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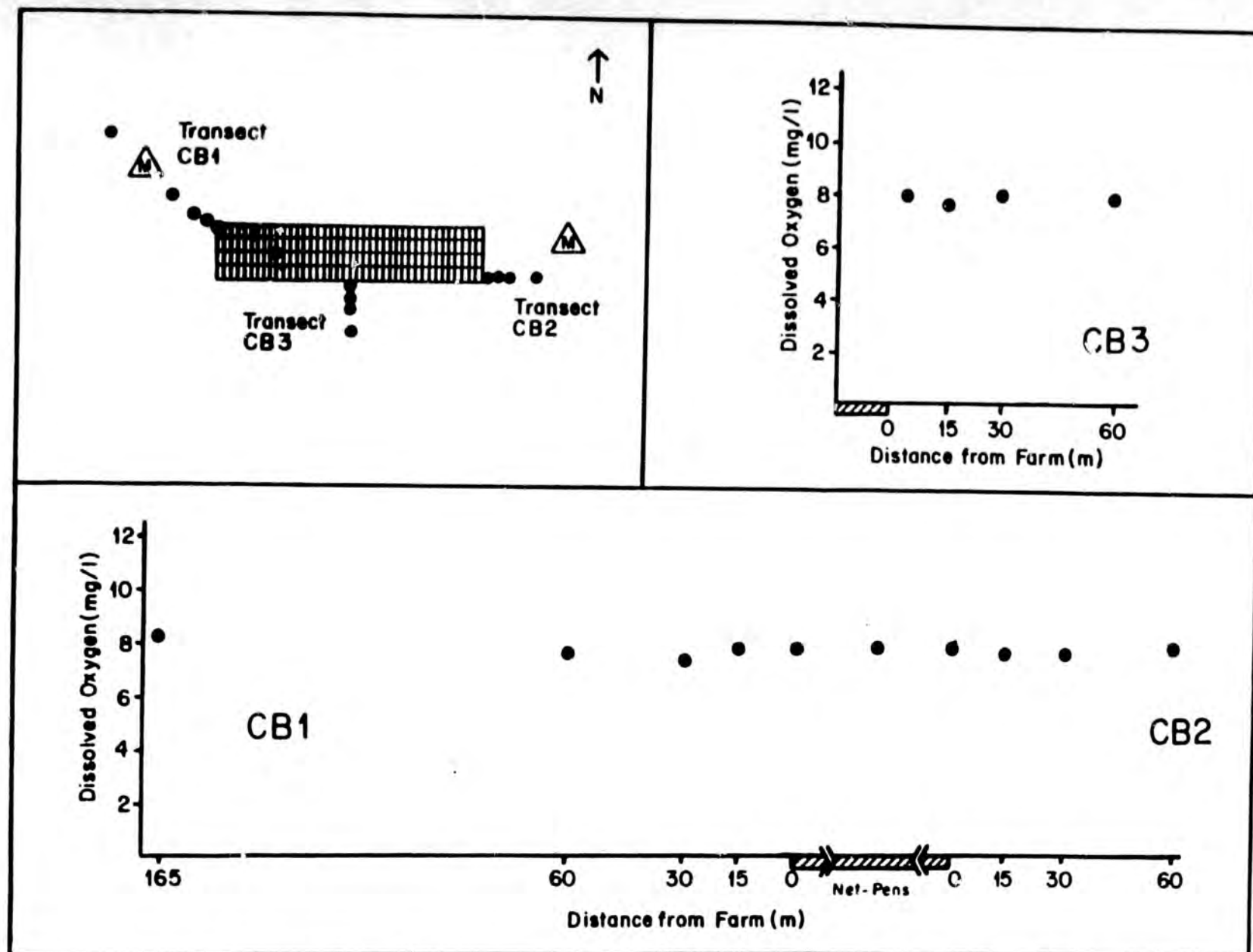


Figure 6. Dissolved oxygen concentrations in near-bottom water surrounding the Clam Bay farm site.

estimated flux of organic carbon to the seafloor was 13.3 kg C·m⁻²·yr⁻¹ under the net-pens and 3.6 kg C·m⁻²·yr⁻¹ at the perimeter.

Macrofauna

Macrofauna were collected one year earlier at four stations along transect CB1 at points close to but not identical to those sampled during the present study (i.e., distances from the farm perimeter of 0, 45, 90, 150 and 450 m vs. 0, 15, 30, 60 and 165 m in the present study). Extensive data analysis is still in progress, so preliminary conclusions are limited to assessment of abundance, biomass, species richness and the density of indicator species.

Figure 7a illustrates typical qualitative changes in species number, biomass and species abundance along a gradient of organic enrichment (Pearson and Rosenberg, 1978). At low levels of organic input, a transition zone develops in which abundance, biomass and species richness gradually decrease from levels typical of the unpolluted environment. In this transition zone there may be a slight species richness and biomass peak attributable to a phenomenon known as "biostimulation". In this area the organic input provides a rich food source, yet the rate of input is not so great that it interferes with the mechanics of suspension feeding nor causes serious oxygen depletion. At a somewhat higher rate of input, total macrofaunal abundance attains a maximum value. Biomass may also be slightly elevated, but the number of species is very low. The increased abundance and biomass results from the proliferation of a few opportunistic species. With still higher rates of organic input there is a complete absence of benthic macrofauna. The rate of organic input is so great that oxygen levels in bottom waters and sediments decrease (or sulfide levels increase) to such an extent that aerobic organisms can not survive.

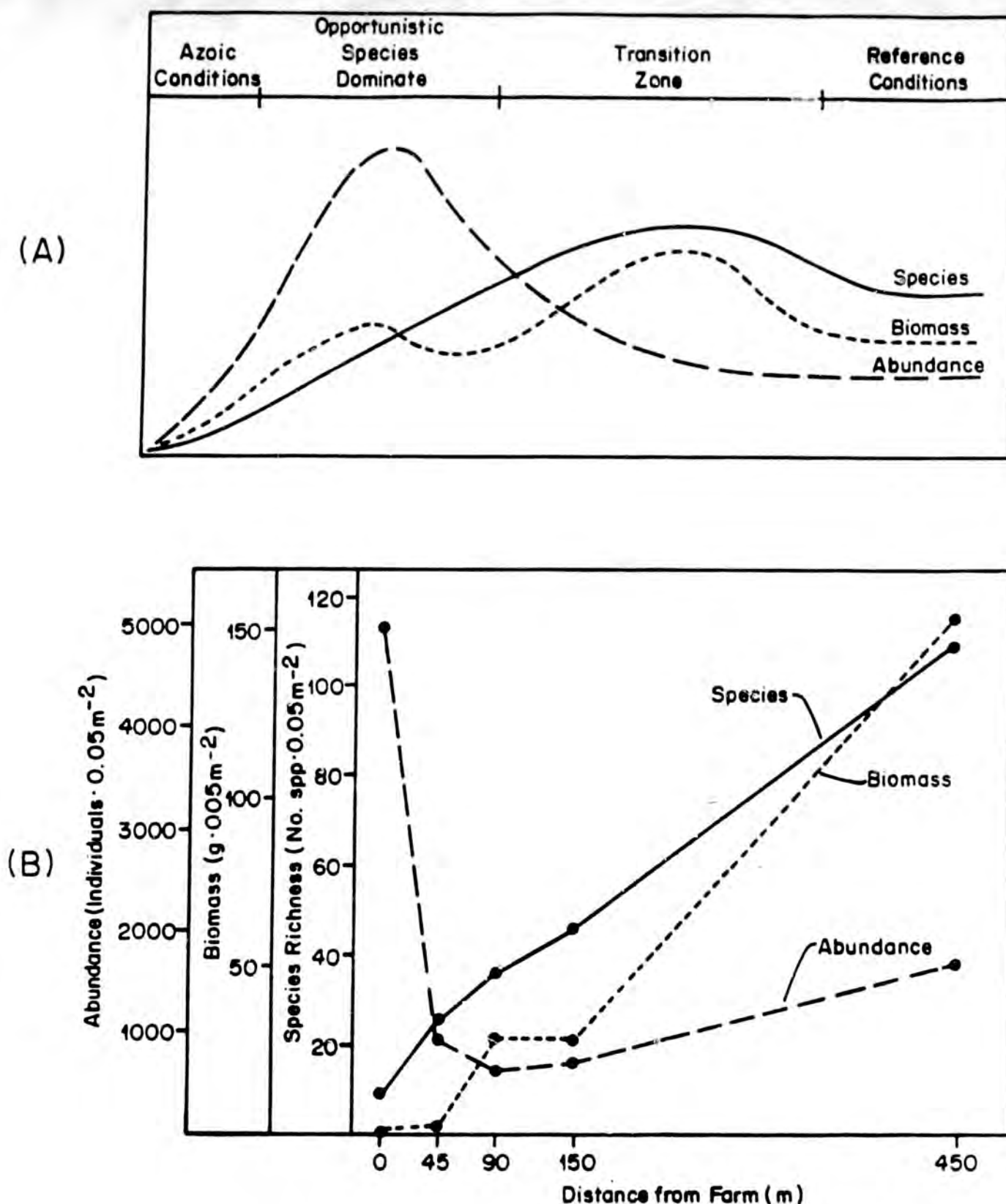


Figure 7. Trends in species richness, biomass and macrofaunal abundance along gradients of organic enrichment. (A) Generalized trends based on Pearson and Rosenberg (1978); (B) Data collected along transect CB1 at the Clam Bay farm site. Inter-replicate variability is not shown in order to simplify the graphical presentation.

Trends in species number, abundance and biomass are shown along transect CB1 for comparison with the ideal model (Figure 7b). Total macrofaunal abundance was elevated 4-fold at the perimeter of the net-pen complex, and decreased to near background levels within 45 m. This peak in abundance was due almost entirely to the contributions of nematodes and the polychaete Capitella cf. capitata. Areal species richness increased consistently along the length of the transect. Biomass was much reduced to a distance of at least 45 m from the pens, for despite the high density of individuals, the organisms were relatively small. Moderate biomass levels were found between 90 and 150 m. The highest biomass was observed at 450 m from the pen site due to the appearance of several large deep-burrowing organisms including bivalves, sipunculans and echiurans.

Capitella cf. capitata is widely recognized as an indicator of organic enrichment and has been found in the vicinity of net-pens throughout the world (Kitamori, 1977; Pease, 1977; Ervik et al., 1985; Brown, 1987). The species was present in densities of over 12,000 indiv. $\cdot$ m⁻² adjacent to the net-pens, and remained in high densities up to 150 m or more from the farm site (Figure 8).

The macrofaunal data from Clam Bay are generally consistent with the ideal model of changes along an enrichment gradient. No azoic conditions were observed, although no samples were collected directly under the pen complex. The data indicate dramatic community alterations beneath the facility perimeter, including the disappearance of most species characteristic of undisturbed Clam Bay habitats. Moderate disturbance with gradually improving conditions was evident between 45 and 150 m from the farm. Normal conditions were reached at some point between 150 and 450 m from the farm.

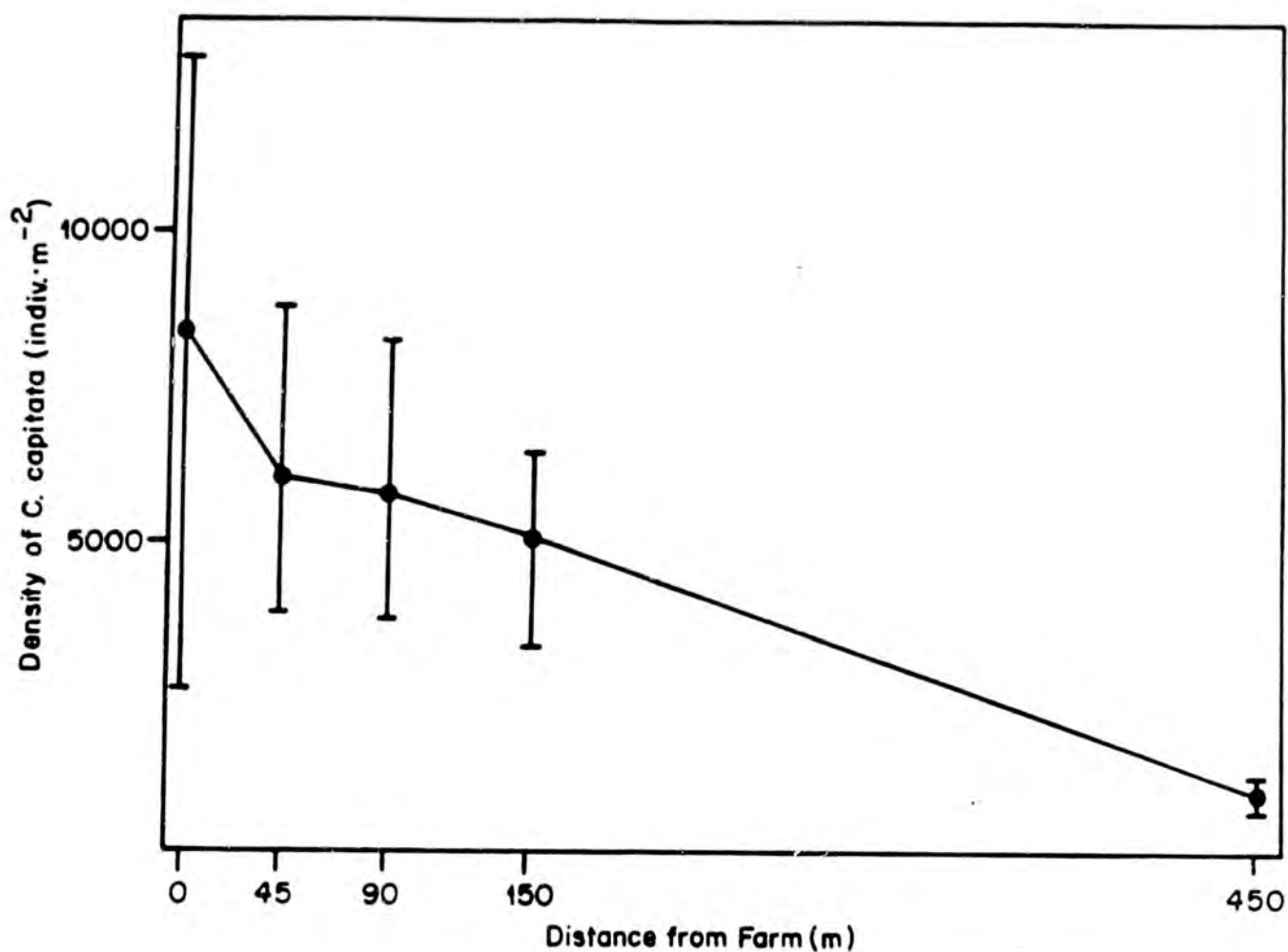


Figure 8. Density of the opportunistic polychaete *Capitella* cf. *capitata* along transect CB1 at the Clam Bay farm site. Vertical bars represent range of the three samples at each station.

Dispersion model

The sedimentation model was run based on the data of Table 3. Standing stock and feeding rate were provided by the farm operator, and based on monthly mean values of these parameters over the previous twelve months. The organic carbon content of the feed was measured directly as was the settling velocity of the feed pellets. Settling velocity of feces was taken from the literature (Warrer-Hanson, 1982). Literature values of feed wastage range from 1 to 30% (VKI, 1976; Penczak, *et al.*, 1982; Braaten, *et al.*, 1983; Gowen, *et al.*, 1985), and lacking a measurement specific to the Clam Bay facility, a wastage of 15% was assumed arbitrarily.

The dispersion model predicted that the area directly under the net-pens would be subject to the greatest rate of solid waste deposition (Figure 9). Areas to the north and west of the farm should receive very little feed and fecal matter, with the vast majority of the material moving towards the south and east. The area delimited by the $1 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ isopleth extends 70 m from the farm at its most distant point.

There is extraordinary agreement between the rate of organic carbon flux predicted by the model and that measured in the sediment traps. Directly under the net-pens at the trap location the predicted rate is $11.1 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$, in comparison to the measured rate of $13.3 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$. At the northwest corner of the farm site the predicted and measured rates were 2.5 and $3.6 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$, respectively.

The dispersion model predicts a deposition rate, and thus a rigorous test of the model would also require rate measurements, such as those obtained from the sediment traps. In the strict sense, a static measurements such as organic carbon concentration or redox potential can not be used to test the model since no information is available on *in situ* post-depositional processes. Nevertheless, since sediment trap data were so limited, the assumption was made that post-

Table 3
Data used in dispersion model at the Clam Bay farm site

Farm size:	280 m by 52 m with long axis oriented east-west
Depth of pens:	4 m
Standing stock:	352 metric tons
Feeding rate:	4409 kg·day ⁻¹
Organic carbon content of feed:	48%
Feed wastage:	15%
Settling velocity of feed:	10 cm·sec ⁻¹
Settling velocity of feces:	4 cm·sec ⁻¹
Water depth (approx. average of MLLW and MHHW along sampling transects):	18 m
Current data:	July 6 - August 4, 1988; meter located 100 m east of farm site.

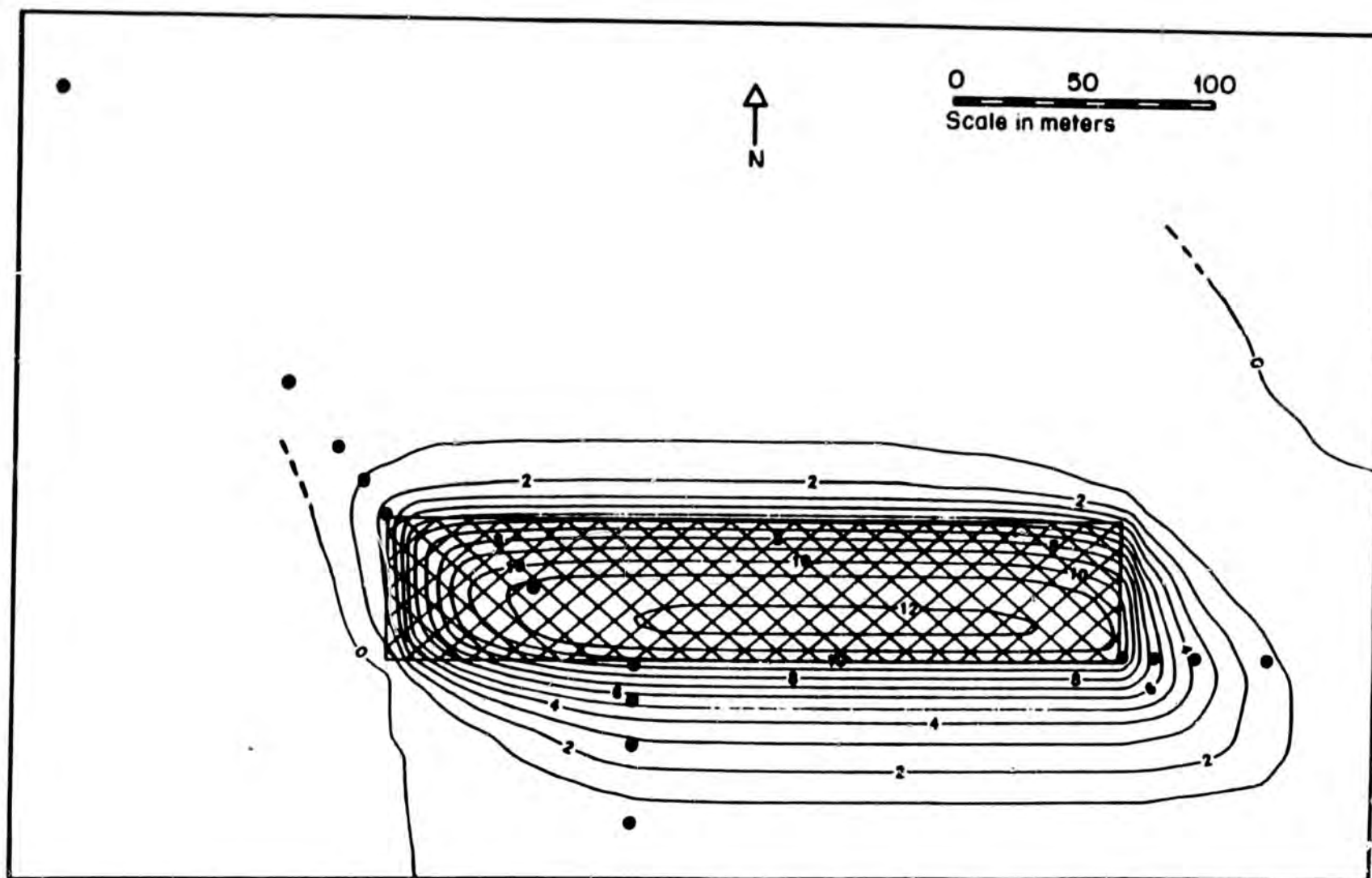


Figure 9. Model predictions of organic carbon loading to the seafloor at the Clam Bay farm site. Contour units are $\text{kg C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$. Dots indicate locations of sampling stations used for model verification.

depositional processes (e.g., mineralization, resuspension) were uniform throughout the study area, and a test was made of the model's ability to identify areas of greatest organic enrichment (Figure 10). There was a significant correlation between predicted deposition rates and measurements of both sediment organic carbon content and redox potential (Spearman rank correlation, $\alpha \gg 0.01$). The model, therefore, performed well in identifying those areas surrounding the Clam Bay farm which experienced the greatest degree of organic enrichment.

SQUAXIN ISLAND SITE

Sixteen stations were sampled in the vicinity of the Squaxin Island farm site (Table 4). Water depths in the area were uniformly 5 m at mean lower low water. The bottom of the net-pens were 2 m above the seafloor at this tidal stage and approximately 6.5 m above the seafloor at mean higher high water. Seawater temperatures were approximately 15°C; salinity was 30.5 ppt.

The substrate was principally silt with varying amounts of sand (14 - 30%) and shell fragments (1 - 40%). Shell debris was so dense at some sites that the collection of undisturbed sediment cores was difficult or impossible. There was no visible evidence of culture-related disturbance on the sediment surface (e.g., Beggiatoa mats, feed or fecal material).

