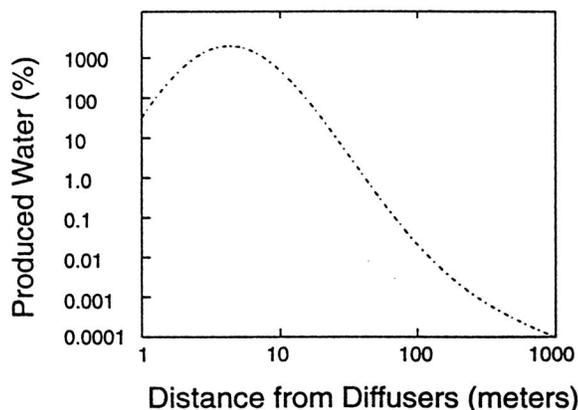
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1000 m from the diffusers (Figure 10.1). Estimates of field concentrations greater than 100% were produced because at distances close to the diffusers performance was much worse than ever found using lab experiments; here field concentrations were extrapolated. These results indicate that the discharge of produced water was much more toxic than could have been predicted from laboratory bioassays.

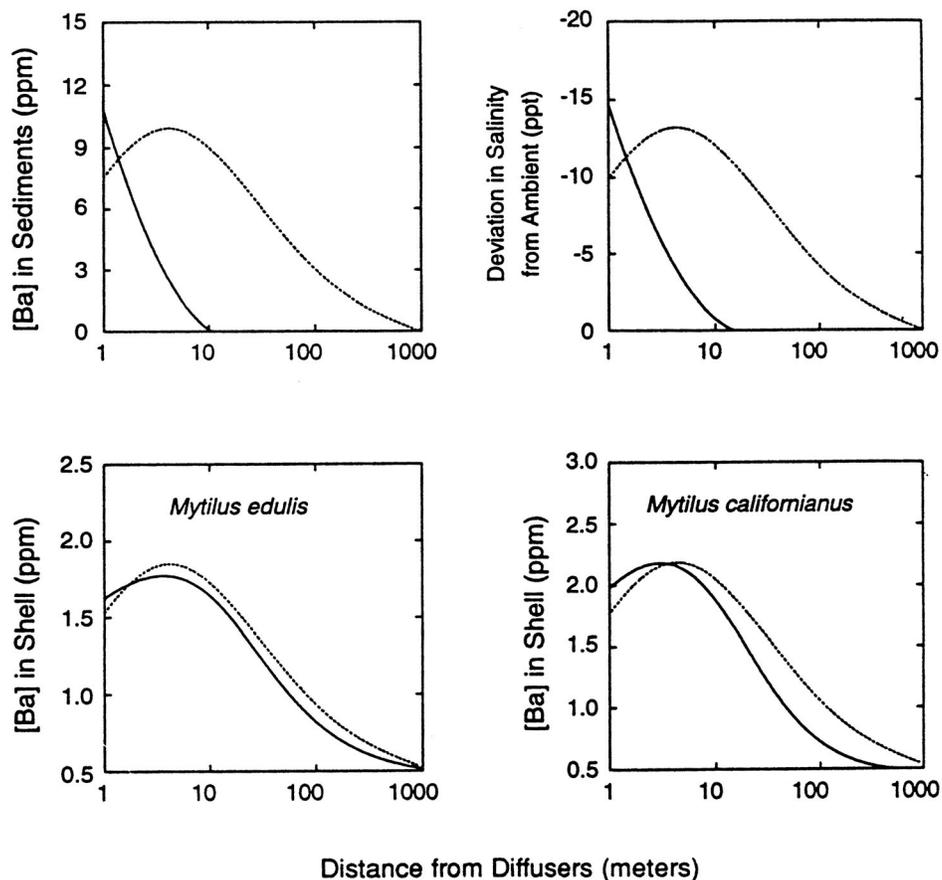
This approach can be used to determine which physical/chemical variables best describe the distribution of the causative agent in the field. In Figure 10.2 we compare the estimated concentration of produced water with the physical/chemical variables that showed a significant correlation with distance from the diffusers (salinity and barium in mussel shells) and with a similar variable that did not correlate with distance (barium in sediments). Only spatial pattern of barium in the shells of the two mussel species was similar to that of produced water estimated from abalone settlement. Interestingly, the spatial pattern of barium in the sediments differed substantially from that of barium in mussel shells. The dissimilarity between the spatial distribution of barium in the sediments and produced water probably reflects the inability of sediments to accumulate toxicants in an open coast environment.

