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

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INTRODUCTION

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

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

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

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

METHODS

Study Site

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

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

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

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

Infaunal Depth Distribution

In a preliminary study, the depth distribution of infauna was examined to determine the appropriate depth for our infaunal sampling survey. On 31 October 1989, divers sampled infauna at four sites that were within 1 km of the Carpinteria outfall. At each site, 10 cores (20.3 cm²/core) were taken to a depth of 25 cm. Each core was sectioned into 5 cm depth intervals, and depth fractions from five cores (all from the same site) were pooled into a single sample, yielding two samples/site, each with five depth fractions. Buffered formalin was added to each sample to bring the total solution to approximately 10% formalin, preserved samples then were washed through a series of 2 mm, 1 mm and 0.5 mm sieves, and each fraction subsequently was stored in alcohol. Organisms were picked from the sediments in each fraction, counted and identified to broad taxonomic categories.

Benthic Survey

An intensive spatial survey of infauna was conducted along the 11 m isobath at the Carpinteria study site. A total of 20 sites was sampled along a gradient upcoast (west) and downcoast (east) of the diffusers. Scuba divers collected samples on 10 February 1991 from 2, 3, 5, 10, 25, 50, 100, 250, 500, and 1000 m west of the diffusers and on 11 February 1991 from 5, 7, 10, 15, 30, 55, 100, 250, 500, and 1000 m east of the diffusers. Infaunal densities were sampled by taking 8 coffee can cores (78 cm²/core to a depth of 10 cm) at each site, and pooling four cores into a single sample. As before, buffered formalin was added to the samples to bring the total solution to approximately 10% formalin, washed through 2 mm, 1 mm and 0.5 mm sieves and stored in alcohol. Organisms were picked from the sediments, counted and identified to broad taxonomic categories. For each site and each taxonomic group a mean based on the two replicates was calculated, and this mean was used in all subsequent analyses.

At the same time that the biological cores were collected, 3 sediment cores also were collected at each site (20.3 cm²/core, 5 cm deep) and frozen. One core was used to determine percent organic matter in the sediments, one core was used for grain size analysis, and the remaining core was archived. Percent organic matter (POM) was determined by thawing sediments, aspirating the overlying water, and drying the sediments for 48 hours at 45° C. Three subsamples (ca. 5 - 6 g) of sediments from each sample were then dried for 3 hours at 60° C, weighed, combusted for 4 hours at 450° C, and reweighed. POM was calculated as 100% X (ash free dry mass / total mass of subsample), and the mean of the three subsamples was determined for each site.

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

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

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

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

Outplanted Mussels

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

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

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

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

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

RESULTS

Infaunal Depth Distribution

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

Benthic Survey

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

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

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

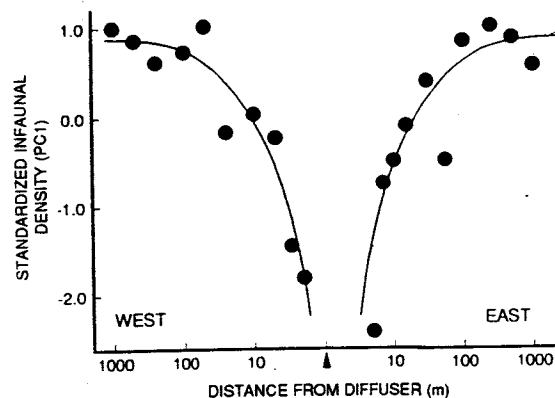


Figure 1. Standardized infaunal density as a function of distance from the produced water outfall. Standardized infaunal density (defined as PC1: Table 1) is an aggregate measure based on the densities of all infauna and is standardized to a mean of 0. The arrow indicates the location of the diffuser.

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

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

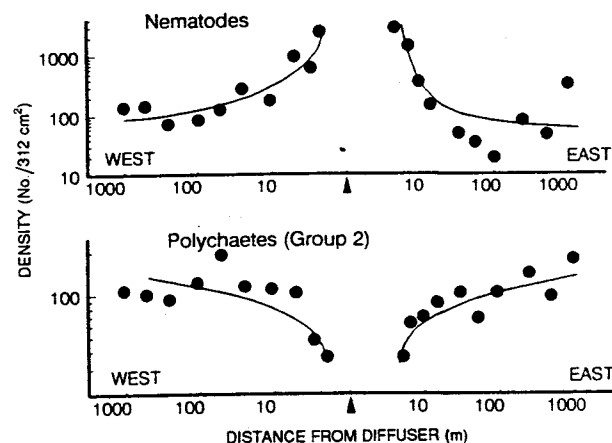


Figure 2. Densities of nematodes and Group 2 polychaetes (see Table 1), as a function of distance from the produced water outfall. Each data point gives the mean of two samples collected at each location. The arrow indicates the location of the diffuser.