Water Currents

Current meters were moored 60 m to the south and to the north of the eastern end of the net-pen complex. Both were positioned 2.5 m above the seafloor and 2.5 to 7 m below the surface (depending on tidal stage), and left in place from June 6 to August 5 1988.

Both meters showed strong north-south tidal oscillations,

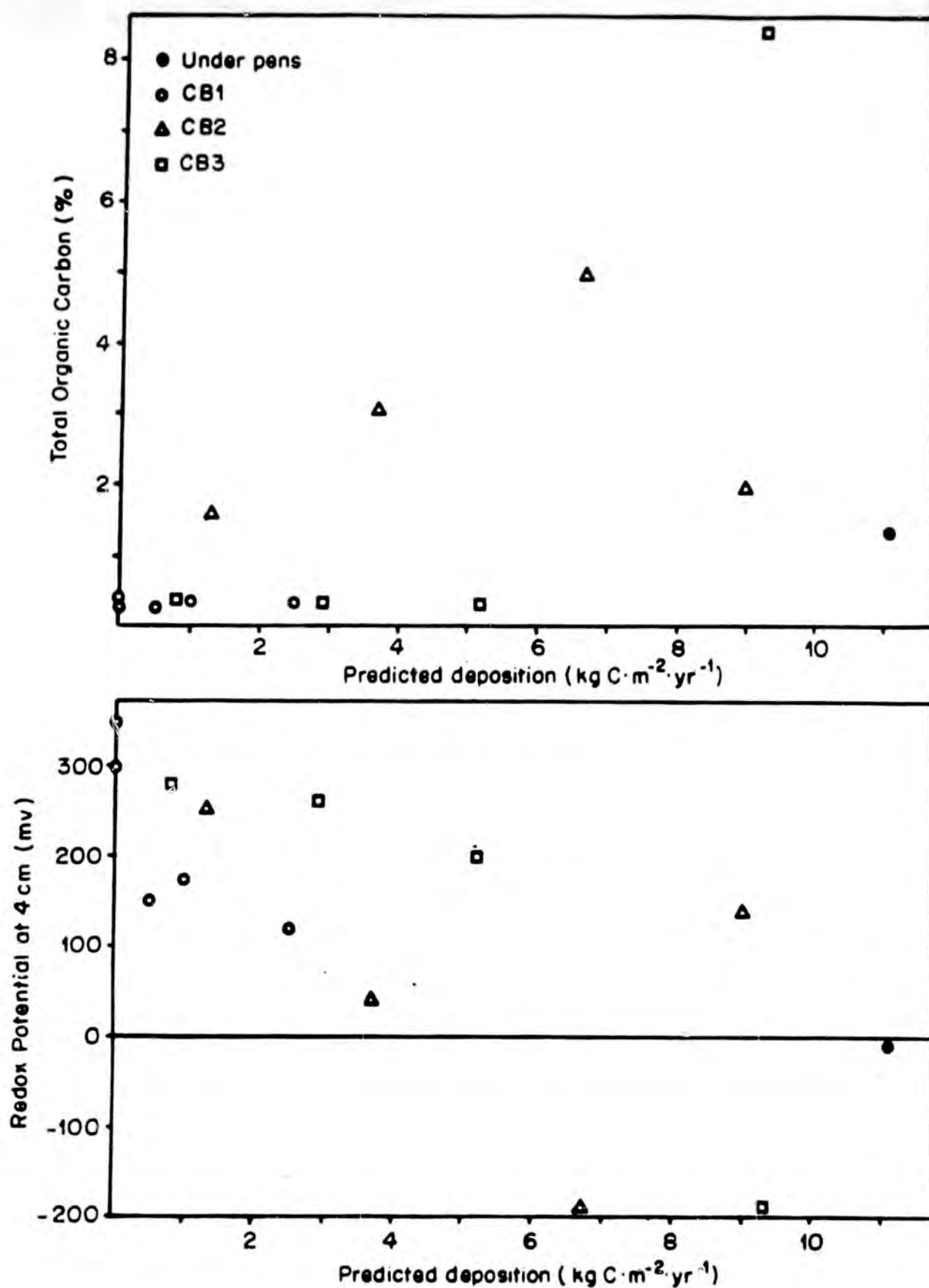


Figure 10. Comparisons of predicted carbon deposition based on the sedimentation model with field measurements of total organic carbon and redox potentials at the Clam Bay farm site.

Table 4
Squaxin Island station summary

<u>Station or Transect</u>	<u>Distance from net-pens (m)</u>	<u>Water depth (m at MLLW)</u>	<u>Substrate</u>
A	0	5	
Transect SQ1	0	5	1.1% shell/gravel 14.3% sand 84.6% silt and clay
	6	5	11.2% shell/gravel 13.6% sand 75.2% silt and clay
	15	5	3.1% shell/gravel 16.9% sand 80.0% silt and clay
	30	5	2.8% shell/gravel 18.0% sand 79.2% silt and clay
	60	5	46.4% shell/gravel 32.2% sand 21.4% silt and clay
	100	5	
Transect SQ2	0	5	
	6	5	
	15	5	
	30	4	41.2% shell/gravel 29.8% sand 29.0% silt and clay
Transect SQ3	0	5	
	6	5	
	15	5	
	30	5	
	60	5	0.4% shell/gravel 17.3% sand 82.3% silt and clay

with the southerly flow predominating (Figure 11). A significant westerly component was evident only in the meter located south of the farm site. The constancy factors were 90.9 and 83.3% for the northern and southern locations, respectively. Current velocities at the northern site averaged $6 \text{ cm} \cdot \text{sec}^{-1}$ over the 60 day deployment with a maximum recorded velocity of $31 \text{ cm} \cdot \text{sec}^{-1}$. Current velocities of 15 to $18 \text{ cm} \cdot \text{sec}^{-1}$ were generally observed at least twice daily. Currents at the southern meter location were slightly slower, averaging $7 \text{ cm} \cdot \text{sec}^{-1}$ with a maximum recorded velocity of $23 \text{ cm} \cdot \text{sec}^{-1}$.

Sediment chemistry

Sediments at the Squaxin Island farm were much finer-grained than at the Clam Bay facility, and thus had considerably greater levels of organic carbon and total nitrogen. Sediment carbon concentrations were typically 2 to 3% at the Squaxin site in comparison to 0.3% in undisturbed areas of Clam Bay. The Squaxin site also differed from Clam Bay in that there were no gradients in sediment carbon or nitrogen concentration that could be attributed to farm activities (Figures 12 and 13). The degree of sediment enrichment was more or less uniform throughout the study area, and the slight variations were unrelated to farm proximity. There was no evidence of sediment enrichment above background levels even directly under the net-cages. One sample collected at the southern pen perimeter showed an organic carbon concentration approximately triple typical values, but this high concentration was not reflected in the two other samples at the same station.

Sediments at a depth of 4 cm (and probably much shallower) were highly reducing due to the fine grain size and reduced porosity of the Squaxin Island sediments (Figure 14). Reduction-oxidation potentials at the sediment-water interface ranged from 50 to 425 mv. This extreme variability is probably a consequence in the sharp gradient of redox potentials

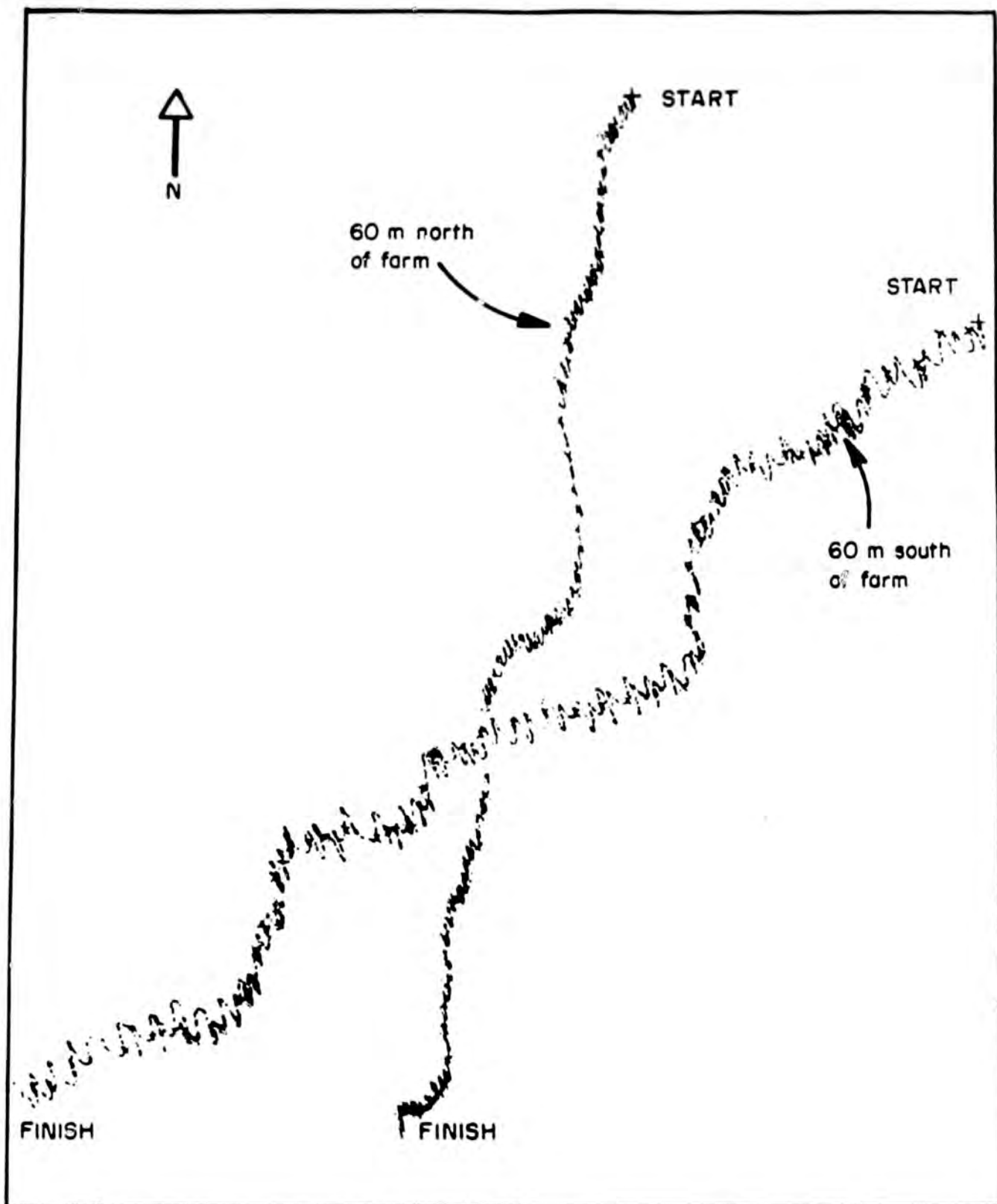


Figure 11. Progressive vector diagrams based on the two current meter records from the Squaxin Island site. X symbols indicate 24-hour intervals.

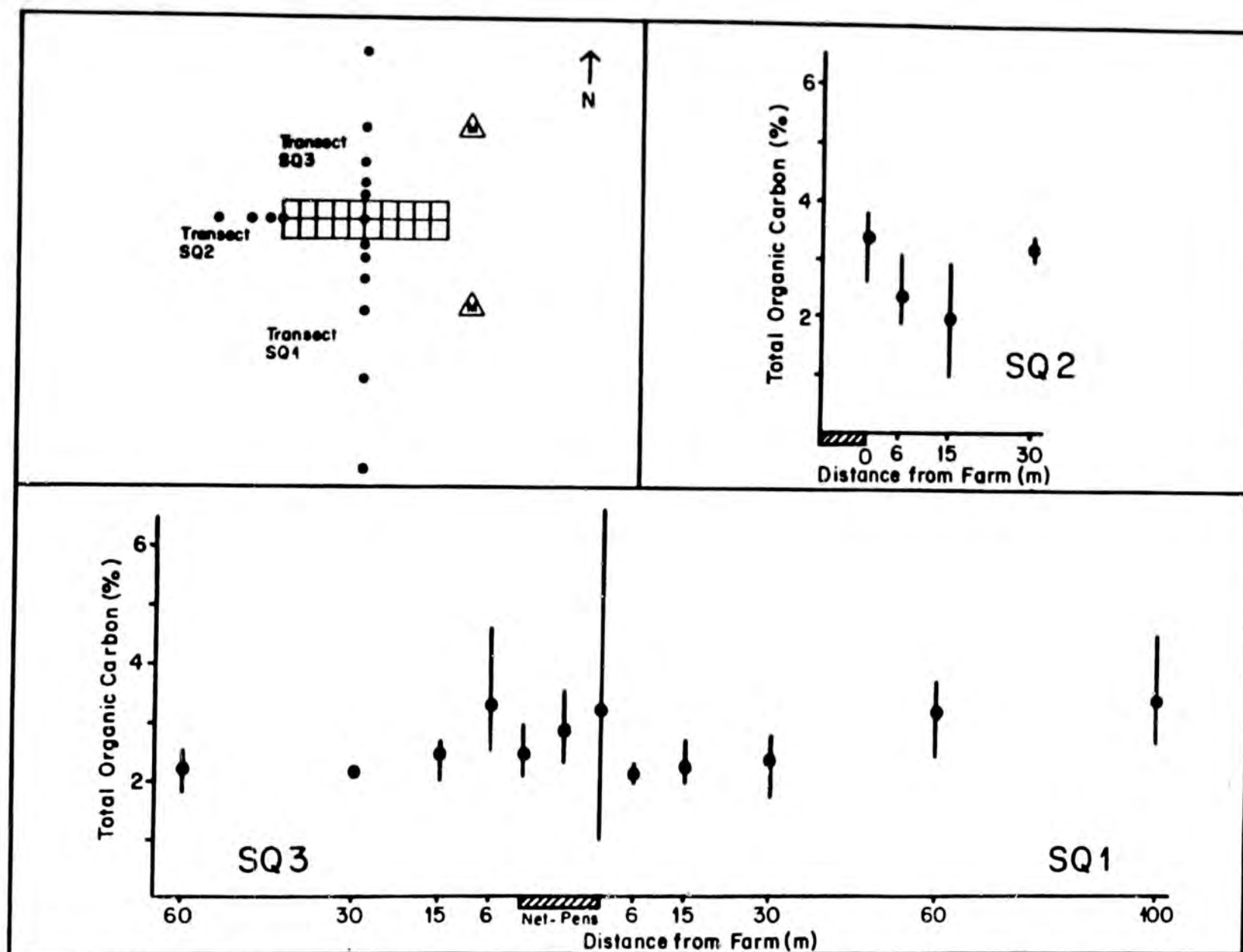


Figure 12. Total organic carbon concentrations in sediments surrounding the Squaxin Island farm. Range bars not shown if the range is less than the size of the mean symbol.

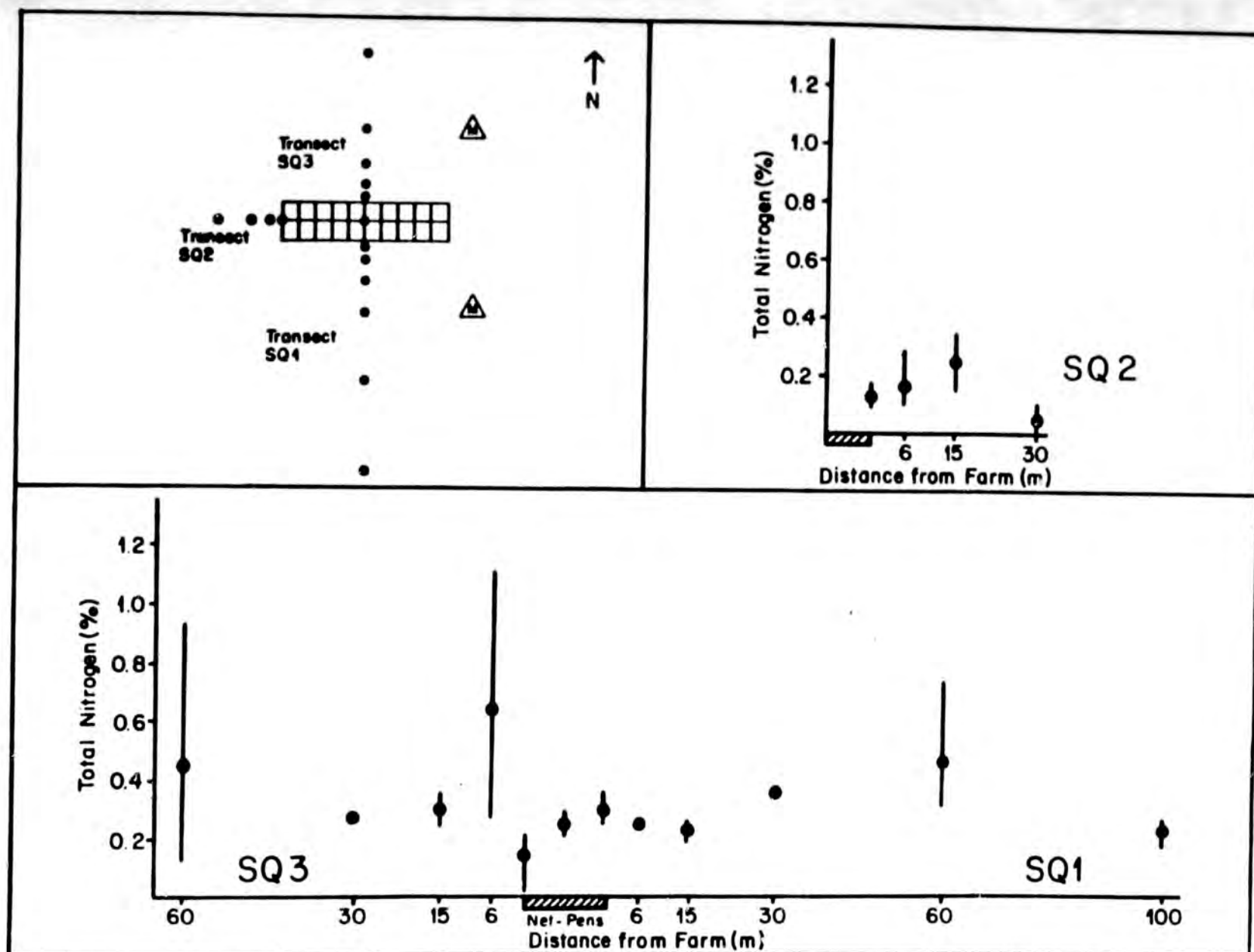


Figure 13. Total nitrogen concentrations in sediments surrounding the Squaxin Island farm. Range bars not shown if the range is less than the size of the mean symbol.

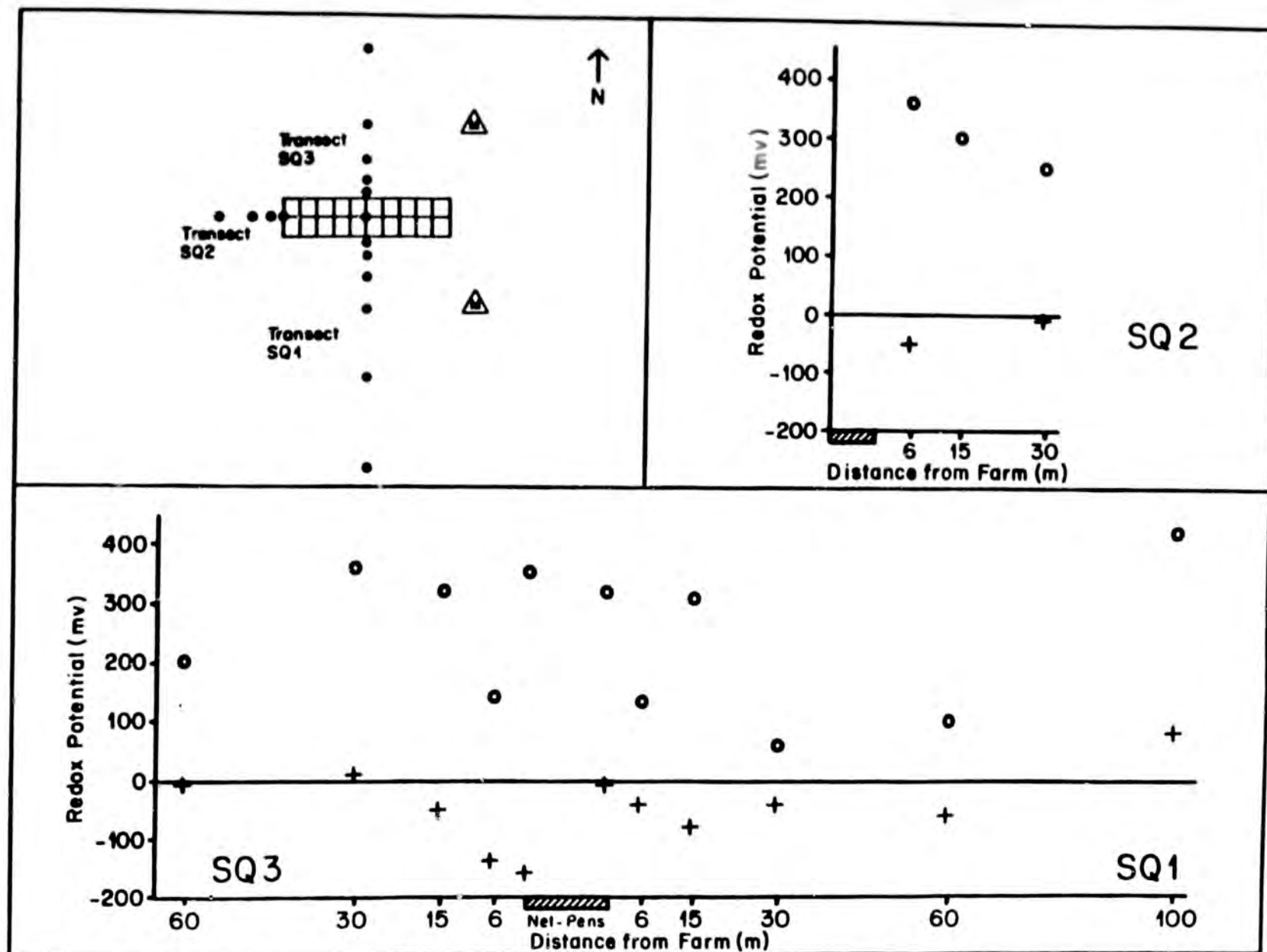


Figure 14. Reduction-oxidation potentials in sediments surrounding the Squaxin Island farm. Open circles represent potentials at the sediment-water interface; cross symbols represent potentials at a depth of 4 cm in the sediment.

in the upper few millimeters of the sediment column, and thus the measured potential can vary widely depending upon whether the probe is held a millimeter or two above or below the ill-defined "interface". Variability of the redox potential at a depth of 4 cm is probably due to the difficulty of collecting an undisturbed core with the high concentration of shell fragments, compounded by the difficulty of inserting the probe into the core without encountering an obstruction and further disturbing the core.