### Life-History Attributes and the Dispersal of Ecological Impacts

Spatial patterns of ecological impacts may be completely uncorrelated with those of physical impacts if individuals have the ability to move from one location to another. Most marine organisms have such mobility at some stage of development. Populations of many pelagic species are sustained by the extensive



**Figure 10.1.** Estimated concentration of produced water as a function of distance from produced water diffusers. Estimate is based upon comparison of laboratory and field studies assessing the settlement of abalone larvae (see text). The curve was generated using a nonlinear curve fitting routine (Systat Inc. 1992).



**Figure 10.2.** Relationships between physical/chemical variables (solid lines) and distance from produced water diffusers. Sources of data are given in Table 10.1. Curves were generated using a nonlinear curve fitting routine (Systat Inc. 1992). For comparison, the curve describing the estimated concentration of produced water (dotted line; see Figure 10.1) is included on each figure.

movement of juveniles and adults that travel in search of food or breeding sites. In contrast, the majority of organisms living in coastal marine habitats have sessile or weakly mobile juveniles and adults. Widespread movement in these species is confined to planktonic propagules, which in many cases disperse considerable distances causing local populations to be strongly reliant on replenishment by propagules produced elsewhere (Scheltema 1971, Keough 1988, Roughgarden et al. 1988). Such movement by individuals is critical in maintaining species distributions and genetic diversity (Scheltema 1971, Slatkin 1987) and can profoundly influence population dynamics and community structure (Connell 1961, Sale 1977, Underwood and Denley 1984, Roughgarden et al. 1985, Raimondi 1990). Thus, localized activities that alter the rates by which individuals (adults or propagules) emigrate from locally disturbed areas or immigrate to more distant nondisturbed sites have the potential to influence population dynamics and community structure over much larger spatial scales

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(e.g., Keough and Black, Chapter 11, Nisbet et al., Chapter 13). Below we discuss the biological attributes of organisms that are most likely to influence rates of movement and how human activities that affect these attributes might cause local ecological impacts to be dispersed to more distant locations.

### Changes in Emigration Rates from Locally Disturbed Sites

Highly mobile species may choose to leave locally disturbed areas, thus causing an increase (usually temporary) in their abundance at some distant location (Hose et al. 1983, Ebeling et al. 1985, Stouder 1987, Breitburg 1992). Most coastal marine species, however, are much less mobile and the extension of local impacts to distant locations occurs via processes that influence propagules, which are the primary source of emigrants. Perhaps the most direct way that localized activities can reduce propagule production at a site is by causing a decrease in adult density (provided that a reduction in the abundance of propagule-producing adults is not fully compensated for by an increase in the fecundity of survivors). Local perturbations that cause a decrease in population size may also indirectly affect rates of propagule production (and hence emigration) by limiting the incidence of syngamy. Species most likely to be affected in this manner include sessile organisms that have internal fertilization (e.g., barnacles that do not self fertilize). Although there can be substantial variation in the length of intromittent organs among individuals, there are presumably limits to the distances separating individuals in which copulation is still possible. Disturbances that increase adult spacing beyond these limits will prevent fertilization from occurring regardless of how many gametes an individual produces. A reduction in population density can also adversely affect fertilization rates in species that broadcast their gametes into the water column. Recent studies indicate that under a wide range of hydrographic conditions, the vast majority of fertilization in broadcast spawners that are sessile or have limited mobility occurs between closely spaced individuals (Pennington 1985, Denny and Shibata 1989, Reed 1990).