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

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

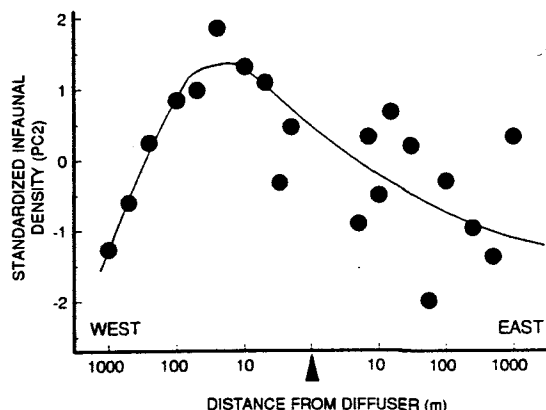


Figure 3. Standardized infaunal density, defined by PC2 (Table 1), as a function of distance from the produced water outfall. The arrow indicates the location of the diffuser. Compare with Figure 1.

Outplanted Mussels

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

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

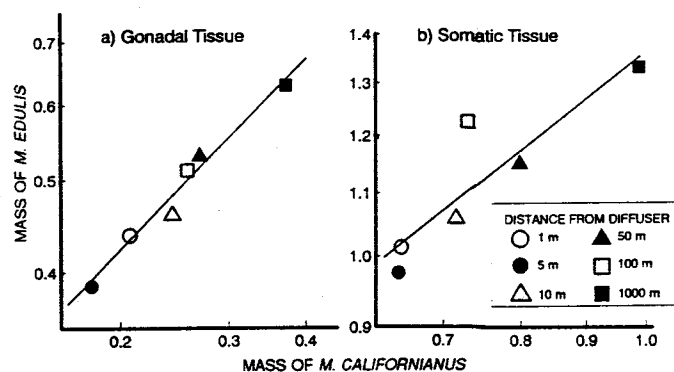


Figure 4. Pattern of covariation in condition (size-specific gonadal mass and somatic mass) of two mussel species (*M. californianus* and *M. edulis*). Each datum gives the response of the two species outplanted to the same site. In general sites close to the outfall yielded low values for each species,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

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

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

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

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

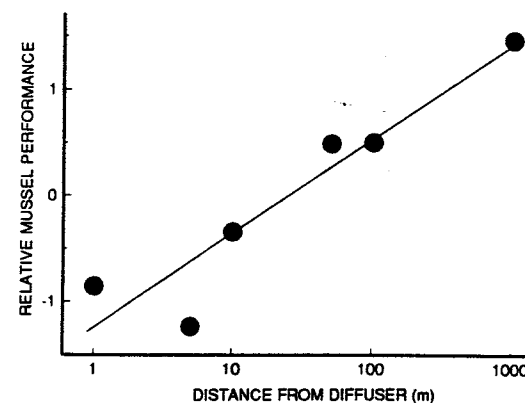


Figure 5. Relative mussel performance as a function of distance from the produced water outfall. Relative mussel performance is defined by the PC1 from analysis of mussel growth and condition (Table 2). Performance and log (distance) were highly correlated ($r = 0.94$, $P = 0.005$).

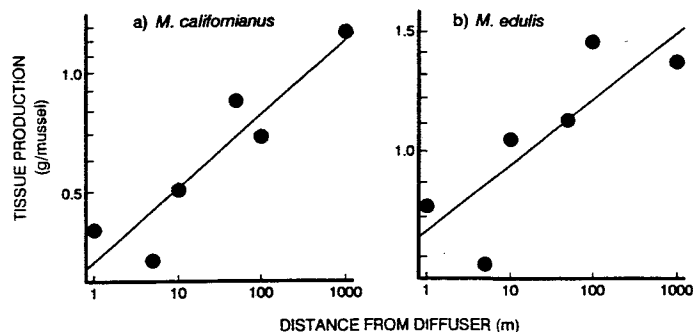


Figure 6. Tissue production for two species of mussel (*M. californianus* and *M. edulis*) as a function of distance from the diffuser. Tissue production and distance were positively correlated for each species ($r = 0.92$, $P = 0.009$ for *M. californianus*; $r = 0.82$, $P = 0.044$ for *M. edulis*).

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

DISCUSSION

Interpretation of Spatial Patterns

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Spatial Extent of Effects

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

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

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

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

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

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

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

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

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