Redox potentials showed no pattern among the sampling stations that could be attributed to the presence of the farm. Unlike in Clam Bay, redox potentials were not consistently lower near the net-cages, except perhaps along transect SQ3.

Dissolved oxygen

Dissolved oxygen concentrations at 5 to 10 cm above the substrate were typically about $10 \text{ mg} \cdot \text{l}^{-1}$, and showed no depression near the farm site (Figure 15). Two samples had an unexpectedly high dissolved oxygen concentration indicative of either analytical error or an abrupt gradient in dissolved oxygen with distance above the seabed. The same qualifications expressed at Clam Bay regarding non-synoptic sampling apply here as well, but it does appear that farm activities are not depleting oxygen in the near-bottom waters.

Sediment traps

Seven sediment trap arrays were placed at the stations along transect SQ1, but only four could be recovered after the 15-day deployment (Table 5). Results from the array directly under the pens are problematic in that these traps captured only half the material of the other traps, and the material retained was comparatively low in organic carbon content. Since the mouths of the traps were only 1.5 m below the bottom of the net-pens at MLLW it was expected that a large amount of

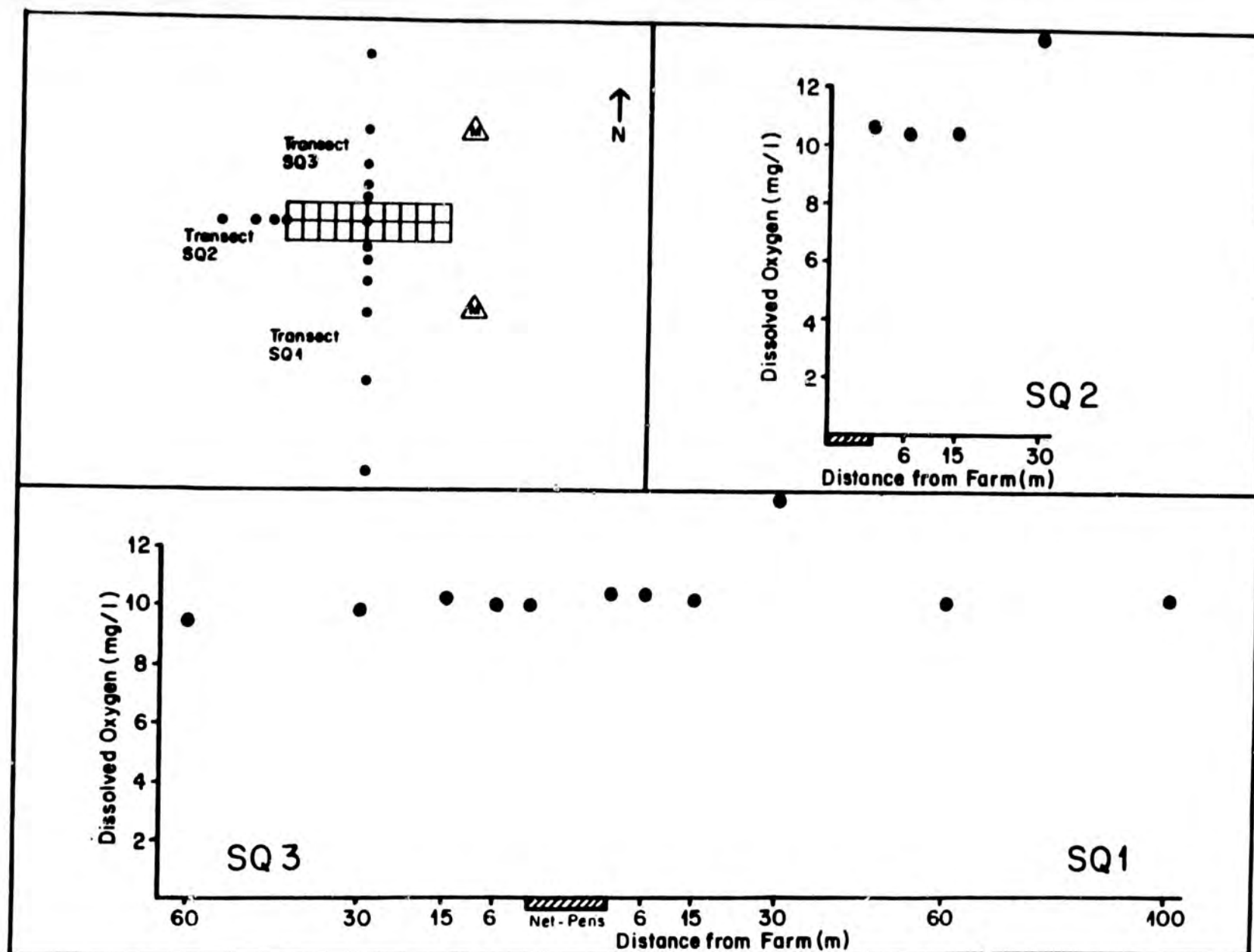


Figure 15. Dissolved oxygen concentrations in near-bottom water surrounding the Squaxin Island farm site.

Table 5
Sediment trap contents at the Squaxin Island farm
(Six measurements at most stations)

Distance from pens (m)	Sedimentation rate (kg·m ⁻² ·yr ⁻¹)	Nitrogen content (%)	Carbon content (%)	Carbon flux (kg·m ⁻² ·yr ⁻¹)
under pens	62.8	0.75	6.12	3.8
	65.2	1.29	7.85	5.1
	59.4	0.56	5.02	3.0
	58.0	0.63	5.19	3.0
	38.9	0.85	7.43	2.9
	<u>41.1</u>	<u>0.99</u>	<u>7.62</u>	<u>3.1</u>
MEANS	54.2	0.84	6.54	3.5
0	115.7	0.49	4.56	5.3
	88.0	0.43	3.95	3.5
	113.4	0.99	8.72	9.9
	<u>119.7</u>	<u>0.66</u>	<u>6.23</u>	<u>7.5</u>
MEANS	109.2	0.64	5.87	6.6
15	109.1	0.42	4.71	5.1
	118.4	0.39	4.21	5.0
	116.9	0.51	4.40	5.1
	111.3	0.64	4.15	4.6
	99.4	0.85	6.79	6.7
	<u>106.0</u>	<u>0.60</u>	<u>5.20</u>	<u>5.5</u>
MEANS	110.2	0.57	4.91	5.3
30	112.4	0.47	3.95	4.4
	98.9	0.35	2.13	2.1
	106.9	0.40	3.70	4.0
	108.7	0.48	5.22	5.7
	109.2	0.34	2.57	2.8
	<u>107.7</u>	<u>0.27</u>	<u>1.64</u>	<u>1.8</u>
MEANS	107.3	0.39	3.20	3.5

organic-rich material would have been collected. It is possible that this array may have become entangled in its own rope or in the nets during a very low tide, and turned on to its side at some time prior to recovery. This was the only array retrieved by a rope rather than by diver, and thus it is not known if it was properly positioned at the time of recovery.

On the basis of data from the other sediment traps, the sedimentation rate was about $110 \text{ kg} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ in the area from the farm perimeter to a distance of 30 m. Lacking data from greater than 30 m from the farm, it is difficult to establish whether the measured sedimentation rate reflects natural conditions or an enhanced sedimentation due to the presence of the farm. The organic carbon and nitrogen content of the trapped material was significantly different among traps (Kruskal-Wallis one way analysis of variance, $\alpha < 0.05$), and, in fact, the traps nearest the pens contained solids with a higher concentrations of both organic carbon and total nitrogen.

In comparison to the Clam Bay site, sedimentation rates at the Squaxin Island farm were two to three times greater (30 to $52 \text{ kg} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ vs. 107 to $110 \text{ kg} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$). The collected material at Squaxin Island, however, contained only half the carbon and nitrogen concentration of the Clam Bay material. The fact that Clam Bay traps contained material with a relatively high organic content (30 to 100 times greater than background levels in surficial sediments) suggests that the collected material was largely farm-derived. At the Squaxin site the measured sedimentation rate was relatively high, yet since the organic content of the trapped material was only slightly greater than background concentrations in surficial sediments (1.5 to 2-fold), it is likely that the bulk of the collected material originated from natural sources.

Macrofauna

Macrofauna samples have been collected at most stations along transect SQ1 as part of routine monitoring by the farm operator. Baseline samples were collected in January 1987 after installation of the pens but prior to stocking with fish. Additional samples were collected in August 1987, January 1988, and August 1988. Intercomparisons among sampling events is complicated by variations in sample design between sampling periods and between replicates. In January 1987 each station was sampled with 3 cores of 5 cm diameter, each of which was sieved through a 0.5-mm screen. In all other sampling periods 10 cm diameter cores were used, two of which were sieved on a 1.0-mm screen and one of which was sieved on stacked 0.5 and 1.0-mm screens. Therefore, when comparing among sampling periods it should be recognized that the January 1987 sampling would tend to under-estimate species richness and abundance because of the smaller area sampled, but over-estimate these parameters because of the finer mesh size. All data are expressed on a "per three sample basis" which, in all but the January 1987 sampling, should be interpreted to include the material retained on a 1.0-mm screen in two samples per station and on a 0.5-mm screen in one sample.

Baseline samples (1/87) and those collected after 6 months of operation (8/87) demonstrated that the five monitoring stations were comparable in species richness and faunal composition and thus suitable for inter-station comparisons (Figure 16). In January 1988 after 12 months of operation there was a dramatic decrease in the species richness and abundance at the station under the perimeter of the pens and, to a lesser extent, at the station 6 m from the pens. This same decrease near the pens relative to the other stations along the transect was evident again after 18 months of operation (8/88). At this time macrofauna present under the pen perimeter included only the polychaetes Capitella cf. capitata (4 individuals), Nephtys cornuta fransiscana

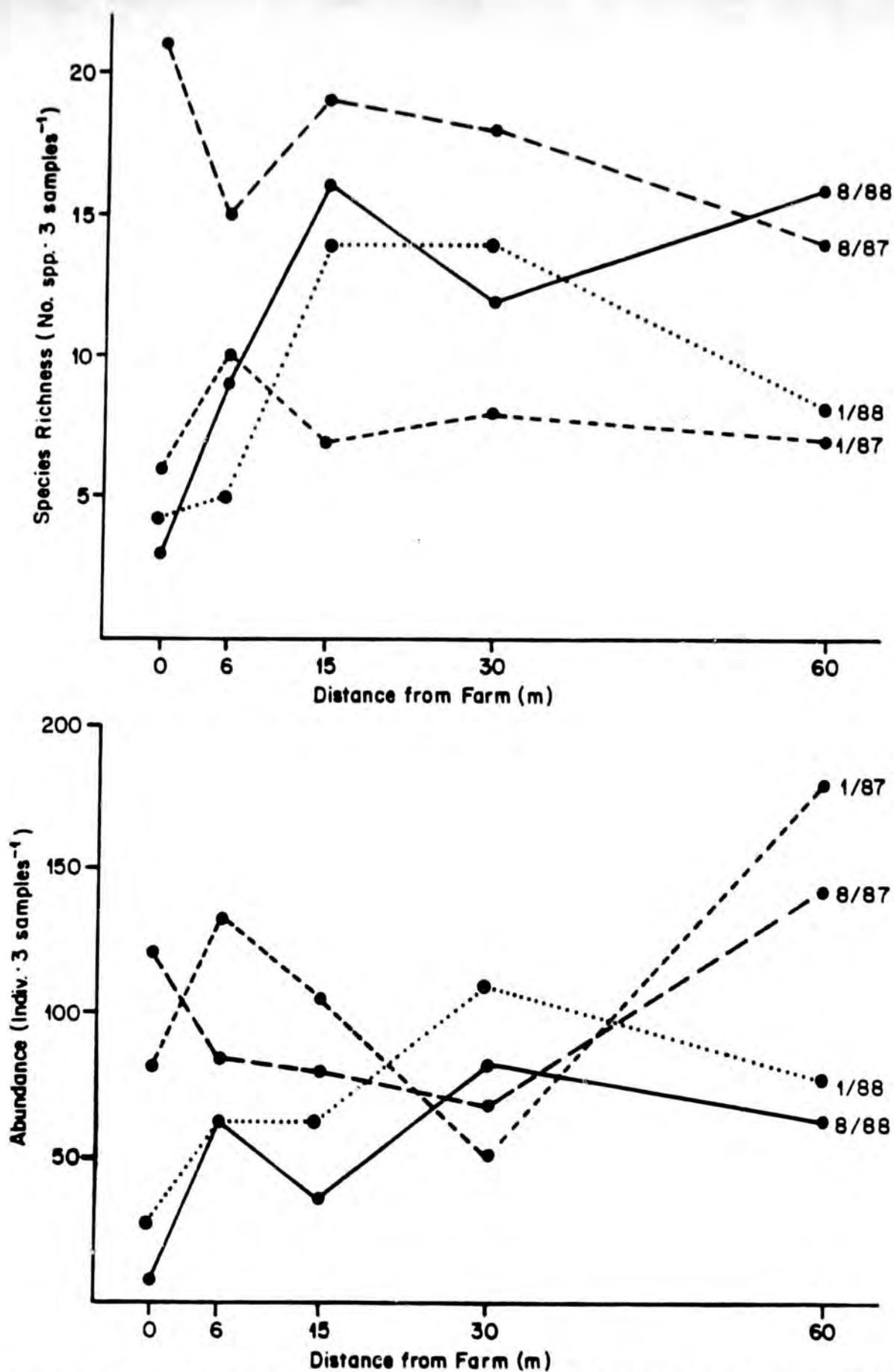


Figure 16. Trends in species richness and macrofaunal abundance along transect SQ1 at the Squaxin Island farm site. Inter-replicate variability is not shown in order to simplify the graphical presentation.

(3 indiv.) and Glycinde picta (1 indiv.). The depauperate fauna 0-6 m from the pens, an area which was formerly comparable to the other sites, indicates a localized impact of farm operation.

The distribution of Capitella cf. capitata (Figure 17) also suggests enrichment 0-6 m from the pen perimeter. No C. capitata were found throughout the sampling transect during the baseline sampling (1/87). In all subsequent sampling periods, however, the species has been found in high densities at the 6 m site and at much lower densities at the pen perimeter and 15 m. Maximum density (945 individuals per three replicates, or $1875 \text{ indiv} \cdot \text{m}^{-2}$) was obtained in the most recent sampling (8/88).

Dispersion model

The dispersion model was run using the data of Table 6. The standing stock value used was based on the biomass present at the time of sampling. This value may seasonally vary by up to 50% depending on stocking and marketing cycles. No data were available on the actual amount of feed provided, so it was assumed that the fish were fed at a rate of 2% of their body weight per day, a rate typical of salmonid net-pen culture and the target rate of the Squaxin pen operators. All other model parameters were determined as described for the Clam Bay farm.

As a result of the shallow water depths, the sedimentation model predicted that the vast majority of feed and fecal waste would reach the bottom directly under the pens or within 5 m of the farm perimeter (Figure 18). A maximum loading rate of $14 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ was predicted for most of the area under the farm. Lesser rates of deposition were predicted to the north and south, with essentially no accumulation of wastes to the east and west. Loading rates of $1 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ or greater were limited to the area 15 m north and 28 m south of the farm site.

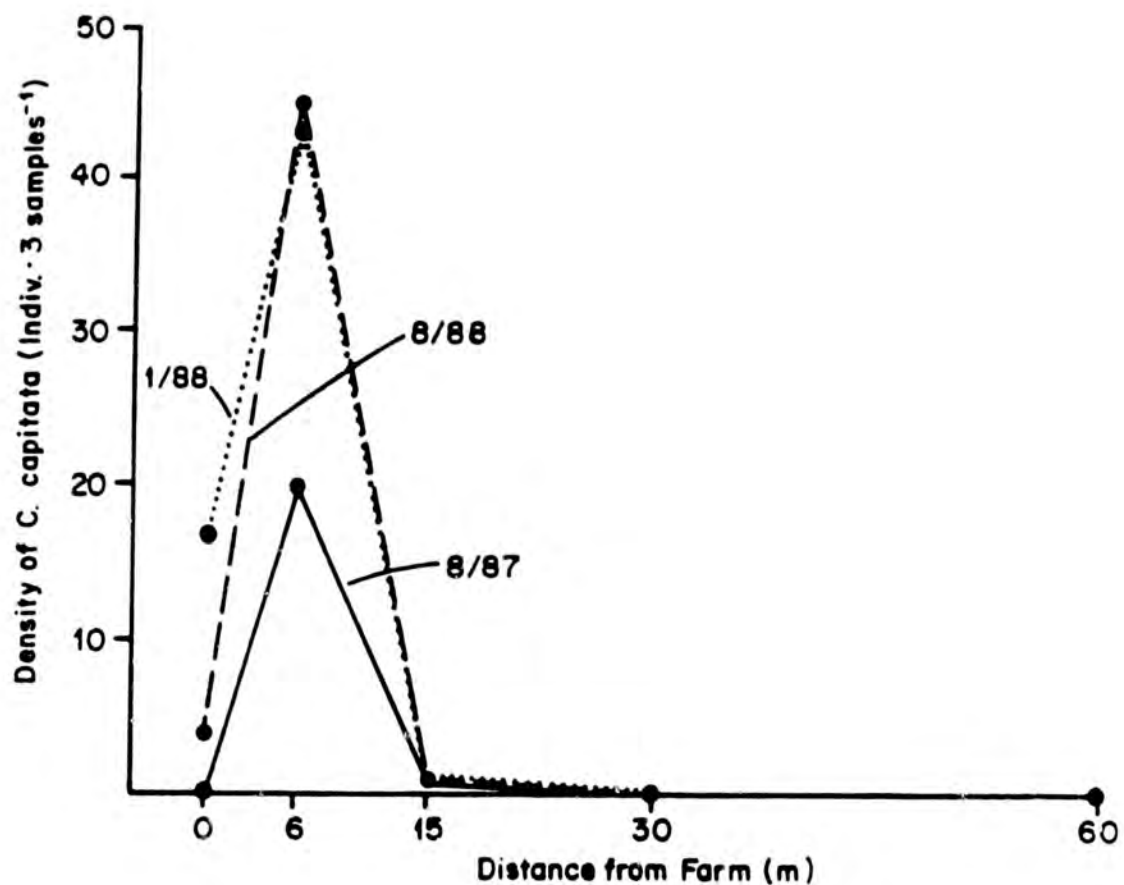


Figure 17. Density of the opportunistic polychaete Capitella cf. capitata along transect SQ1 at the Squaxin Island farm site. Inter-replicate variability is not shown in order to simplify the graphical presentation.

Table 6
Data used in dispersion model at the
Squaxin Island farm site

Farm size:	74 m x 16 m with long axis oriented east-west
Depth of pens:	3 m
Standing stock:	20 metric tons
Feeding rate:	400 kg·day ⁻¹
Organic carbon content of feed:	50%
Feed wastage:	15%
Settling velocity of feed:	14 cm·sec ⁻¹
Settling velocity of feces:	4 cm·sec ⁻¹
Water depth (approx. average of MLLW and MHHW along sampling transects):	7 m
Current data:	June 6 - August 5, 1988; meter located 60 m north of farm site.

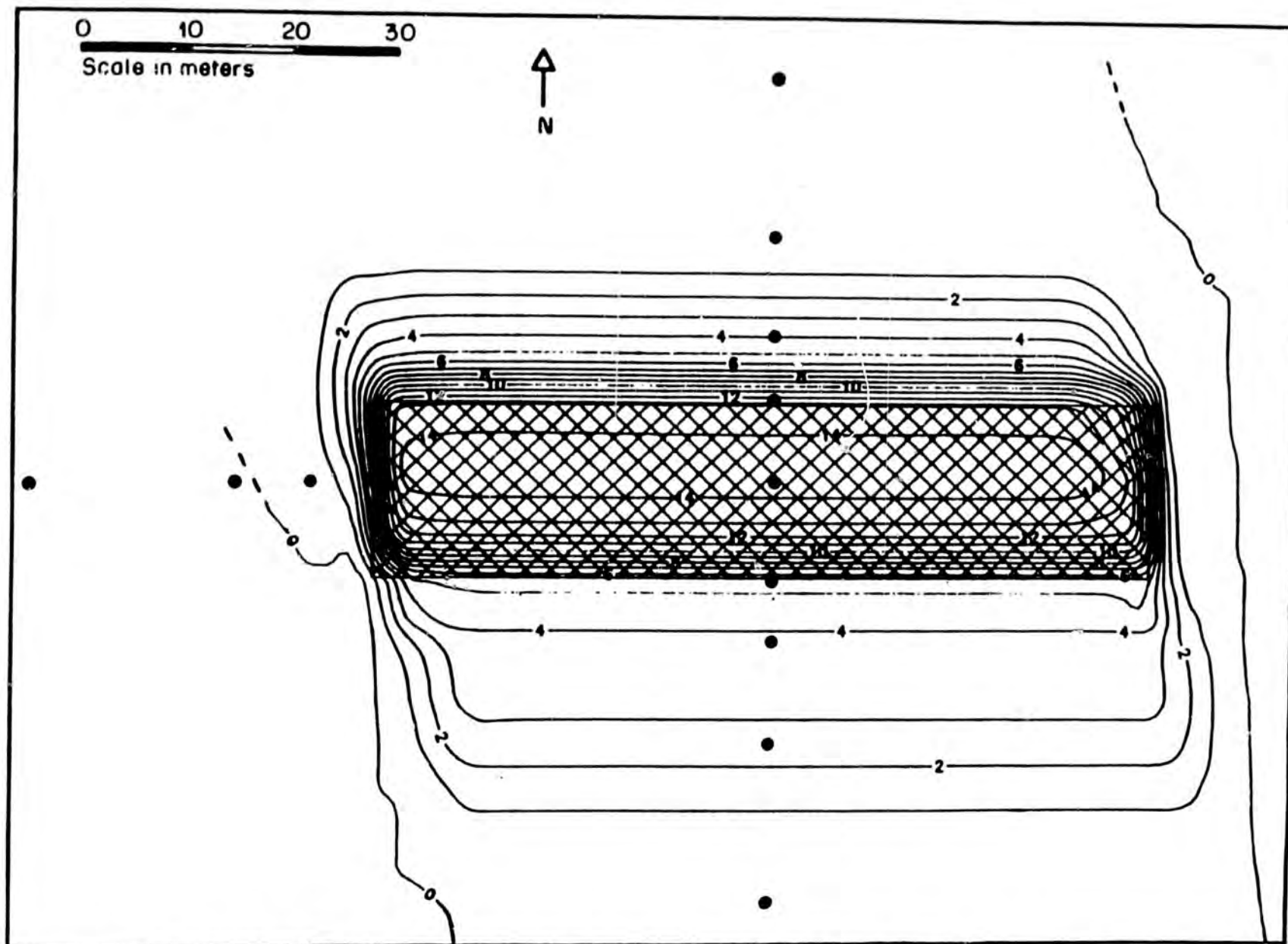


Figure 18. Model predictions of organic carbon loading to the sea-floor at the Squaxin Island farm site. Contour units are $\text{kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$. Dots indicate locations of sampling stations used for model verification. Stations 60 and 100 m from farm are not shown.