Sublethal effects of anthropogenic activities also have the potential to affect emigration rates from locally disturbed sites. Although most marine organisms have some capacity for tolerating increased levels of toxicants, emigration rates can be affected if toxicants alter per capita gamete production and/or viability. Pollutants are known to cause a reduction in both the quality (Hogan and Brauhn 1975, Westernhagen et al. 1981, Fan et al. 1992) and quantity (Linden 1976, Osenberg et al. 1992) of gametes produced by adults living in disturbed areas. Typically, nonpolar organic compounds cause the most harm to gametes because of their tendency to bioaccumulate (i.e., be taken up and concentrated by living systems) in gonads and other body tissues that are high in lipid (highly polar compounds are less likely to enter the body system, and when they do, tend to be quickly excreted: Manahan 1992). Not surprisingly, elevated levels of polar compounds (e.g., chlorinated hydrocarbons) in gonads have been listed as a

cause of serious abnormalities in gametes and embryos of a variety of species. Notable examples that have resulted in reproductive failure include eggshell thinning in birds (Ratcliffe 1967, Anderson et al. 1975) and premature births in marine mammals (De Long et al. 1973, Addison 1989). Some organisms may actually cleanse tissues sequestering toxins by shunting the toxins to the fatty tissues of eggs. Not surprisingly, several studies have found major differences between males and females in the bioaccumulation of environmental toxicants; males continuously accumulate contaminants with age while females periodically reduce their contaminant levels when reproducing (Gaskin 1983, Britt and Howard 1983).

The effects of toxicants on gametes are not necessarily restricted to processes that occur *in vivo*. Numerous studies have shown that spawned gametes may be particularly sensitive to waterborne pollutants (Allen 1971, Kobayashi 1980, Krause et al. 1992). For obvious reasons, fertilization rates of species that broadcast spawn are more likely to be affected by toxicants in the water column than species that transfer gametes via intromittent organs.

Even if the toxicity of released substances is low, the potential for fertilization rates to be significantly reduced still exists. This is because fertilization in virtually all organisms is dependent on chemical recognition (sperm is guided to the egg via a specific pheromone) and changes in water column chemistry that arise from a local impact have the potential to disrupt the chemical communication system between male and female gametes. In many brown algae, for example, a pheromone is produced by the female plant that not only guides the sperm to the egg but also triggers the release of sperm from male plants (Müller et al. 1985). Reed et al. (1994) found that an effluent of 1% produced water hampered the ability of male gametophytes of the giant kelp *Macrocystis pyrifera* to detect female pheromone. This in turn caused a threefold reduction in the quantity of sperm released by these males relative to that of male plants placed in seawater lacking the effluent. Contaminants can also change the sexual behavior of adults. Linden (1976) found that concentrations of crude oil  $\geq 0.002\%$  completely suppressed copulatory behavior in the amphipod *Gammarus oceanicus*. Such chemical disruption that alters sexual behavior of gametes or adults can greatly influence emigration rates in species whose propagules disperse after fertilization.

The number and caliber of gametes that an individual produces are generally highly dependent on its nutritional status. Consequently, local anthropogenic activities can *indirectly* affect propagule emigration rates (via changes in per capita fecundity) if they alter the quality and/or quantity of an organism's food resources. When fecundity is altered via changes in food, the effect and extent of the impact should vary among species with different nutritional requirements. For example, ocean discharges that cause an accumulation of organic matter on the bottom might be expected to positively influence suspension feeders and deposit feeders that are able to use the material for food. Changes in the abundance of these secondary consumers may in turn cause corresponding increases in the abundance and fitness of species at higher trophic levels. In con-

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trast, species that do not rely on heterotrophic nutrition may be negatively influenced by the increased flux of organic matter. Plants use light energy to fix gaseous carbon into organic carbon and increased turbidity resulting from the discharge of organic matter may reduce primary production (both in the water column and on the bottom) by decreasing irradiance. Substantial reductions in plant biomass may in turn negatively influence the fitness of primary consumers that actively graze micro- and macroalgae. Obviously the nature of the impact will determine which species are affected. Discharges of municipal wastes that are rich in nutrients may increase primary production to a level where macrophytes exclude sessile invertebrates (Banner 1974, Gameson 1975, Rastetter and Cooke 1979), whereas the discharge or spillage of petroleum products may favor chemotrophic organisms (Atlas et al. 1978, Cowell et al. 1978, Spies and Davis 1979, Bakke et al. 1982).