The predicted carbon fluxes at 0, 15 and 30 m south of the farm were 5.7, 2.5 and $<1 \text{ kg C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$, respectively. Over the same distance actual rates of carbon flux as measured by the sediment traps were 6.6, 5.3 and $3.5 \text{ kg C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$. It should be recognized, however, that the model predicts deposition of solid wastes originating from farm activities, whereas the sediment traps do not differentiate between farm-derived particulates and deposition unrelated to the culture operation. It was noted above that the bulk of the material collected in the traps at the Squaxin Island site probably originated from natural sources such as resuspension of bottom sediments or terrigenous run-off. A true test of the model would require that the contribution of the natural sources be subtracted from the total material retained in the traps. The strength of the natural "noise", however, is so great compared to the pen "signal", that many more traps would have to be deployed to discriminate between these two sediment sources. The traps located farthest from the farm (60 and 100 m), which presumably would best represent the natural carbon flux, were not recoverable, so the magnitude of carbon flux typical for the area may be substantially less than the $3.5 \text{ kg C}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ measured at the 30 m trap. Model predictions vary from actual measurements by a factor of about 2 or less, depending on the particular trap and the assumed magnitude of the natural carbon flux.

The model was further evaluated by comparing predictions of deposition with sediment organic carbon concentration and redox potential (Figure 19). It should again be recognized that this comparison requires that the untested assumption be made that post-depositional processes are similar at all sampling sites. There was no significant relationship between model predictions and sediment carbon content, although predicted carbon flux was correlated with sediment redox potential (Spearman rank correlation, $\alpha > 0.05$). This correlation was largely attributable to the influence of the 0 and 6 m stations along transect SQ-3, an area which the model

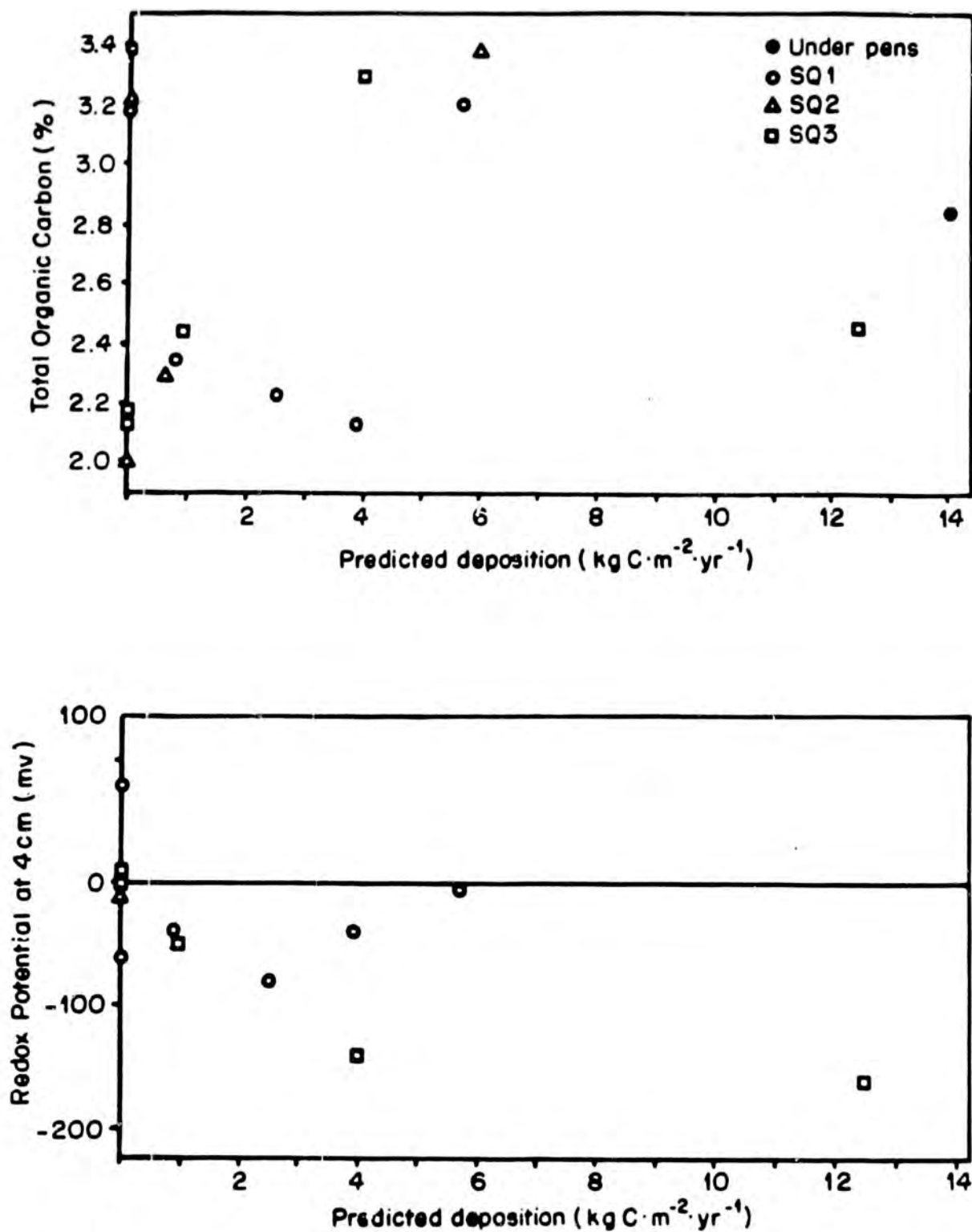


Figure 19. Comparisons of predicted carbon deposition based on the sedimentation model with field measurements of total organic carbon and redox potentials at the Squaxin Island farm site.

predicted to be among the most enriched and which also was found to have the lowest redox potentials.

DISCUSSION

CLAM BAY SITE

The various chemical parameters used to measure the effects of the farm operation on the sediments of Clam Bay showed good agreement, and provided a clear picture of the areal extent of impact. The culture of fish at the Clam Bay site has resulted in a measurable enrichment of the sediments directly under the pens and to a distance of approximately 30 m from the perimeter of the pens. The exact distance of impact depended upon the direction and the chemical parameter used as indicator of impact, but in all cases varied from 15 to 60 m. Within this area deposition of feed and feces has resulted in increased concentrations of total organic carbon and total nitrogen. Degradation of these organic wastes has depleted pore water oxygen, resulting in more negative reduction-oxidation potentials. At a few sites reducing conditions were found throughout the entire sediment column, although at most stations oxidizing conditions persisted to a depth of at least 4 cm. The enriched sediments did not measurably decrease oxygen concentrations in the overlying water at a height of 5 to 10 cm above the seafloor, although this does not exclude the possibility that reduced dissolved oxygen levels may be observed if measurements had been made on a scale of millimeters rather than centimeters.

The biological data (available only to the northwest of the farm) showed a severely disturbed community at the pen perimeter. This assemblage was comprised almost entirely of nematodes and Capitella cf. capitata. If the assumption could be made that a similar fauna existed wherever organic carbon concentrations were comparable (and given similar substrate type), then such a community might also be found 15 to 30 m south of the pens and at least 60 m to the east. Beyond this zone of severe disturbance, moderate effects of the farm were

evident in communities 45 to 150 m from the site. This area was characterized by reduced species richness, biomass, and *C. capitata* densities in excess of 5000 indiv. $\cdot$ m⁻².

All previous studies of the benthic effects of net-pen farming have reported localized impacts comparable to those found at Clam Bay. Brown *et al.* (1987) found normal conditions appearing at some point between 15 and 120 m from a farm in Scotland. In a survey of numerous Scottish farms, effects such as depressed reduction-oxidation potentials and appearance of *Beggiatoa* were commonly found up to 30 m from the farm site (Earll *et al.*, 1984). Doyle *et al.* (1984) found effects extending 25 to 45 m from a site in Ireland. The extent of effects at the Clam Bay site is comparable to these earlier studies based solely on sediment chemistry as a measure of effect. The biological indicators of disturbance, however, suggest effects extended to at least 150 m from the farm, approximately five times the distance typically reported.

SQUAXIN SITE

The effects of the Squaxin Island site on the benthos were more subtle and evident almost entirely on the basis of the biological data alone. There was little or no evidence of farm effects in the sediment organic carbon, nitrogen, redox, and dissolved oxygen data. One of the three samples collected below the southern pen perimeter showed a three-fold enrichment in organic carbon, but since such enrichment was not evident in the other samples at this site, the effects were presumably very patchy. The best physical/chemical evidence of farm impacts was provided by the elevated concentrations of organic carbon and nitrogen in material collected by the sediment traps nearest the pens. The macrofaunal data indicated reduced species richness and/or abundance from the pen perimeter to a distance of 6 m, and a peak in *C. capitata* abundance at a distance of 6 m. *C. capitata*, an indicator of enriched sediments, first appeared in the area 6 months after

culture began, and was increasingly abundant 12 and 18 months after the initiation of culture. For comparison, Brown et al. (1987) reported changes in sediment chemistry and appearance of *C. capitata* 3 months after initiation of culture in a Scottish loch. Mattson and Linden (1983) monitored benthic conditions after installation of mussel longlines and found a period of 6 to 15 months were required for replacement of the original fauna with an assemblage characteristic of enriched conditions.

The limited measurable physical/chemical effects of culture and the highly localized biological effects at the Squaxin site are surprising given that the bottom of the pens are only 2 m above the seafloor at low tide. There are two possible explanations for this observation. First, the current velocities at the Squaxin site are surprisingly high, and only slightly less than near-bottom currents at Clam Bay. The 15 to 18 cm·sec⁻¹ measured twice daily at the Squaxin site may promote dispersal of the solid wastes, particularly if the narrow distance between the nets and the seafloor promotes a channeling effect and an acceleration of currents directly under the pens. Secondly, the Squaxin pens have only been in place for 18 months in comparison to approximately 13 years for the Clam Bay farm. Benthic conditions at the Squaxin site may continue to deteriorate with time, but a recent change in farm operation may slow or halt this deterioration. The operators of the Squaxin site have recently decided to curtail aquaculture operations and use the pens primarily for delayed release. The seasonal nature of use should minimize further effects on the benthos.

ASSESSMENT TECHNIQUES

It should be noted that at both Clam Bay and the Squaxin sites, the macroinfaunal data showed evidence of alteration in areas where sediment chemistry data failed to show farm effects. Biological data appeared to be a more sensitive

indicator of disturbance, and therefore suggest that chemical information alone can not adequately define the extent of benthic impacts from net-pen culture. The biota are certainly better integrators of temporal variation, and they also undoubtedly are responding to chemical and physical parameters unmeasured in conventional surveys.

The reduction-oxidation measurements proved valuable, particularly at the Clam Bay site where redox potentials closely mirrored gradients in organic carbon and nitrogen. Redox measurements have several advantages over carbon and nitrogen analysis, most notably the fact that results are obtained in the field immediately after sampling and there are no analytical costs once the pH meter and redox electrode are acquired. Redox measurements worked well in the sandy sediments of Clam Bay where vertical gradients in sediment redox potentials were gradual, but were more problematic in the muddy sediments of the Squaxin sediments. In these fine-grained sediments much of the vertical change in redox potentials occurs in a thin veneer of sediments at the sediment-water interface. Measured potentials (and the identification of culture effects) become highly susceptible to minute variations in the extent to which the probe is inserted. Conventional redox probes are approximately 1 cm in diameter and do not permit the fine-scale resolution necessary in muddy sediments. Micro-electrodes are available, but are expensive and subject to frequent breakage.

MODEL EVALUATION

The **dispersion** model was tested on its ability to predict: 1) the absolute loading of particulate wastes; and 2) the relative loading among numerous sites. The former evaluation was done by comparing the quantity of material retained in the sediment traps with the quantity that the model predicted would accumulate at the trap location. At the Squaxin site natural deposition made discrimination of the pen

contribution difficult, but the predicted loading appeared to differ from measured values by factors of 2 or less. In Clam Bay the predictions were in good agreement with the measured loadings, differing by factors of 1.2 and 1.4 in the two trap arrays.

It should be recognized that there are inherent difficulties of measuring actual carbon flux and there are many potential artifacts associated with the use of sediment traps (Butman, 1986). Nevertheless, if the sediment trap data can be taken as an accurate representation of actual carbon flux, then model errors of only 1 to 2-fold demonstrate remarkably good predictive capability of the model.

The second test of the model was to compare predicted loadings at all stations with measured values of sediment carbon content and redox potential. At both farm sites the model predictions of carbon flux showed a statistically significant correlation with redox potential. Sediment carbon content was correlated with model predictions only at the Clam Bay site. The better model performance at Clam Bay than at Squaxin Island was probably due to the greater amount of sediment resuspension at the latter site. The sediment trap data and the lack of pronounced physical/chemical gradients with distance from the farm both suggest a high degree of sediment transport and resuspension. Under such conditions it is not surprising that the sites predicted to have the greatest carbon input rate failed to show the highest levels of sediment enrichment. This illustrates the shortcomings of using static measurements (sediment carbon concentration) as a test of rate measurements (model predictions of carbon flux).

The model predictions were correlated with sediment redox potential at both of the sites examined, and with carbon concentration at one of the two sites. The performance of the model in Puget Sound is in general agreement with tests of the model in Scotland where, out of six farms, the model predictions correlated with measured redox potentials at all six farms and with carbon at two farms (Gowen *et al.*, 1988).

Ideally, the model should be capable not only of predicting the extent of chemical change in sediments but also the degree of biological disturbance. Such predictions are much more difficult for the biological effects of a given rate of carbon flux is likely to be habitat specific. Communities of sandy substrates may not respond to a given flux in the same manner as mud bottom communities. The model has not been refined to the point where reliable biological predictions are possible, but some preliminary observations have been made. In model tests in Scotland, it was found that severely disturbed sites, defined as containing four or fewer macro-faunal species, had predicted loadings of at least 1.8 to 4 kg C·m⁻²·yr⁻¹ (Gowen et al., 1988). At the Squaxin Island farm four or less species were found where the predicted loading was 5.7 kg C·m⁻²·yr⁻¹ and undisturbed communities were present where predicted loadings were 2.5 kg C·m⁻²·yr⁻¹ or less. At Clam Bay the fewest number of species (9) were found where the predicted deposition was 2.5 kg C·m⁻²·yr⁻¹, although more moderate impacts were evident at lower rates of carbon flux. Other than order-of-magnitude approximations, the existing data base is inadequate to determine a threshold carbon flux beyond which biological effects are likely. Nevertheless, such estimates may be possible with additional refinement of the model and recognition of the habitat-dependence of biological impacts.

The model has performed well in both Puget Sound and Scotland, yet it has inherent limitations which should be recognized:

- 1) As discussed earlier, the model does not take into consideration any post-depositional processes that may occur, or differences in the rate of these processes among sites. For example, the model only predicts the point at which a settling particle impacts the bottom and not any reuspension or transport that may occur later. Model predictions would be invalidated if the degree of

resuspension varied throughout the study area as might be the case with differing substrate types or pen-related alteration of current flow.

- 2) The model, as currently formulated, is incapable of coping with variations in bottom topography. At the Clam Bay site a single water depth (18 m) had to be assumed despite the fact that water depths varied from 10 to 30 m over the predicted area of deposition. The net effect is that in shallow areas the model over-estimates the lateral extent of deposition, and under-estimates the lateral extent in deep areas. This shortcoming could be remedied but would dramatically increase the computational requirements of the model.
- 3) An arbitrary assumption had to be made that feed wastage at both farm sites was 15%. Puget Sound farm operators typically claim a wastage factor of about 5%. The magnitude of predicted organic carbon loading to the bottom is dependent upon the degree of wastage assumed as demonstrated by Figure 20. (Note also in this figure the depth-dependence of the loading with reference to the limitation under point #2 above.) A reduction in the wastage factor from the 15% employed in this analysis to the 5% claimed by Puget Sound operators would result in a decrease in predicted organic carbon loading of about 15%. In fact, there is probably no reliable estimate of wastage in Puget Sound or throughout the industry in general. Until there are reliable estimates of feed wastage, neither this nor any other model will be able to predict loading with a high degree of accuracy. The wastage-dependence of loading shown in Figure 20 also illustrates the effect that the operator's feeding practices can have on waste production, and the environmental benefits to be gained by reducing wastage.

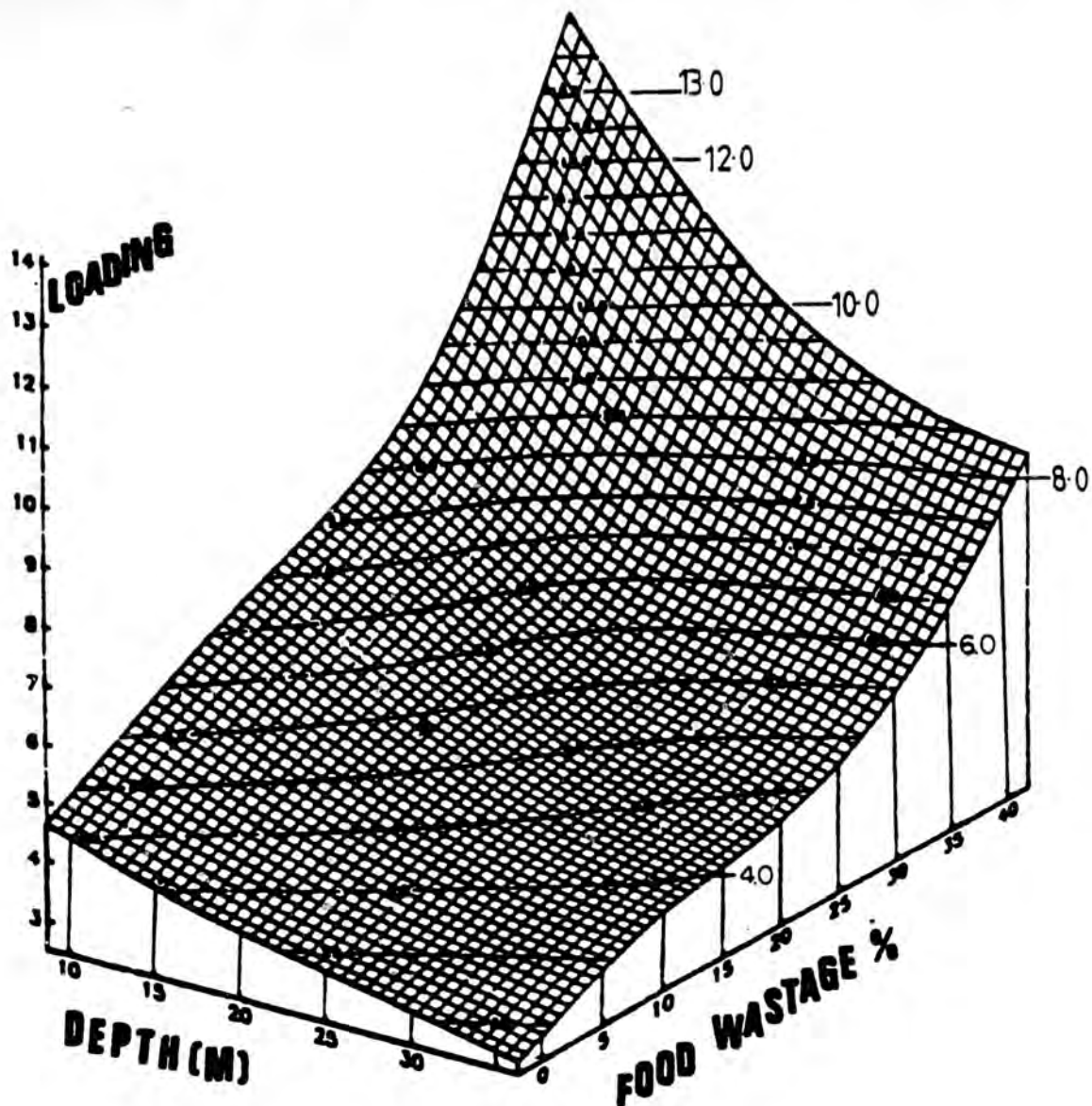


Figure 20. The relationship between food wastage, depth and organic loading to the sediment for a hypothetical set of current data. Loading rates are given as $\text{g C} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$. (From Gowen *et al.*, 1988).

- 4) The model requires the designation of a single settling velocity for feed and a second settling velocity for feces. As noted by Thomson (1986), however, the size and density of particulates released from a net-pen are likely to depend upon the species and size of the fish, the type and pellet size of the feed, and the amount of physical disturbance induced by either water current or fish activity. In addition to these potential variables, particulate wastes are not uniform in size or density, and thus can not be adequately characterized by a single settling velocity, or even the two velocities of the model. Settling velocities of culture wastes are best described by a frequency distribution, and the model therefore requires that a major oversimplifying assumption be made.
- 5) It is not possible to describe variations in the flow field attributable to the presence of the pen structures, and how these variations may influence deposition. If the model is used for siting in a pre-development stage, the installation of the pen structures may modify the predicted magnitude and distribution of waste loadings. The effect of pen installation on waste dispersion is, however, likely to be small in most cases particularly when the pens occupy a small proportion of the total water column.
- 6) The hydrographic input to the model is based on current records at a single point, and there is no allowance for changes of the flow field along a particle's trajectory. The current velocity and direction measured at the meter mooring may not be representative of current regimes on other sides of the farm complex or over the entire area of deposition. At the Clam Bay farm the two current meters gave very different pictures of current patterns. The model was run using data from the eastern meter since

this site would be less subject to the complications of bay geomorphology, but the model results would have been somewhat different had data from the other meter been used. The effects of lateral variation of currents on model predictions are especially pronounced at Clam Bay because of the atypically large size of the farm and shoreline effects on current patterns. At the Squaxin Island site and at other farms of small to moderate size the effect of lateral variations in current patterns may be negligible and ignoring these variations in the model may be justified.

- 7) The model, as presently formulated, is incapable of dealing with depth-related variation in current flow. With increasing water depth, the probability increases that currents measured at a single depth are unrepresentative of the multiple current regimes a particle encounters during settling to the seafloor. In the present study the meters were positioned at a depth approximating half the distance between the bottom of the pens and the substrate. In some situations multiple current meters at several depths may be necessary to accurately predict waste dispersal.
- 8) The model has no mechanism by which to consider duration of culture. The magnitudes of organic carbon loading were comparable at the Squaxin and Clam Bay sites, yet the effects on sediment chemistry were much greater at Clam Bay. This difference, which may be due to the short period of time that the Squaxin site has been in use, can not be incorporated into the model.

Despite the limitations of the model, it represents the best means currently available to predict the magnitude and extent of culture impact on the seafloor. The model has performed well both in the present study and in previous tests

in Scotland, yet because of its limitations and the necessary simplifying assumptions, the model should be used cautiously as a predictive tool. As indicated by the probable high degree of transport and/or resuspension at the Squaxin Island site, consideration must be given to site-specific conditions which violate model assumptions and therefore make predicted loadings subject to error. On a fine scale (predicting carbon flux at a specific point), model loading predictions appear to be reliable to within a factor of two in most cases. While this is generally adequate for site assessment, the potential error should be recognized and compensated for in identifying areas of potential impact.

On a broader scale (predicting the affected area), model predictions appear very reliable, for at five sites examined in Scotland, the model predicted that the $1 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ isopleth would extend up to 15 to 30 m from the farm perimeter. In the present study, the model predicted this rate of loading up to 28 and 70 m from the Squaxin and Clam Bay sites, respectively. Areal extents of impact of this magnitude are consistent with observations from the present study and other studies reported in the literature.

The current information collected as input to the model is extremely useful both from the perspectives of environmental protection and farm husbandry. The current meters deployed in this study measured velocity and direction every 15 minutes for a period of 60 days. Such information is invaluable not only to predict the dispersal of solid wastes but to determine mooring requirements, rates of water renewal in the pens, and duration of slack water (which may be the limiting water quality parameter in maintaining suitable growing conditions).

The model is useful in condensing a massive data set of current measurements into a single summary figure interpretable by non-specialists. It should therefore be helpful in

explaining probable impacts in public hearings and similar forums.

The model is also helpful in identifying sites which would be clearly unsuitable for culture. Given the current state of knowledge it is not possible to define the impacts of a given loading rate, however severe biological disturbance has been observed in Scotland where predicted loadings were as low as $1.8 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$. At some unquantified level below this loading, development of a site is unlikely to have significant effects on the benthos. At most farms examined in Washington and Scotland, loading rates directly under the pens range from 6 to $14 \text{ kg C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$. Where loading rates are far in excess of these values, the generation of hydrogen sulfide by enriched sediments and the consequent effects on the cultured fish themselves would be of serious concern.

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APPENDIX B

Modeling of Particulate Deposition Under Salmon Net-Pens

**MODELING OF PARTICULATE DEPOSITION
UNDER SALMON NET-PENS**

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INTRODUCTION

One of the observed effects of some salmon net-pen facilities on the environment has been organic enrichment of the underlying soils from the deposition of fish feces and uneaten feed. Under separate report to the Department of Fisheries, Drs. Donald Weston and Richard Gowen have examined deposition rates, chemical changes and biological impacts under two net-pens sited in Puget Sound. Their evaluation included a predictive deposition model developed by Gowen.

A separate modeling approach has been used in the evaluation of several proposed net-pens recently by Parametrix, Inc. The purposes of this report are: (1) to run the Parametrix model for the same sites modeled by Gowen and comment on the comparative results, and (2) modify configuration, orientation and density of the pens and evaluate the sensitivity of predicted deposition rates to these variables.

MODEL DESCRIPTION

The Parametrix model is a modification of a model developed by EPA (1982) to predict the deposition of particulates from sewage treatment plant outfalls in coastal waters. This model has already been applied to several proposed net-pens in the State of Washington. The model relies on average speed and frequency along the principal and minor axes to predict excursion distances from the pens and areal deposition rates for settleable materials of distinct settling velocity. A sloping bottom can be accounted for in the model. Comparison of the Parametrix model with Gowen's model will be saved for the Discussion.

The Parametrix model includes evaluation of post-depositional processes related to decay of the organic material. The organic material will decay as it accumulates on the sea floor. When the rate of deposition matches the rate of biodegradation, a steady-state accumulation of organic material will result. Decay of organic material will create an oxygen demand in the bottom waters in the vicinity of the net-pens. The EPA methods are used to predict the resulting steady D.O. depletion in the near-bottom waters.

RESULTS: CLAM BAY SITE

Currents. Like Gowen's model, the Parametrix model is based on the current meter located 100 m east of the farm site (#F2053). The current rose from this meter is shown in Figure 1. Each of the current measurements has been catalogued into one of eight 45° directional "bins." The percentage of currents falling within each bin and the average speed of those currents are indicated by the rose. The length of each rose petal is proportional to the percentage of currents in that direction. The current rose for the Clam Bay site used in the model indicates the predominant current direction falls within the bin from 90° to 135° (ESE). The average currents along the major and minor axes of the net-pens used in the model are adjusted for the frequency and strength of currents

in each direction (for example, the fastest currents occur from 315° to 360° but are not very frequent, thus are not weighted as high as the easterly currents).

Wasteload and Settling Velocities. The method of determining wasteload and settling velocities used by Gowen is different than that used for this model. The wasteload assumed for this model study is consistent with the wasteloads assumed in earlier modeling efforts by Parametrix for net-pens in Puget Sound (Harding Creek, Discovery Bay, and North Skagit Bay). The Gowen model is based on a carbon mass balance, whereas the Parametrix model accounts for total solids. The results modeled herein are converted to carbon for comparison with Gowen's results in the summary results, based on 48 percent carbon content for feed, and 80 percent for feces.

The wasteload and settling velocities assumed for the Parametrix model are based on an annual production of 617 metric tons, Gowen's published data for European farms, and other European researchers (Gowen 1987). Lost feed would be about 10 percent of the total feed. The data used in this modeling are tabulated below:

<u>Waste Component</u>	<u>Total Solids Loading</u>	<u>Total Carbon Loading</u>	<u>Percent</u>	<u>Settling Velocity</u>
Uneaten Feed	430 kg/d	206 kg/d	40%	10 cm/s
Large Feces	325	260	30	5
Small Feces	325	260	30	2
Total	1,080 kg/d	726 kg/d	100%	

By comparison, Gowen's model assumed 317 kg C/day lost feed and 540 kg C/day feces, for a total loading of 857 kg C/day.

Model Runs. The model was run for the existing pen size and orientation and four other configurations as summarized below:

<u>Run</u>	<u>Description</u>
1	Existing pen size and orientation
2	Rotate existing pens 90°
3	3 round pens with same total surface area
4	Increase pen width by 50 %
7	Decrease fish production 50 %

The results of each model run are summarized below and in the Figures attached at the end of this report (except Run 7 which would have the same areal coverage as Run 1, with 50 percent of the deposition rate). The table below includes only the maximum predicted impact, which would be concentrated directly under the pens. Total accumulation is based on a steady-state decay of organic material. The model output is provided at the end of this report for all runs.

Run	Total Deposition kg/m ² /yr	Organic Deposition kg C/m ² /yr	Total Accumulation kg/m ²	D.O. Depletion mg/L
1	14.7	9.3	4.0	0.13
2	9.8	6.1	2.7	0.04
3	10.6	6.5	2.9	0.08
4	10.8	6.9	2.9	0.11
7	7.4	4.6	2.0	0.07

RESULTS: SQUAXIN SITE

Currents. The model runs for the Squaxin site are based on the meter #F2057 located 60 meters north of the existing pens. The current rose for this meter is shown in Figure 2. The rose reveals a predominant NNW by SSE current axis. The SSE component is slightly less frequent, but much stronger than its counterpart. Current speeds used in the model are handled similar to the Clam Bay site.

Wasteload and Settling Velocities. The Squaxin site is much smaller than the Clam Bay site. The assumed wasteloads and settling velocities are tabulated below:

Waste Component	Mass Loading	Mass Percent	Settling Velocity
Uneaten Feed	40 kg/day	40 %	10 cm/sec
Large Feces	30	30	5
Small Feces	30	30	2
Total	100 kg/day	100 %	

Model Runs. The model was run for the pen configurations as summarized below:

Run	Description
5	Existing pen size and orientation
6	Rotate existing pens 90°

The results of each model run are summarized below and in the figures and printouts attached at the end of this report.

Run	Total Deposition kg/m ² /yr	Total Accumulation kg/m ²	D.O. Depletion mg/L
5	11.9	3.3	0.02
6	17.2	2.7	0.05

DISCUSSION

Comparison with Gowen Model. The basic calculation in both models, horizontal displacement of settleable particles, is governed by the same function of settling velocity and current velocity. However, Gowen's model simulates individual particle trajectories for each current velocity from the current meter data, whereas the Parametrix deals only with averages. Accordingly, the Gowen model requires more input data and is capable of producing a larger number of sediment contours and more precise deposition pattern.

Unlike the Parametrix model, Gowen's model does not consider sloping topography, multiple settling velocities or post-depositional biological processes. However, Gowen's model could certainly be modified to include these additional features. Although these features are desirable, the uncertainties regarding wasteload and post-depositional processes exceed the precision achieved by these features.

Neither model accounts for dissolution, suspension or resuspension of particles by high currents. However, a very detailed field investigation of the spatial distribution of currents near each site would be necessary to predict impacts at remote locations if material is resuspended. Resuspension and maintenance of fish feces in suspension under turbulent conditions would be a valuable future research topic.

Comparison of Model Results. Gowen's model produces a more detailed map showing deposition contours ranging from 0 to 12 kg C/m²/yr for Clam Bay, with 1 kg C/m²/yr intervals. The Parametrix model reveals only 3 contours, with an average of 9.3 kg C/m²/yr centered under the pens (Run 1). Gowen's assumed wasteload was greater than that assumed for the Parametrix model. Given the uncertainties regarding wasteload assumptions (discussed below) and the greater resolution possible with Gowen's model, the results appear compatible.

As discussed in the model description, the Parametrix model accounts for the post-depositional process of decay. The assumptions made are generally conservative, but may be useful for management uses. The accumulation mass and thickness would be masked in areas where natural deposition is also occurring. In each case modeled, the predicted D.O. impact would be negligible, which is consistent with the observations of Weston. The D.O. function in the model would be useful to flag potential problems, but could not be relied on for an accurate prediction if oxygen problems are anticipated.

Sensitivity Analysis. The results of Runs 1 through 4 and 7 indicate the sensitivity of deposition to various factors. Runs 1 through 4 represent an equal number of fish, or wasteload. Run 7 reveals that the most effective way to reduce deposition is to reduce the wasteload.

For a given wasteload, the greatest mitigation is achieved by orienting the pens perpendicular to the predominant current direction (a reduction of 33 percent in this case: see Runs 1 and 2). In this respect, the Squaxin site is already mitigated, as evidenced by a like increase by rotating the pens by 90 degrees (see Runs 5 and 6). Of course, engineering and navigation considerations may not allow this mitigation measure.

Separation of the pens into several pods may also mitigate deposition. Essentially, the impact would be spread over a greater area. In this example, a reduction of 28 percent would be achieved by the configuration chosen for Run 3. The reduction at any proposed site would be site specific and a function of the size and separation of the individual pens.

Run 4 increased the dimension perpendicular to the predominant current by 50 percent. A reduction in deposition of approximately 25 percent is anticipated. Again, the reduction obtained at any site would be specific to that site. An increase in the dimension parallel to the predominant current would have little effect.

CONCLUSIONS

The primary conclusions of this analysis follow:

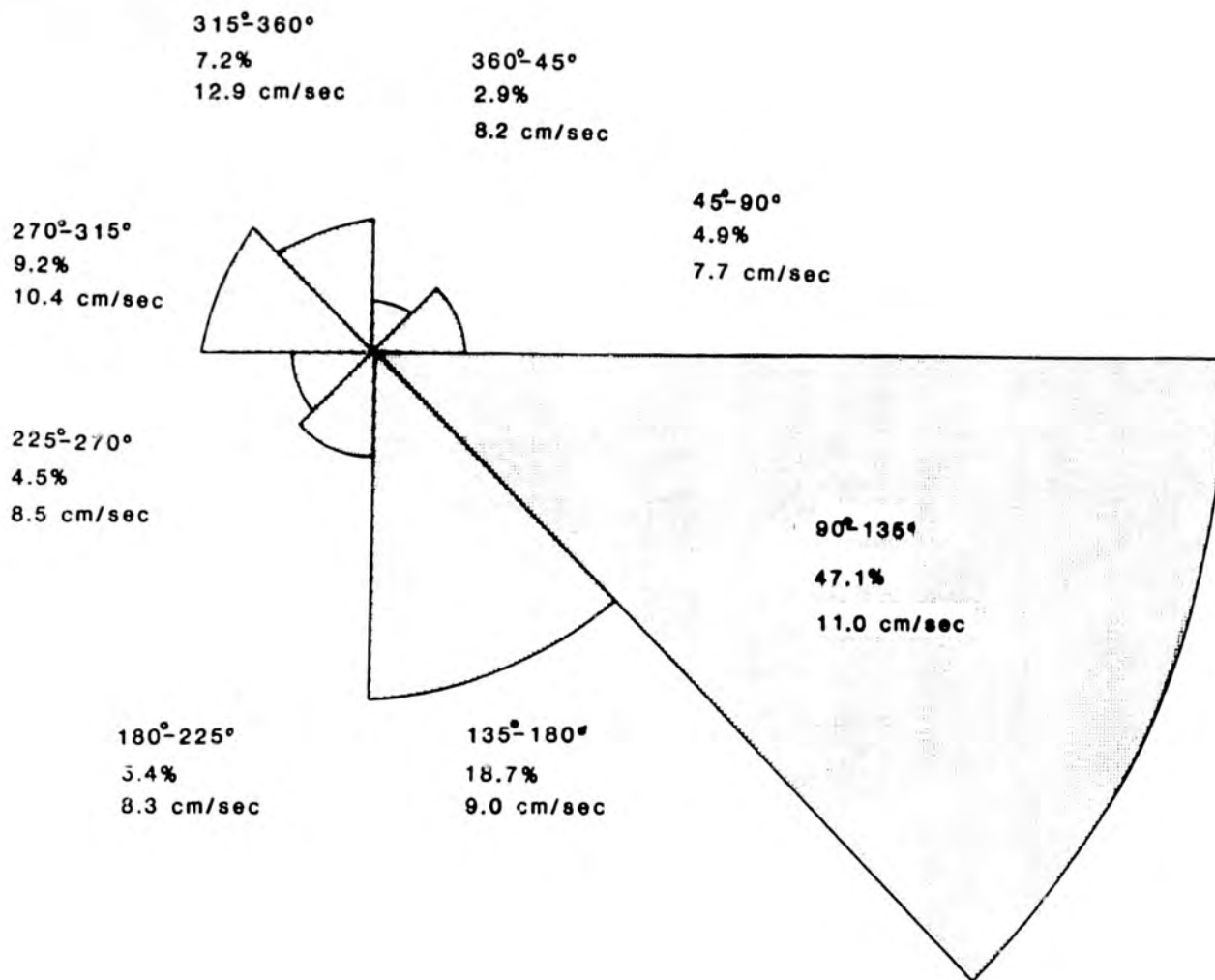
1. Gowen's model produces a deposition map of better resolution than the Parametrix model. The Parametrix model crudely accounts for uniformly sloping bottom topography and post-depositional decay, and can accept any number of settling speeds or vertical variation of current speed.
2. Improvements could and should be made to both models regarding suspension and resuspension of depositional materials.
3. Current speed and direction relative to the pens are critical to deposition. Fecal material from the pens may remain suspended at some threshold current speed. If current speeds are below this threshold level, pen orientation to the axis of predominant currents is critical.
4. The greater resolution of Gowen's model or any other modeling improvements (such as multiple settling velocities or complex topography) are secondary to the need for more defined criteria regarding wasteload, as discussed below.

The most settleable particles are the uneaten feed. However, lost feed ratios reported in the literature range from 1 to over 30 percent of the applied feed. Run 7 revealed

the importance of the predicted wasteload in predicting deposition rates. Until more accurate criteria for wasteload are developed, which seems unlikely, less subtle improvements to the models are ineffective. The models may provide useful management tools, particularly when comparing alternative sites and pen configurations, or establishing best management practices to limit feed wastage.

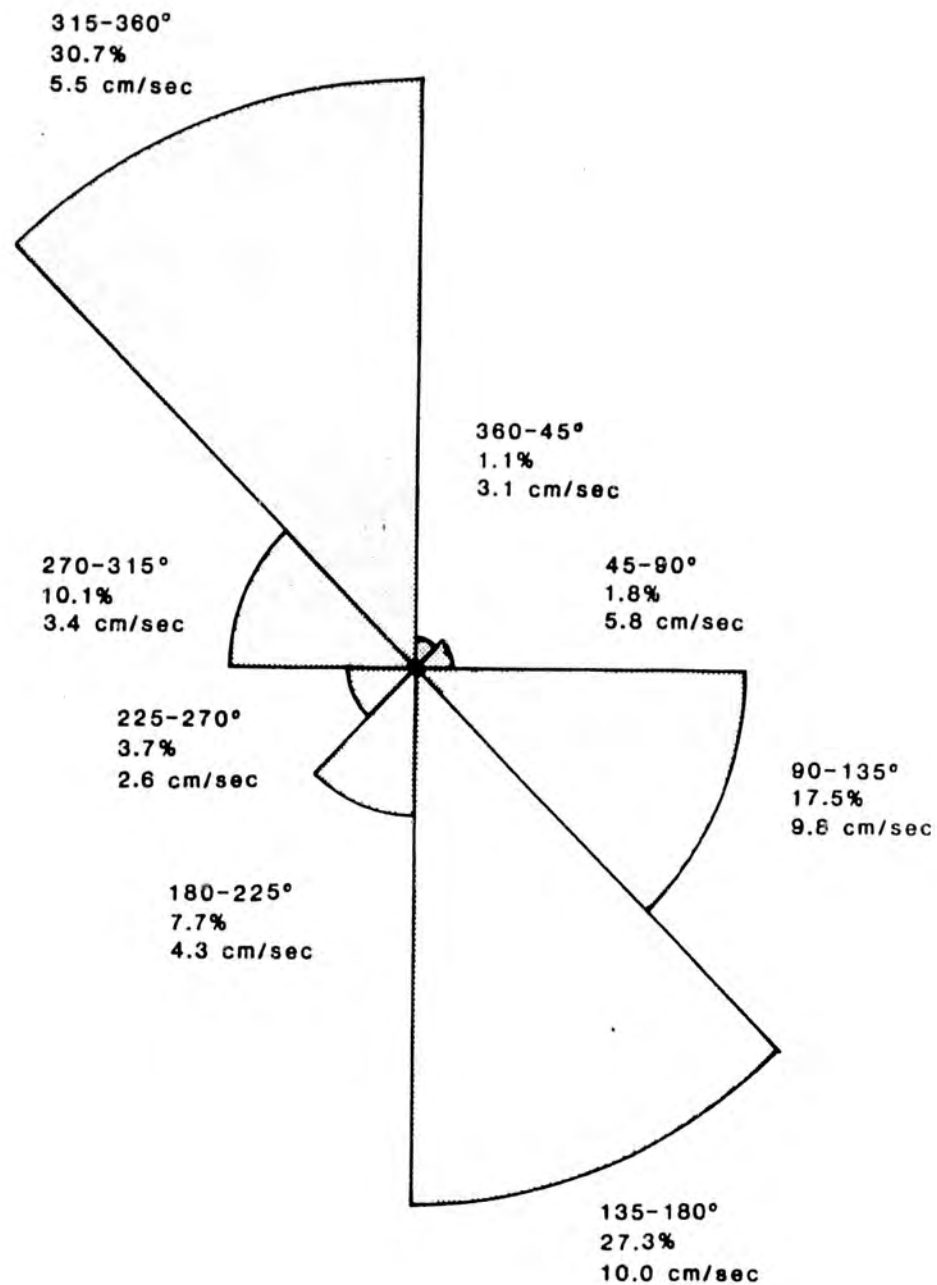
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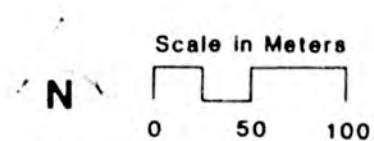
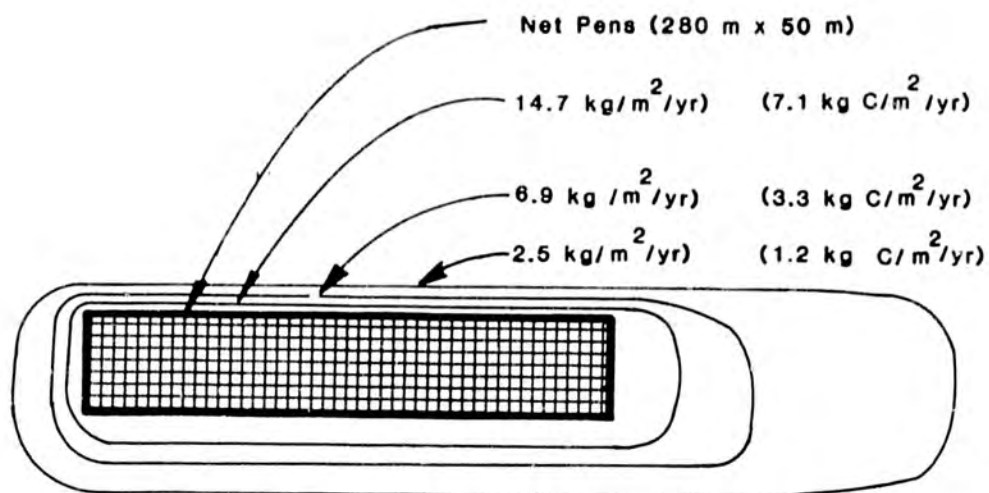
Note: Current Rose Indicates
Percentage and Mean
Speed of Currents Within
Directional "Bina." Length
of Rose is Proportional
to Percentage.