Such patterns were observed near a coastal nuclear generating station in San Onofre, California. Widespread increases in the abundance of benthic invertebrates living in or on the sediments were observed near the power plant and were attributed to an increase in organic matter on the bottom that fell out of the discharge plume (Murdoch et al. 1989). Increases related to the discharge generally extended 3–6 times farther for species that fed on and just above the surface of the sediments compared to species that fed in the sediments, presumably because the latter are affected mainly by heavier particles that fall out of the discharge plume earlier, while surface feeders are more influenced by lighter particles that fall out of the plume over a larger distance downcurrent. Similar increases in the abundance of more mobile benthic feeding fish were also observed. In contrast, adult and subadult densities of giant kelp, the lone autotroph examined in this study, were reduced ~60% below the densities expected in the absence of the power plant (Bence et al., Chapter 8). The local reduction in kelp was attributed to an increase in turbidity and a decrease in bottom irradiance caused by the discharge plume, which suppressed the development and survival of microscopic stages of kelp. Significant declines in sea urchins and many species of fish that use the kelp bed for food and shelter were also observed (Ambrose et al., Chapter 18).

### Changes in Immigration Rates to Distant Undisturbed Sites

Local anthropogenic activities that influence mortality of mobile stages during transport or cause sublethal effects that render an individual incapable of settling and/or fully developing upon its arrival to a new site will potentially alter immigration rates at distant locations, thus extending local impacts over much greater spatial scales. Most marine organisms have multiple free-living life-history stages. Perturbations affecting any stage can cause ecological impacts to be extended in space. Because different stages typically use different resources, live in different habitats, are of different size and shape and may even prey on one another, their susceptibility to a given perturbation is likely to vary. There is

considerable evidence that propagules (gametes, spores and larvae) are among the most sensitive life stages to hazardous wastes (see above) and other changing environmental conditions that may arise from anthropogenic activities (Conner 1972, Rosenberg 1972, Wilson 1977, Weis and Weis 1989).

Propagules of many marine organisms undergo several different developmental stages during dispersal (e.g., barnacle larvae typically have six distinct naupliar larval stages that feed in the plankton, followed by a nonfeeding cyprid stage, which ultimately attaches to a hard surface and makes the metamorphic transition to a sessile, feeding adult). Evidence from field experiments indicates that different larval stages of the same species may be affected differently by the same perturbation. Raimondi and Schmitt (1992) found that the produced water outfall off Carpinteria differentially influenced the survivorship of precompetent and competent larvae of the red abalone, *Haliotis rufescens*; survivorship of pre-competent larvae was negatively affected while that of competent larvae was unaffected. Although encountering the discharge plume did not prove fatal to competent larvae while in the water column, it did significantly impair their ability to settle and metamorphose into the sessile adult form relative to larvae that did not encounter the plume. Such effects could potentially influence immigration rates to areas down current outside the zone of physical impact. Metamorphosis in red abalone larvae typically occurs 1 to 3 weeks after competency is achieved and during this period individuals remain as swimming larvae. This is a sufficient amount of time for affected larvae to be transported considerable distances along an open coastline.

Not all anthropogenic causes of propagule mortality necessarily result from the discharge of toxic compounds. The dispersal potential of spores and larvae of many species depends on the availability of exogenous energy sources and there is some evidence that food limitation during the planktonic dispersal phase may influence recruitment success in a wide variety of marine organisms, including fishes (Richards and Lindman 1987), invertebrates (Connell 1961, Olson and Olson 1989, Morse and Morse 1991) and algae (Reed et al. 1990). Localized activities that influence water-column productivity, therefore, may affect the dispersive potential of propagules by adversely affecting their food supply. The effect of a local reduction in propagule food supply on the dynamics and structure of distantly located populations has not been examined. However, such effects are likely related to the ratio of the length of time a propagule spends in the area of reduced food to the total duration of its planktonic feeding phase.