Figure 1:
Current Rose For
Clam Bay Site,
Meter F2053



Note: Current Rose Indicates
Percentage and Mean
Speed of Currents Within
Directional "Bins." Length
of Rose is Proportional
to Percentage.

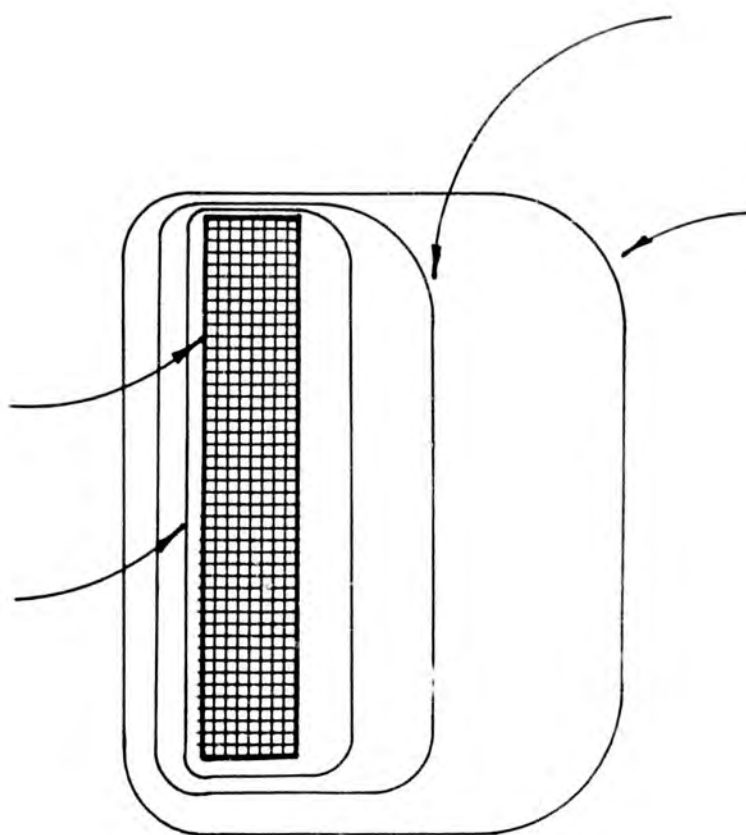
Figure 2:
Current Rose
For Squaxin Site,
Meter F2057



Run 1:
Clam Bay,
Existing Configuration

Net Pens
(50m X 280m)

$9.8 \text{ kg/m}^2/\text{yr}$
 $4.7 \text{ kg C/m}^2/\text{yr}$



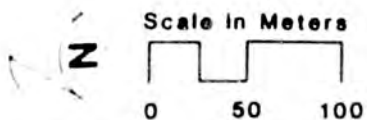
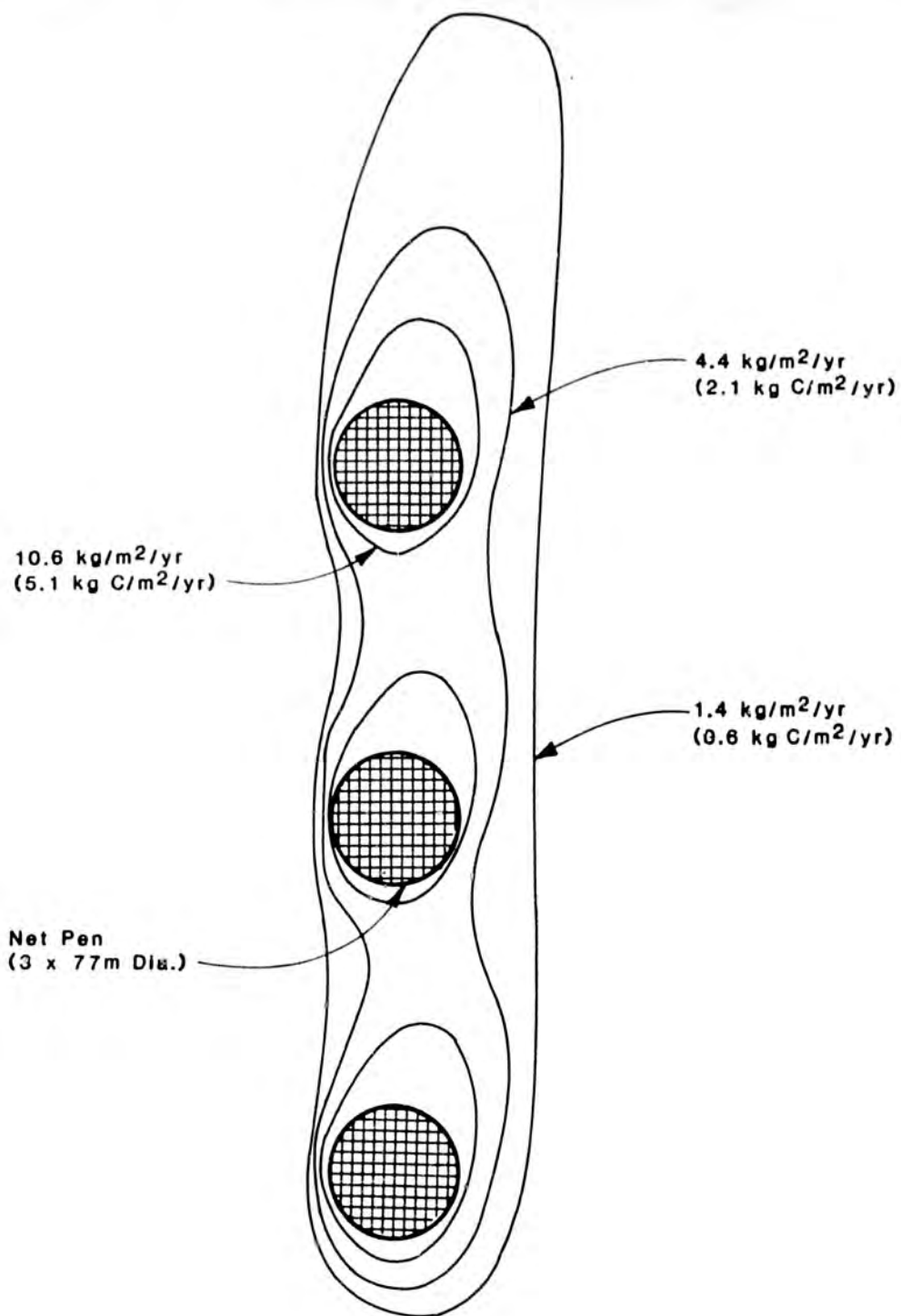
$4.2 \text{ kg/m}^2/\text{yr}$
 $(2.0 \text{ kg C/m}^2/\text{yr})$

$01.3 \text{ kg/m}^2/\text{yr}$
 $(0.6 \text{ kg C/m}^2/\text{yr})$



Scale in Meters
0 50 100

Run 2:
Clam Bay
Rotate 90°



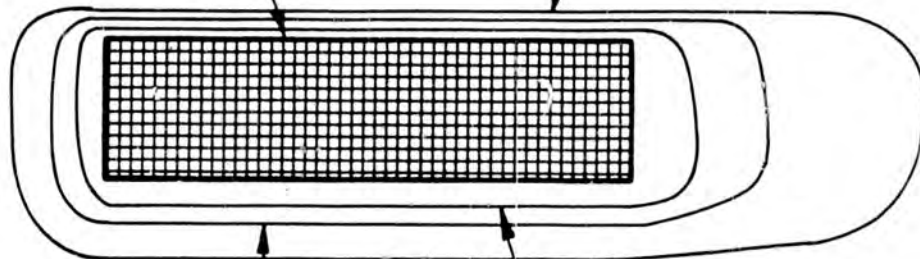
Run 3:
Clam Bay.
Round Pens

Net Pens
(280 m x 75 m)

2.0 kg/m²/yr
(0.9 kg C/m²/yr)

5.3 kg/m²/yr
(2.5 kg C/m²/yr)

10.8 kg/m²/yr
(5.2 kg C/m²/yr)



Scale in Meters
0 50 100

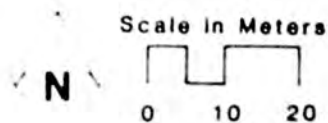
Run 4:
Clam Bay,
Double Wide Pens

Net Pens
(75 m x 17 m)

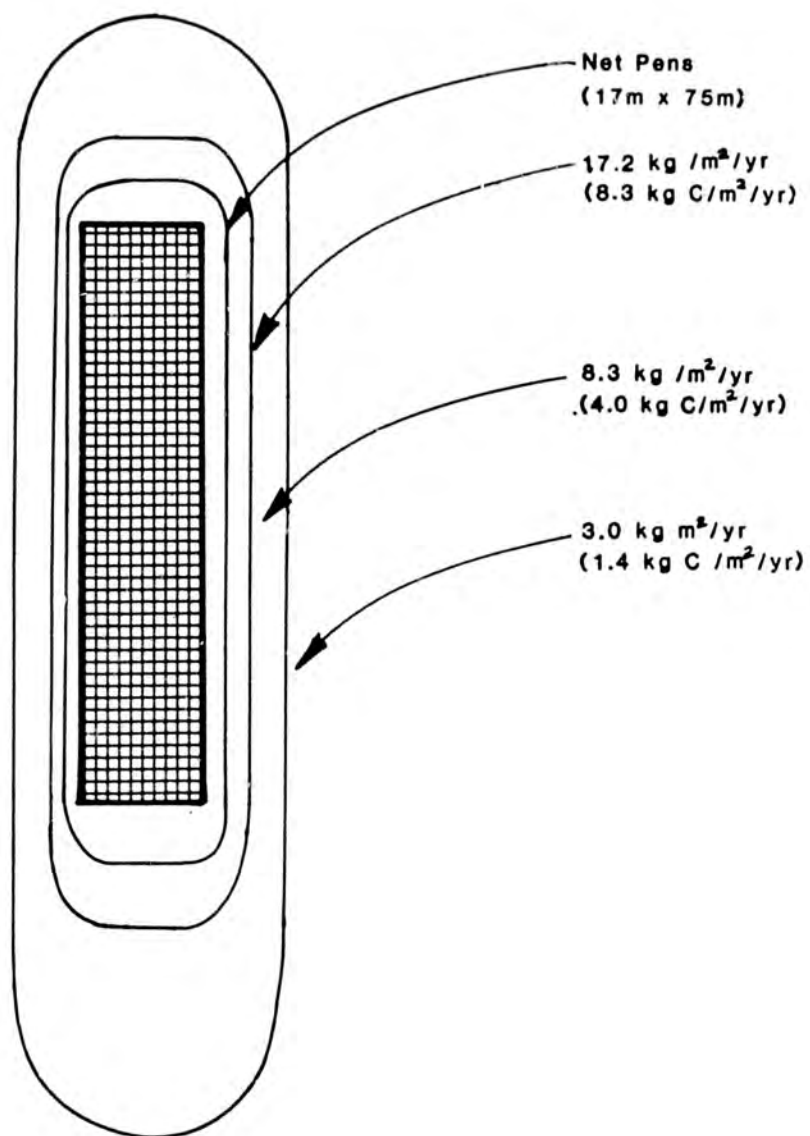
11.9 kg/m²/yr
(5.7 kg C/m²/yr)

5.2 kg/m²/yr
(2.5 kg C/m²/yr)

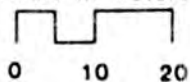
1.7 kg/m²/yr
(0.8 kg C/m²/yr)



Run 5:
Squaxin,
Existing Configuration



Scale in Meters



Run 6:
Squaxin Rotate 90°

APPENDIX C

Phytoplankton and Nutrient Studies Near Salmon Net-Pens

At Squaxin Island, Washington

PHYTOPLANKTON AND NUTRIENT STUDIES
NEAR SALMON NET-PENS
AT SQUAXIN ISLAND, WASHINGTON

by

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Rensel Associates
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for

The Washington Department of Fisheries
and the
Technical Appendices of the
Programmatic Environmental Impact Statement:
Salmon Aquaculture in Puget Sound

January 6, 1989

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Abstract

The effect of a salmon net-pen farm on nutrient concentration, phytoplankton density and primary productivity was investigated in a shallow passage of southern Puget Sound, Washington. The farm was relatively large and situated in surface waters that are depleted of dissolved nitrogen for unknown periods during the summer. The assessment was conducted during a period of maximum net-pen fish biomass and one month later during similar tidal and weather condition, after release of 60% of the fish. Monitoring of reference stations at both ends of the passage, beyond the immediate effect of the net-pens, was conducted to assess source water conditions.

During the period of maximum fish biomass, minor increases in dissolved nitrogen ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4^+$) were seen downstream of the pens during one tidal period, but not during the next. Total ammonia was significantly elevated within the pens compared to ambient concentrations, but concentrations were well below the chronic exposure concentration for salmonids and other sensitive coldwater fish. A distance of 30 m downstream approximately 80% of the ammonia had been oxidized to nitrate, presumably by microbial nitrification.

Before fish release, the rate of phytoplankton primary productivity (growth rate estimation by the C-14 method) was greater near the net-pens than at the reference stations during the morning tide, but not different during the following tide. After release of most of the fish, a similar trend was seen, but there were no significant differences of productivity between tidal periods or among stations. Phytoplankton density (chlorophyll a concentration) followed a similar trend before, but not after fish release.

The results indicate that phytoplankton density and growth rate fluctuated with tidal stage and time of day and were not directly related to nutrient discharge from the salmon farm. Surface waters throughout the passage were, at times, low in dissolved nitrogen content, but total nitrogen to phosphorus (N:P) ratios indicated that there was an adequate reserve of nitrogen in non-dissolved forms (e.g., plankton). The N:P ratio data suggest that nitrogen cycling processes (e.g., remineralization of organic matter in plankton) were adequate to prevent limitation of phytoplankton growth during the period of most likely impact by the net-pen farm.

Introduction

Salmon reared in marine net-pens produce solid and dissolved wastes including various forms of nitrogen. Dissolved nitrogen wastes from salmon are as much as 60 to 90% ammonia, with lesser amounts of urea and amino acids (Stickney 1979). Nitrogen is the nutrient most likely to be limiting to the growth of marine phytoplankton. Therefore, the potential for localized nutrient enrichment and increased algal abundance near salmon farms exists, and will depend mainly on the total size of the farms in the restricted water body and existing hydrographic conditions (Gowen et al. 1985, Gowen and Bradbury 1987).

Nutrient limitation of surface waters is a key consideration of the State of Washington's Recommended Interim Guidelines for the Management of Salmon Net-pen Culture in Puget Sound (SAIC 1986). That document conservatively, but somewhat arbitrarily, designated the nutrient limitation status of sub-areas of Puget Sound based on a threshold of 0.1 mg/l, two and one-half times greater than the literature value of 0.04 mg/l (URS 1986a). As discussed below, simple measurement of dissolved nutrient concentrations is inadequate to determine if nutrients are inadequate for algal growth.

There have been relatively few attempts to monitor nutrient enrichment near salmon net-pens and even fewer studies of the effects upon phytoplankton dynamics. Prior studies of nutrients from net-pens strongly suggest there is little measurable effect beyond the immediate area of the pens (near-field). While nutrient concentrations are relatively easy to monitor, phytoplankton studies (far-field second and third order effects) are more difficult to conduct and have typically relied on measurement of chlorophyll *a* concentrations, the primary photosynthetic pigment in phytoplankton.

In the only study conducted in the Pacific northwest on this topic, Pease (1977) surmised that no measurable impact on phytoplankton populations occurred under worst-case conditions. The study area at Henderson Inlet, Washington had limited circulation during summer months, exacerbated by the net-pen site location within a shallow log dumping and storage area. Intense dinoflagellates blooms occurred at the site during the summer of 1974 which killed farmed salmon and prawns (Rensel and Prentice 1980). The 1974 blooms, which appeared to be exceptional in abundance, occurred throughout large parts of Puget Sound and also killed salmon in net-pens at Squaxin Island. These conditions were not seen in the previous year at either Henderson Inlet (Snyder et al. 1974) or in several previous or one later year at Squaxin Island (Fraser 1976).

Pease (1977) found increased density of phytoplankton (chlorophyll *a*) during summer months with increasing distance

into the log rafting area. The net-pens were located near the outer, seaward edge of the log rafting area. Reference areas (controls) were located in the main channel of Henderson Inlet, outside the log rafting area, and further inside the log rafting area. From my analysis of his monthly dissolved nitrate data (Table 5 and Fig. 8 of Pease 1977), it appears that nitrogen-depleted conditions could have occurred only in July when some of the samples had less than 0.04 mg/l dissolved nitrogen. Since both total nitrogen and phosphorus concentrations were not monitored, there is no way to know for sure. Low concentrations of dissolved nitrogen coincided with chlorophyll *a* maxima, but that does not mean that nitrogen availability limited phytoplankton growth. A generalized, inverse correlation between dissolved nutrients (nitrate and orthophosphate) and phytoplankton density was apparent over the entire summer period.

Phytoplankton standing crop during Pease's study was consistently greater at a reference station inside the log rafting area and at the net-pen site, compared to an outside, midchannel reference area in the open water of Henderson Inlet. However, Pease (1977) concluded that there were no abnormally high concentrations of phytoplankton anywhere in the area, and that phytoplankton activity throughout the inlet was unrelated to the net-pen rearing.

The second conclusion is possible, but neither supported or refuted by his data, as the inside reference area was too close (a few hundred feet) to the net-pens to be considered as separate and unaffected. Since water currents are weak and often imperceptible at the Henderson Inlet site, the inside log-rafting reference areas can not be considered as separate from the net-pen site, with regard to phytoplankton populations. These criticisms do not invalidate Pease's conclusions regarding other water quality conditions, but suggests that a greater standing stock of phytoplankton existed near the net-pens and that the effect of location and nutrient impact of the net-pens could not be sorted out given the experimental design. Several years after removal of the Henderson Inlet facility Rensel (unpublished data, 1988) found that chlorophyll *a* concentrations in midchannel were about twice those found at the now vacant net-pen site, opposite the condition that prevailed throughout Pease's year long study (2.72 versus 1.71 ug Chl. *a*, SD = 0.273 and 0.412 respectively, *n* = 6). However, these samples were taken too late in the fall to be representative of optimum algal growing conditions and indicated relatively low phytoplankton density at both areas.

There are numerous difficulties in determining perturbations of phytoplankton from fish-farms or from other of man's activities. Foremost, as discussed above, once the net-pens are established, there is usually no adequate means to establish baseline conditions. Although nutrient uptake by phytoplankton may be rapid, there is a lag time of up to a day or so between the addition of nutrients and a measurable increase in phytoplankton biomass (Parsons et al. 1984). Knowledge of local hydrodynamic

processes of dispersion, such as distance of tidal excursion, are required for interpretation of these types of data. Simply monitoring upstream and downstream of a net-pen farm site may reveal near-field nutrient effects, but may not be adequate to monitor the relatively slow response of phytoplankton populations to the increased nutrient concentrations. Monitoring of phytoplankton abundance and dynamics in the field is also difficult due to natural variations in time and space of the phytoplankton. A number of discrete or loosely interacting measures of water column ecology must be assessed, as no single measure provides all the necessary information.

Nitrogenous wastes from net-pen reared fish or other sources are unlikely to increase phytoplankton abundance in most of the main channels of Puget Sound since nitrogen is already in abundance (Collias and Lincoln 1977, Anderson et al. 1984, SAIC 1986). Accordingly, this study focused on a worst-available-case of nutrient enrichment from net-pens in what appeared to be nutrient-depleted waters, at least during some tidal, seasonal and weather conditions. To establish baseline conditions, the present study was conducted before and after the release of large numbers of fish at a public benefit, salmon rearing and release net-pen site. In addition to having spatially separate reference areas, "before fish release" water quality samples were used as the experimental data, and "after fish release" monitoring served as a baseline.

Study Site: Description and Hydrography

The net-pen study site, located in Peale Passage east of Squaxin Island in southern Puget Sound (Fig. 1), is a cooperative Washington Department of Fisheries and Squaxin Island Indian Tribal sponsored program. Coho (*Oncorhynchus kisutch*) and other species of salmon have been reared at the site since the early 1970's (STOWW 1974) and the nearby beaches have abundant littleneck clam and planted oyster populations. The Squaxin Island net-pens are presently the largest public benefit facility in Washington state and produces substantial numbers of fish for commercial and sport fisheries (Rensel et al. 1988). There are three adjacent sets of net-pens, two for delayed release of salmon and, in recent years, a third rearing facility to the north operated by the tribe for normal commercial purposes (pen system 3 in Fig. 2). All of the fish reared at the first two facilities are destined for release into Puget Sound, and are held in the net-pens for only a few months of the year after attaining smolt condition.

Peale Passage is a shallow tidal channel connecting Dana Passage on the south and Pickering Passage to the north (Fig. 2). The Squaxin Island Indian reservation forms the west boundary of Peale Passage, Harstene Island the east boundary. The source waters for Peale Passage are relatively well-mixed by strong tidal currents, although only one year of sampling data were available for the Dana Passage sampling station (unpublished WDOE

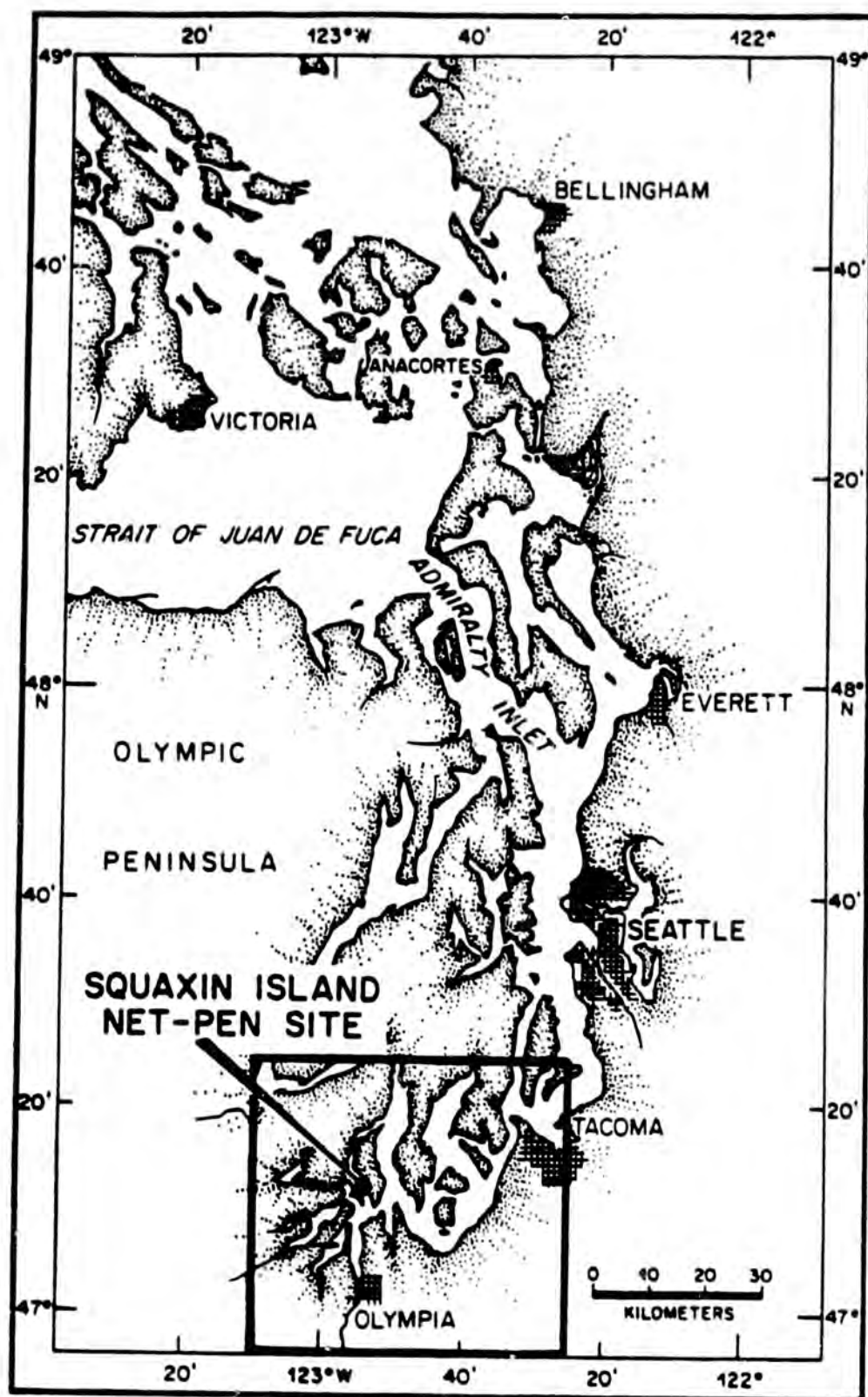


Figure 1. Map of the Puget Sound region with Squaxin Island net-pen site located in South Puget Sound within outlined box.

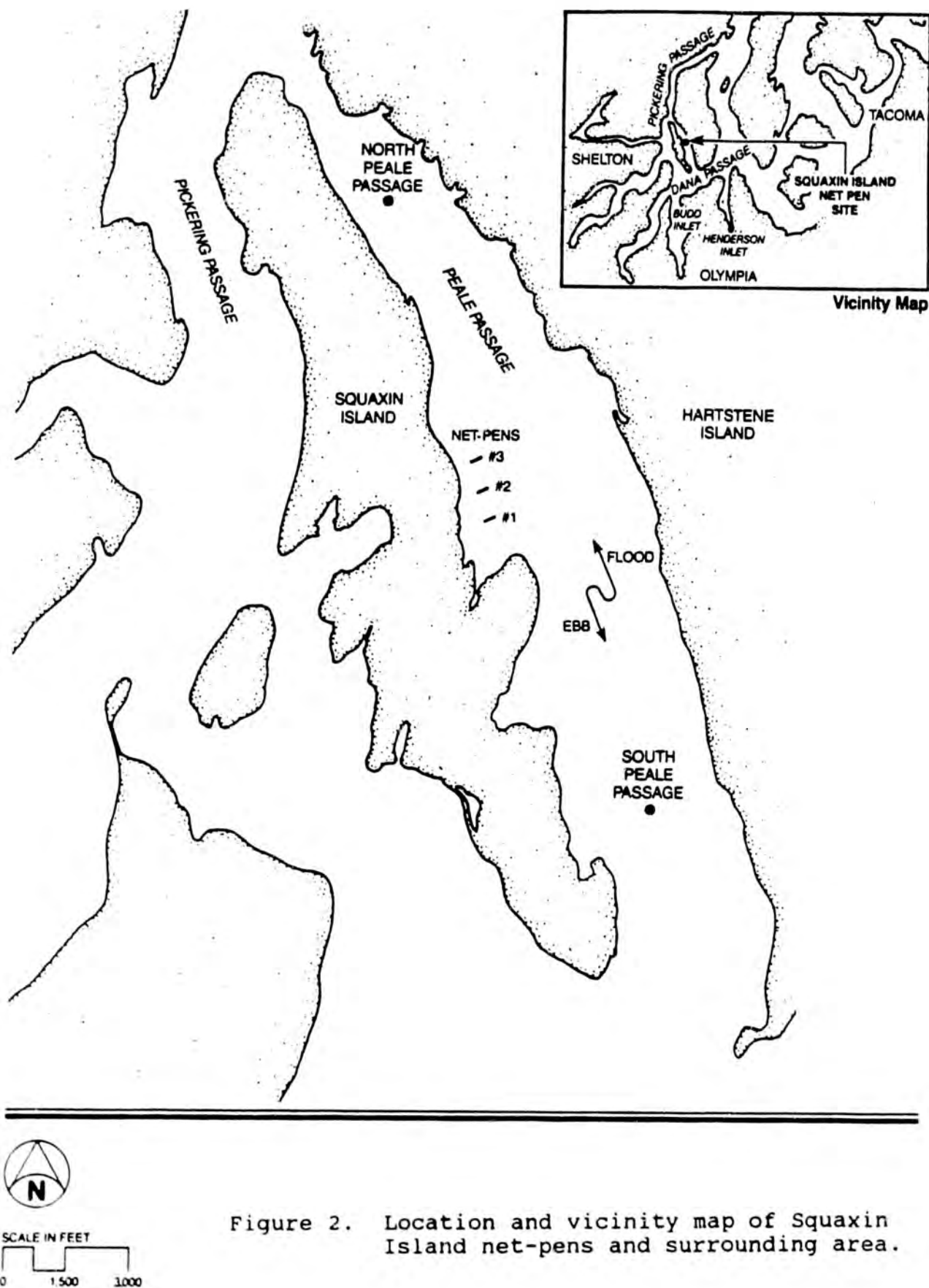


Figure 2. Location and vicinity map of Squaxin Island net-pens and surrounding area.

water quality data DNA001). These data indicate low dissolved nitrogen in surface waters occurred 15% of the period April to November. Pickering Passage data show low dissolved nitrogen in surface waters about 39% of the time (SAIC 1986).

Recent studies of circulation and nutrients in deep southern Puget Sound (URS 1986b) indicate that additional source waters for Peale Passage are the inlets at the west end of Dana Passage, especially Budd and Eld Inlets, due to clockwise circulation of surface and deeper waters in western Dana Passage. These inlets both exhibit strong vertical stratification and nutrient limitation during clement weather and undoubtedly influence Peale Passage surface waters at times during calm weather.

An early study of hydrographic conditions in southern Puget Sound measured vertical profiles of physicochemical parameters in Peale Passage on a few occasions (Oclay 1959). Moring (1973) noted that there was little background information concerning water quality in the Peale Passage area. His studies provide some basic information concerning conditions at the net-pen site. In subsequent years, fish culturists of the Squaxin Island Tribe collected additional information at the site that, combined with the earlier information, is adequate to characterize the vicinity. These data indicate that the area is well-mixed in the late fall to spring months but has a gradual thermal gradient and very minor salinity gradient in the clement weather periods of summer. There are no significant freshwater sources in Peale passage and no sharp discontinuities of water column characteristics.

Unpublished drogue (drift object) data collected for this study and recording current meter data collected for a related study (Weston and Gowen 1989) suggest that water passing through the net-pens does not exit Peale Passage on the same tide. This situation potentially could lead to an increased abundance of phytoplankton, since phytoplankton will rapidly assimilate dissolved nitrogen during growth-limiting, nitrogen depletion.

With an average depth of about 5.0 meters at mean lower low water (MLLW), depths in the vicinity of the net-pen site are shallow compared to other existing net-pens in Western Washington. Mean water current velocity near the most northern set of net-pens is about 6 to 7 cm sec⁻¹ with a net directional flow to the south (Weston and Gowen 1989). Currents diminish an undetermined amount in the vicinity of the other two net-pens and may be affected by the presence of a small cove that tends to slow water movement and form a gyre, particularly on the flood tide (unpublished survey data of B. Wood, Squaxin Island Tribe, 1982).

Although no historical nitrogen data were available from Peale Passage, two days prior to the first sampling date of this study I found dissolved nitrogen concentrations less than 0.04 mg/l, and a total molar nitrogen to phosphorus (N:P) ratio of 16.6 at 0 to 2 m depth in south Peale Passage. Use of a simple numerical

value of dissolved nitrogen may be misleading for representation of the actual threshold of nutrient depletion (Welch 1980); examination of the total N:P ratio is more useful in determining nitrogen depletion and the status of the complex nitrogen cycle. Recent studies in nearby Budd Inlet suggested that nutrient limitation in surface waters occurred during summer months when the concentration of dissolved nitrogen was less than 0.04 mg/l and N:P ratios were 5 to 1 or less (URS 1986a).

Experimental Design

Two types of experiments were conducted. The goal of experiment A was to see if rates of primary productivity, as measured by the uptake of radiocarbon isotope C-14, were enhanced in mid-Peale Passage near the net-pen site. Reference area stations were selected to be remote enough from the net-pens to avoid having waters that had passed through the net-pens on any single, moderate amplitude tide. Sampling was conducted during morning and early afternoon hours that coincided with the early and late portions of the flood tide, essentially low and high tide. The experiment was conducted before and after release of most of the delayed-release fish (experiments A-1 and A-2, respectively), about a month apart, during similar tidal exchange and timing. Both dates had relatively calm, warm weather and were during the peak algal growing season in Puget Sound (Winter et al. 1975, URS 1986a). Other direct measures of phytoplankton density (chlorophyll *a* and species cell counts for relative abundance) as well as indirect, surrogate measures (Secchi disk depth and dissolved oxygen concentration) were made as time allowed.

During sampling in late May 1988, the three sets of net-pens had a total of 118,600 kg of fish distributed 38% within system number 1, 45% in system number 2 and 17% in system number 3. Most of the fish in system one and two were released in early June. During the second sampling date in late June there was approximately 47,200 kg of fish in the net-pens, 55% within system number three and 45% in system two.

Experiment B was an assessment of water quality upstream and downstream of the net-pens, similar to nutrient monitoring described in the Interim Guidelines (SAIC 1986). The monitoring was conducted at low and high tide in late May, before release of the fish. The goal of experiment B was to monitor the near field effect of the net-pens on nutrient and ammonia concentrations. Table 1 summarizes the measurements and replication conducted for experiment A and B.

Worst-possible-case conditions were ensured by timing the experiments during a period with: the greatest amount of fish in the pens (experiment A-1) and with relatively small tidal

Table 1. Sampling parameters and number of replicates per depth.

	¹⁴ Carbon isotope -----	Chloro- phyll <u>a</u> -----	Dissolved nutrients -----	Dissolved oxygen -----	Secchi Disc -----	Temper.- Salinity -----	Phytop. counts -----	Total N & P -----
<u>Experiment A-1:</u> pens versus reference areas, May 25th, before fish release								
Low Tide	3	1	1	1	1	1	-	1
High Tide	3	1	1	1	1	1	1	1
<u>Experiment A-2:</u> pens versus reference areas, June 21st, after fish release								
Low Tide	3	3	3	1	1	1	1	1
High Tide	3	3	3	1	1	1	1	1
<u>Experiment B:</u> upstream and downstream of pens, May 25th								
mid-flood	-	-	3	1	1	-	-	3
early ebb	-	-	3	3	1	-	-	1

Codes: ¹⁴Carbon isotope = primary productivity assessment, phyto. counts = microscope identification and enumeration of phytoplankton, total N and P = total nitrogen and phosphorus (sum of dissolved and particulate) Total N and P from center of the net-pens only on ebb tide, experiment B. pH was sampled concurrent to collection of nutrient samples.

exchange. The mean tide for nearby Dofflemeyer Point is 10.4 feet, and the diurnal range is 14.4 feet. Sampling on May 25 and June 21st was conducted on the beginning and end of 6.3 and 4.9 foot flood tides, respectively.

Methods

Water velocity at the net-pen site was measured with a Scientific Instruments Price Meter, fitted with an Swoffer optical sensor and remote, digital readout unit. Surface drift sticks and 2 meter deep drogues were used to monitor current direction immediately downstream of the net-pens. Salinity and temperature were measured with a YSI SCT-33 meter carefully calibrated to standard seawater solution. All water samples were collected with a 2 liter Scott-Richards sampling bottle. Dissolved oxygen was measured by a modified Winkler titration method with an accuracy of 0.02 mg/l. pH was recorded in the field using a VWR model 55 probe. Chlorophyll *a* samples were collected by filtering 50 ml of water through Whatman GF/F filters. Filters were folded, packaged and iced for analysis the next day.

Nutrient samples were collected in acid washed and sample-water rinsed, polyethelene bottles, iced and frozen later the same day. No filtering or acidification was conducted to avoid introduction of broken cells and other artifacts, and to avoid destruction of nitrite (APHA 1985). This is standard research methodology used for dissolved nitrogen analysis of seawater samples at the University of Washington. Kjeldahl nitrogen, a measure of the concentration of combined organic nitrogen and ammonia, was not determined as it is a relatively useless measure in phytoplankton studies since it yields a combination of available and unavailable forms of nitrogen for cell growth. Separate determination of dissolved nitrogen ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4^+$) and total nitrogen was conducted as well as determination of orthophosphate and total phosphate at the University of Washington Routine Chemistry Laboratory using a technicon autoanalyzer and a modification of the acid digestion method (APHA 1985), respectively. Dissolved nutrient and oxygen concentration were normally reported in mg/l (ppm), for ease of comparison to Weston (1986). Total nitrogen and phosphorus were reported as mg at./l, for comparison to other results of other studies using ratios. Identification and enumeration of larger phytoplankton (greater than 5-10 microns) was performed by an experienced phycologist using sedimentation chambers to concentrate samples and an inverted microscope (Unesco 1978).

Relative rates of phytoplankton production were estimated using a modification of the carbon 14 productivity method (Steemann Nielsen 1952), using water from the same water bottle cast that provided chlorophyll *a*, nutrients and other measures mentioned above. Samples were collected, during the morning and afternoon, from 2 m at three sites: directly next to the net-pens and at the north and south entrances to Peale Passage (Fig. 2). Triplicate

120 ml samples were put into acid-cleaned 125 ml BOD bottles and transported in the dark to the net-pens for incubation.

On May 25, 1988, 1.1 ml of the ^{14}C stock (20 $\mu\text{Ci/ml}$ NaHCO_3 solution) was added to each sample, and then 100 μl was immediately removed and placed into a liquid scintillation cocktail containing phenethylamine for total activity determination. The BOD bottles were incubated next to the pens at 2 m depth within 20 minutes of sample water collection. Care was taken to insure that the incubating samples were never shaded by the pens. At the end of the incubation period (5.67 hrs for the morning samples and 2.67 for the afternoon samples), duplicate 20 ml aliquots were removed from each BOD bottle and placed into a glass scintillation vial containing 1.8 ml of a 37% formalin. Upon return to the laboratory, the formalin-killed aliquots were filtered onto glass fiber filters (Whatman GF/F), fumed over 12 N HCL for 15 seconds, and placed in scintillation vials. Seven ml of liquid scintillation solution was added to the vial, and shaken overnight. The samples were counted in a Beckman LS1800 liquid scintillation counter for 15 minutes. Carbon uptake was calculated according to Strickland and Parsons, (1968). Reported values are not corrected for time-zero controls or dark bottles.

On June 21, 1988, the same initial procedures were followed. Additionally, single aliquots for time zero controls were removed and placed into glass liquid scintillation vials containing 1 ml 6 N HCL. At the end of sample bottle incubation, duplicate 5 ml aliquots were removed from each BOD bottle and placed into glass scintillation vials containing 1 ml of 6 N HCL. Upon returning to the laboratory, 8 ml of liquid scintillation solution was added to the vials, and they were shaken overnight. The samples were counted in a Beckman LS1800 liquid scintillation counter for 15 minutes. Reported values were corrected for time zero controls but not for dark bottles.

Statistical analysis of hydrographic data utilized one way and two-way analysis of variance and T-test procedures (Zar 1984).

Results

Experiment A

On sampling dates before and after the fish were released all stations had weak thermal stratification with little difference between the salinity at the surface and bottom (Tables 2 and 3). The highest water temperatures usually occurred at the southern entry to Peale Passage, not at the mid-channel areas near the net-pens. No trends in dissolved oxygen concentrations were seen on either sampling date. Secchi disk values (water transparency) were slightly lower at the net-pen site on both sampling dates.

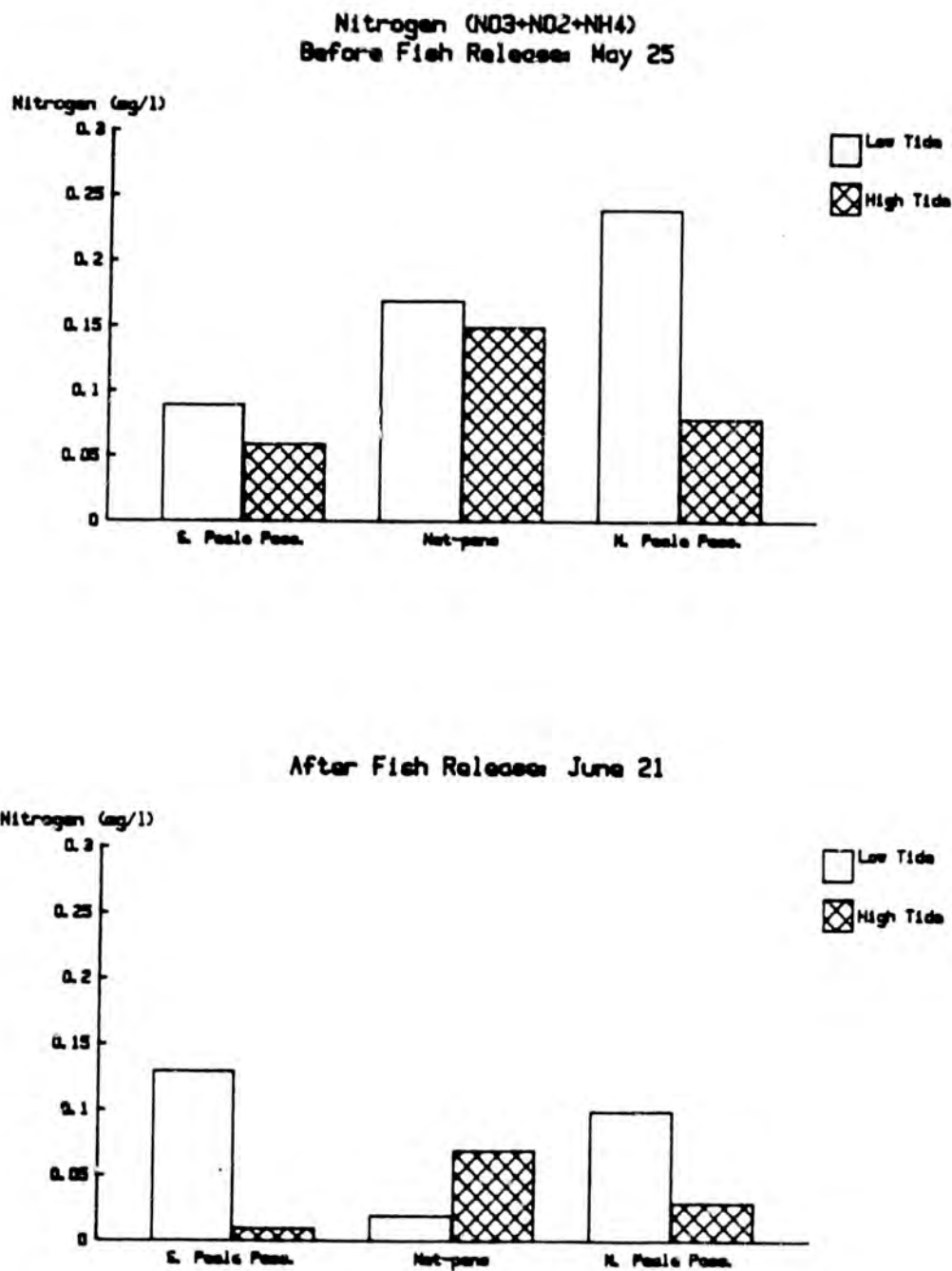


Figure 3. Dissolved nitrogen concentration before fish release (top, Fig. 3a) and after most of the fish were released (Fig. 3b). Standard deviation bars are omitted due to the very small amount of variance.