Presently, there are very few types of anthropogenic impacts that cause a detectable reduction in the planktonic food supply over large spatial areas. Thus, unless hydrographic features increase retention time in the impact area (see Keough and Black, Chapter 11), propagules are not likely to be subjected to limited food supplies for lengthy periods of time (indeed, significant ecological impacts would not be expected at distant sites in situations where propagules are retained). It is unlikely that a temporary reduction in propagule food supply will lead to significant ecological changes in distant populations for species with

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propagules that spend relatively long periods feeding in the plankton. For such species, the ecological consequences of a temporary reduction in food may be ameliorated by renewed and lengthy feeding outside the zone of food reduction. Moreover, a lengthy planktonic phase greatly reduces the probability that propagules from a local source will disperse to and settle in the same area, thereby decreasing the odds they would contribute significantly to any one population outside the zone of food reduction. This may not be the case for species with propagules that feed for shorter periods in the plankton. For such species, there is less chance that a temporary reduction in food will be compensated for and a greater probability that propagules of similar origin will disperse to and settle in the same area. Moreover, the odds that a greater proportion of propagules will be adversely affected by a temporary reduction in food increases as the ratio of the duration of the food limitation to the duration of planktonic feeding period increases. For these reasons, it may be easier to detect ecological changes in distant (i.e., outside the zone of physical impact) populations that arise from a local reduction in propagule food supply for species that have propagules with relatively short feeding durations.

Sublethal effects of local activities may affect the behavior of mobile stages, and thereby influence their ability to disperse and settle at distant locations. Stimulation of a neuro- or chemoreceptor initiates most behaviors (Pawlik 1992) and local perturbations may interfere with both the receptor (direct effect) and the stimulus or cue (indirect effect). Of the two, direct effects have been investigated the most thoroughly in the marine environment. There have been a number of studies that have demonstrated detrimental effects of various anthropogenic activities on mating and other behaviors of vertebrates (Anderson 1978, Garshelias and Garshelias 1984, Sokolov et al. 1990). Much less is known concerning how such activities affect invertebrate behavior. Whether or not the interference of behavior at one location causes effects to be manifested at more distant locations probably depends in part on whether exposure to a perturbation permanently alters behavior or simply changes it during the period of exposure. If the behavior is permanently altered there may be effects resulting from exposure at other times or places where "normal" manifestation should have occurred. If the behavior is altered only during exposure then the potential for local impacts to be dispersed is less likely, unless the affected behavior decreases the probability that the individual will leave the locally affected area.

There is some evidence from the field that local activities can influence larval behavior in ways that could alter immigration rates to physically undisturbed sites. Raimondi and Schmitt (1992) found that some behaviors of the larvae of red abalone were permanently altered (e.g., settlement behavior) and others temporarily so (e.g., swimming behavior) following exposure to a produced water-discharge plume; larvae observed outside the plume showed no such changes. Both alterations in behavior could cause ecological effects at distant locations. In the case of settlement behavior, large numbers of individuals that were temporarily exposed to produced water were unable to settle, even when they

were outside the physically affected area. Such behavior could lead to reduced settlement and subsequent adult numbers in locations down current of the plume. The change observed in swimming behavior was a complete suppression in swimming by individuals during exposure to the discharge. Although this effect was sometimes reversible (i.e., ~50% of affected larvae resumed swimming after being placed in nonplume water), it too could cause an ecological impact at distant locations if a loss of swimming ability reduces the odds that an individual will leave the area affected by the discharge. Such may be the case for species like abalone that have negatively buoyant larvae. These larvae quickly sink to the bottom once they stop swimming, thus greatly reducing the chance of their being transported substantial distances by prevailing currents.

Any changes in population dynamics and community structure that arise from reductions in rates of propagule immigration are more likely to be detected in systems that are limited by the recruitment of new individuals rather than by interactions among established residents. The likelihood that a population will be limited by recruitment rates may vary with particular life-history characteristics. For example, because clonal organisms can also reproduce asexually, the dynamics of their populations might be expected to be much less dependent on rates of propagule immigration than those of asexual organisms (i.e., species that can only reproduce sexually). Consequently, local impacts that reduce rates of propagule immigration to more distant sites may have a greater effect on populations of asexual species than clonal species. Similar arguments can be made concerning ecological dominance. The percent of the substrate that a species occupies should be more tightly linked to rates of propagule immigration in asexual species than clonal species simply because of the latter's potential for unlimited clonal growth.

## Conclusions

A goal in the field of impact assessment is to be able to predict general ecological impacts from investigation of a few variables (see also Jones and Kaly, Chapter 3). This would result in a tremendous savings in the cost and effort required to accurately assess impacts resulting from anthropogenic disturbances. Our analysis of results from studies of a local impact at Carpinteria, California indicates that it may be difficult to attain this goal. The effectiveness of using biological variables to predict general ecological impacts is likely to be limited by differences in responses among species, ontogenetic stages and life-history attributes. Some physical/chemical variables may be well correlated with the maximum extent of ecological impacts, such as we found for barium in mussel shells. However, unless it can be determined *a priori* that a particular physical/chemical variable correlates well with biological variables, its usefulness for predicting the extent of ecological impacts is limited.

Detecting the extent to which ecological impacts are dispersed to locations

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removed from the area of physical impact is perhaps the most difficult challenge facing scientists responsible for ecological impact assessment in coastal marine habitats. Tracking individuals (especially small propagules) in a habitat as vast and complex as the nearshore marine environment can be extremely difficult. Assessing the contribution of a particular population affected by an impact to the ecology of populations and communities at distant sites is typically confounded by complex circulation patterns and other numerous, but unaffected, source populations.

Due to the difficulty of detection there is little empirical evidence to date that implicates local impacts as the causative agent producing significant ecological change at distant locations. Examples in which local impacts have been charged with producing larger-scale effects are restricted primarily to modeling studies, which are forced to make inherent assumptions that are difficult to validate (e.g., the assumption that compensatory mechanisms are not important in the planktonic propagule stage: Nisbet et al., Chapter 13). However, there should be little disagreement that local impacts have the potential to significantly alter the composition and dynamics of nearshore marine communities over larger spatial scales.

Detecting effects dispersed in complex habitats such as the coastal marine environment is critically dependent on the ability to make accurate predictions. Detection, hopefully, will become easier if there is a clear sense of what characteristics of species contribute to ecological impacts being dispersed over larger spatial scales. In this chapter, we present some ideas as to which attributes of species are the most likely to lead to broad-scale population changes as a result of a localized disturbance. Many of the ideas we discuss still need to be rigorously tested. Even if verified, however, knowledge of which species characteristics lead to dispersed effects will not, by itself, allow dispersed impacts to be detected in most situations. Equally important is knowing *where* impacts are likely to be dispersed to. Details of oceanographic transport and larval behavior under different transport regimes are critical to the accurate prediction of where dispersed impacts will most likely occur (Keough and Black, Chapter 11). Complementary information on “sources and sinks” (sensu Pulliam 1988) of population replenishment will aid in predicting where and how much change can be expected from a given local impact. Such information may require the use of modern techniques in molecular biology and in the analyses of stable isotopes to develop genetic and environmental markers for distinguishing source and sink populations. Moreover, population effects may not be limited to numerical changes; changes in source populations can alter the genetic makeup of sink populations. Such genetic alterations can result in delayed effects by reducing the stability and resiliency of a population during times of environmental change (Soulé and Simberloff 1986, Gilpin 1991). All such information that serves to improve the accuracy of predicting the scale over which localized effects are dispersed will undoubtedly aid in the detection of such effects.

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### 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.