Table 2. Partial results of experiment A-1 from May 25, 1988.

Station	Depth (m)	Temp. (C)	Sal. PPT	Secchi Disk (m)	Dissolved Oxygen mg/l	Percent Oxygen Saturation	Chl. a ug/l	Dissolved Nitrogen* mg/l
<u>LOW TIDE</u>								
South	0	13.5	28.9	5.8				
Peale	2	13.5	29.0		10.8	124%	6.81	0.09
Passage	5	12.0	29.0					
	10	11.4	29.1					
Net-	0	13.1	29.0	3.6				
Pen	2	12.2	29.0		8.7	98%	23.91	0.17
Site	5	11.4	29.0					
North	0	12.1	28.9	4.0				
Peale	2	12.1	28.9		10.3	116%	6.52	0.24
Passage	5	11.9	29.1					
<u>HIGH TIDE</u>								
South	0	15.1	28.1	4.5				
Peale	2	13.5	28.4		13.7	158%	13.58	0.06
Passage	5	12.5	28.7					
	10	11.1	29.7		14.1	155%	15.25	0.70
Net-	0	13.1	29.0	3.5				
Pen	2	12.2	29.0		12.1	134%	15.56	0.15
Site	5	11.4	29.0					
North	0	13.6	29.0	3.9				
Peale	2	12.9	29.0		10.4	119%	9.76	0.08
Passage	5	12.1	29.0					

* Dissolved nitrogen values represent the total of nitrate, nitrite and ammonium. Nitrogen limitation for diatoms may occur below 0.04 mg/l.

Table 3. Partial results of experiment A-2 from June 21, 1988. Standard deviation shown in parenthesis.

Station	Depth (m)	Temp. (C)	Sal. (PPT)	Secchi Disk (m)	Dissolved Oxygen (mg/l)	Oxygen Saturation Percent	Chl. a (ug/l)	Dissolved Nitrogen*
<u>LOW TIDE</u>								
South	0	14.9	29.2	3.9				
Peale	2	13.8	29.1		9.6	116%	6.99	0.13
Passage	5	13.0	29.0				(0.082)	(0.019)
	10	12.6	29.0					
Net-	0	15.1	29.3	3.8				
Pen	2	14.9	29.0		12.0	143%	4.73	0.02
Site	5	14.1	28.8				(0.759)	(0.004)
North	0	15.0	29.0	3.4				
Peale	2	14.4	28.9		9.6	129%	6.84	0.10
Passage	5	13.8	28.7				(0.798)	(0.005)
<u>HIGH TIDE</u>								
South	0	15.3	29.0	3.7				
Peale	2	14.8	29.0		11.8	141%	3.95	0.01
Passage	5	13.5	29.0				(1.777)	(0.001)
	10	12.8	29.0					0.49 (---)
Net-	0	17.5	29.1	3.0				
Pen	2	15.0	28.8		12.1	144%	4.20	0.07
Site	5	14.1	29.0				(0.950)	(0.006)
	8	13.8	28.9					
North	0	16.4	28.5	3.5				
Peale	2	15.2	28.5		11.0	131%	5.47	0.03
Passage	5	14.0	28.7				(1.800)	(0.006)
	10	13.6	28.8					

* Dissolved nitrogen values represent the total of nitrate, nitrite and ammonium. Nitrogen limitation for diatoms may occur below 0.04 mg/l.

Table 4. Total nitrogen and phosphorus concentration, and T:N molar ratio. Mean and standard deviation of concentration when available from May 25, 1988 samples, expressed in ug-at/l.

<u>Site</u>	<u>Total N</u>	<u>Total P</u>	<u>N:P Ratio</u>
Experiment A-1: net-pen vicinity versus reference areas			
Low Tide			
south Peale	19.70	1.22	16.1
net-pen site	26.79	1.64	16.3
north Peale	31.06	1.91	16.3
High Tide			
south Peale	26.84	1.40	19.17
net-pen site	27.83	1.59	17.50
north Peale	26.83	1.58	16.98
Experiment B: upstream and downstream of pens			
Mid-flood			
upstream	24.95 (1.284)	1.50 (0.036)	16.63
downstream	30.83 (3.844)	1.87 (0.078)	16.49
Early ebb			
inside pens	47.35	1.93	24.53

Nutrients: Figure 3 shows dissolved nitrogen (defined as $\text{NO}_3 + \text{NO}_2 + \text{NH}_4^+$) at the three sampling stations on both sampling dates and tides. A gradient of increasing dissolved nitrogen from south to north was apparent at low tide. At high tide waters from central Peale Passage had moved toward the north Peale station to reduce dissolved nitrogen concentrations. Although dissolved nitrogen was relatively low at all stations on May 25th, it was not below the 0.04 mg/l threshold value for nitrogen limitation of diatoms (URS 1986a). Additionally, the total nitrogen to total phosphorus ratios (table 4) were not indicative of nitrogen limitation (Redfield 1958, Ryther and Dunstad 1971, McCarthy 1980, URS 1986a). Variation among replicates nitrogen samples was very small, thus statistical error bars were omitted in figure 3.

After release of most of the fish, dissolved nitrogen concentrations were lower at all stations than before the fish release, and not significantly different among stations ($p < 0.05$). Even on this later date, the dissolved nitrogen concentrations were not consistently lower than the growth limitation concentration. On both sampling dates the reference stations had lower dissolved nitrogen concentrations at low tide.

Chlorophyll a: The concentration of chlorophyll a was much greater at the net-pen station at low tide on May 25 but only slightly greater by high tide (Fig. 4a). Since replicates were not obtained, statistical significance is not known. However, the concentration at low tide near the net-pens was nearly four times greater than the reference areas and likely to be significantly greater. After most of the fish were released, on June 21 (Fig. 4b), there was significantly less chlorophyll a ($p < 0.05$) at the net pen site, compared to reference stations at low tide. Again, by high tide no differences were apparent as suggested by the overlapping standard deviation bars of that tide shown in figure 4b.

Primary Productivity: On May 25, primary productivity at the net-pen site was not significantly different at low tide versus high tide ($p < 0.05$). Compared to the reference stations, productivity was greater at low tide, but not at high tide (Fig. 5a). There were no statistical differences among stations on June 21, due to some of the replicate samples, possibly statistical outliers (see replicate values in appendix table A-2, pens). This data was not excluded from the analysis. Comparison of the trends of May 25 and June 21 data (Fig. 5a and 5b) suggests a similar trend with increased productivity during the morning low tide on both dates.

Due to methodological differences in processing the samples, the absolute values were considerably different on each sampling date, thus comparisons were not appropriate. Yet the

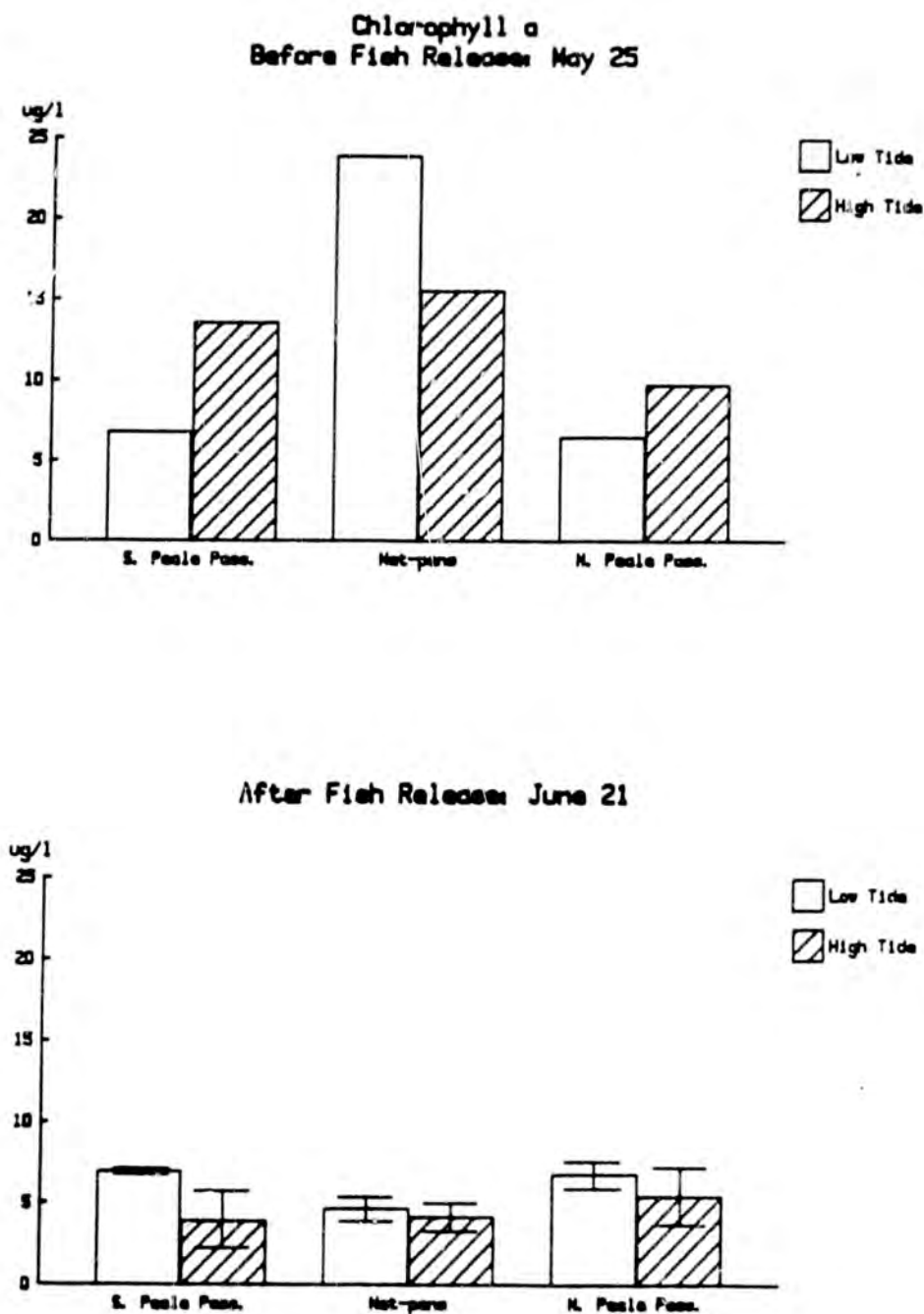


Figure 4. Chlorophyll a concentration before fish release (top, Fig. 4a) and after most of the fish were released (bottom, Fig. 4b). Standard deviation bars are shown where appropriate.

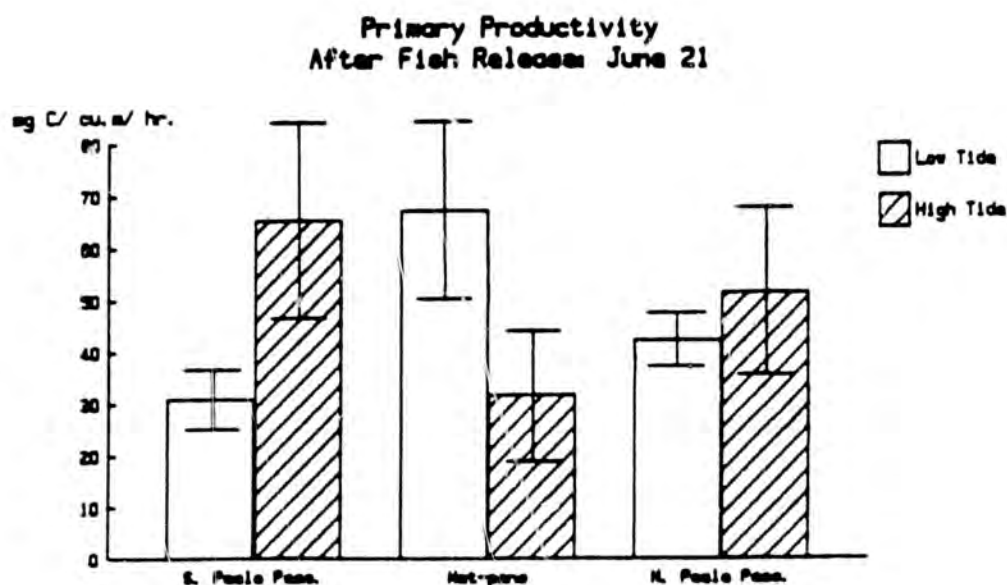
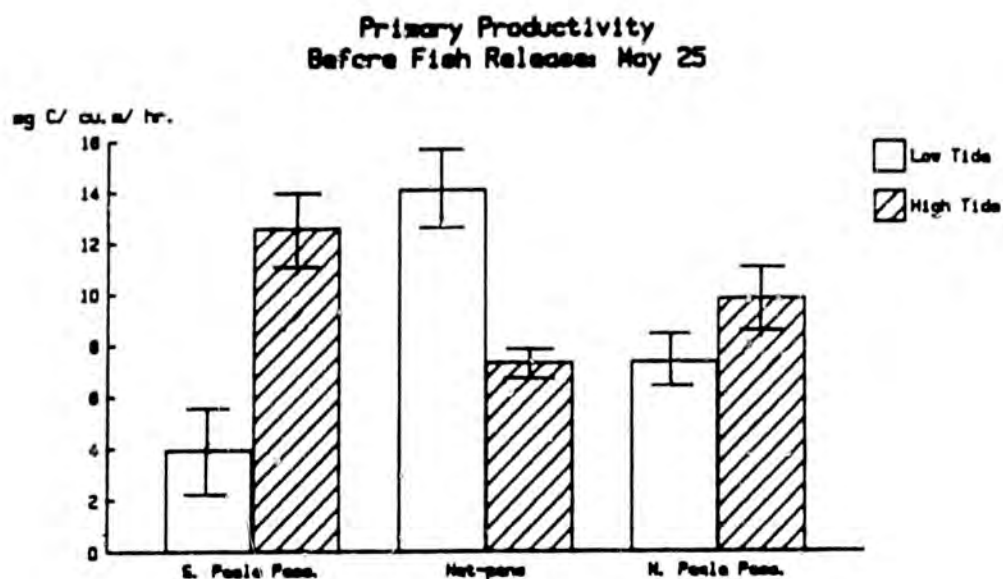


Figure 5. Primary productivity before fish release (top, Fig. 5a) and after most of the fish were released (bottom, Fig. 5b). Standard error bars are shown where appropriate. N = 6 for each station.

same relative trends on the two dates are apparent through inspection of figures 5a and 5b. Note that standard error bars were shown here, to save space, not standard deviation bars. Standard error is the standard deviation divided by the square root of the number of replicate measures. If standard deviation was shown on figure 5b, there would be greater overlap of bars.

Cell Counts: A summary of the species assemblage present during portions of the study is shown in table 5. On both sampling dates diatoms were by far the dominate phytoplankton. During the May sampling Coscinodiscus spp. and Thalassiosira spp., common chain forming species were prevalent. By late June Chaetoceros, also a very common diatom group, were most abundant. The Chaetoceros spp. present were members of the subgenus Hyalochaete, thought not to be directly responsible for fish kills in net-pens in the past as have been members of the subgenus Phaeoceros (Gaines and Taylor 1986). However, reports from Canada (Bell et al. 1974) and studies in Washington State (Rensel et al. in press), indicate that these species may contribute to mortality of net-pen salmon when abundant. There were not enough phytoplankton samples to make a rigorous comparison between tide stage or to other environmental factors. However, the slightly greater number of cells at the net-pens versus the reference station on May 25 appeared to correlate with nutrient and chlorophyll a values at high tide. The net-pen site had greater counts at high tide of June 21, which correlated with increased nutrient concentration, primary and specific productivity, but not with chlorophyll a concentration.

Table 5. Summary of cell counts from 2 m depth in cells per milliliter.

BEFORE FISH RELEASE: May 25, 1350-1420 hr.

	<u>S. Peale</u>	<u>Pen site</u>	<u>N. Peale</u>
<u>Chaetoceros Hyalochaete</u> spp.	51	0	not sampled
<u>Coscinodiscus onusta-lineata</u>	118	191	
<u>Thalassiosira</u> spp.	24	16	
total diatoms	231	278	
total dinoflagellates	22	131	
-----	----	----	
total phytoplankton (c/ml)	536	664	

AFTER FISH RELEASE: June 21, 1220-1310 hr.

<u>Chaetoceros Hyalochaete</u> spp.	536	1,639	740
<u>Skeletonema costatum</u>	6	25	80
Misc. pennate diatoms	2	59	22
total diatoms	645	1,825	952
total dinoflagellates	28	52	52
-----	----	----	----
total phytoplankton (c/ml)	806	2,069	1,109

Experiment B

During the morning flood tide, the concentration of dissolved nitrogen immediately upstream of the net-pens averaged 0.07 mg/l (table 6). The dissolved nitrogen (DN) was composed of nitrate (78%) and ammonia (22%). Just downstream of the net-pens, the concentration of dissolved nitrogen had doubled to 0.15 mg/l, which was significantly greater than upstream ($p < 0.005$). However, most of the dissolved nitrogen was in the form of nitrate ($\text{NO}_3^- = 86\%$ of DN), not ammonia ($\text{NH}_4^+ = 12\%$ of DN). A similar picture emerges if the data is viewed on a gram atom weight basis; the downstream nitrate was about 2.5 times greater than the ambient concentration. There was no statistical difference in the concentration of ammonia during this tide (on either unit basis) from upstream to downstream, but nitrate was significantly greater downstream ($p < 0.001$).

Total ammonia concentration in the plume of the net-pen during the morning flood averaged 0.0163 mg/l which is equivalent to an un-ionized ammonia concentration of 0.00037 mg/l at pH 8 and temperature 13 C. The EPA (1986) chronic exposure criteria (four day average concentration) for un-ionized ammonia under these conditions is 0.030 mg/l, about 80 times greater than the concentration observed downstream from the net-pens.

During the early portions of the ebb tide, the concentration of total dissolved nitrogen was significantly greater within the net-pens compared to upstream values ($p < 0.001$ for NH_4^+ and NO_3^- , $p < 0.005$ for NO_2^-). However, there was no significant difference in the upstream and downstream values of total dissolved nitrogen. Within that total, there was a significant increase in the concentration of ammonia (table 7). The proportion of toxic, un-ionized ammonia in the downstream area was about 90 times less than the EPA's (1986) chronic exposure criteria.

The concentration of un-ionized ammonia within the center of the net-pens during the ebb tide was 23 times greater than the upstream, ambient concentration. However, this amounted to only about 10% of the chronic exposure criteria mentioned above. The within-pen results were from the widest set of net-pens (three pens abreast), under maximum loading, and minimal current conditions. Ammonia loading within other net-pen systems at Squaxin Island was probably less due to the two abreast configuration which allows greater dilution with surrounding waters.

Table 6. Results of experiment B from May 25, 1988. All data collected from 2 meters depth where the water temperature was 12.2 C °. pH remained at 8.0 throughout this sample collection.

Tidal Stage & station	Dissolved Oxygen (mg/l)	Oxygen Saturation (%)	Dissolved Nitrogen** (Mean & SD) (mg/l)	Total Ammonia (mg/l)	Un-ionized Ammonia (mg/l)	Current Velocity (cm sec ⁻¹)
MID-FLOOD: 1200-1300 hours						
upstream:	11.7	132%	0.07 (0.020)	0.0155	0.00036	11.4
downstream	11.6	131%	0.15 (0.011)	0.0163	0.00037	5.2
EARLY EBB: 1500-1625 hours						
upstream:	12.2	137%	0.03 (0.006)	0.0069	0.00014	8.1
within pens***	10.4	117%	0.23 (0.006)	0.1606	0.00337	3.0
downstream	12.6	142%	0.03 (0.015)	0.0155	0.00033	7.9

* Total net-pen salmon biomass was 118,600 kg for the flood and 97,970 kg for the ebb, due to differing sampling location. Ebb tide samples were collected downstream (south) of the larger WDF pens (number 2).

** Dissolved nitrogen values represent the total of nitrate, nitrite and ammonium. Nitrogen limitation for diatoms may occur below 0.04 mg/l.

*** within pens site was in center of the pen system (number 2, WDF) that were 3 cages wide by 11 cages long with 52,640 kg of fish.

Table 7. Ammonia concentrations at the Squaxin Island net-pens during the upstream/downstream analyses (experiment B). Distance to downstream sampling station was 30 m from facility number 3. Percent change in concentration is relative to ambient, upstream concentration. Chronic exposure concentration is the four day average recommended by EPA (1986).

Tidal Stage	Influencing Fish Biomass (Kg)	Total Ammonia Concentration (mg/l) with standard deviation in parenthesis		
		Upstream	Within Pens	Downstream
Flood tide	118,600 kg	0.0155 (0.00686)	----	0.0163 (0.00202)
percent increase of total ammonia		-->		5%
percent increase of un-ionized ammonia		-->		2.3%
percent of chronic toxicity concentration		->		1.2%

Ebb tide	97,970 kg	0.0069 (0.00038)	0.1606 (0.00337)	0.0155 (0.00262)
percent increase of total ammonia		-->	2,337%	224%
percent increase of un-ionized ammonia		-->	2.1%	2.1%
percent of chronic exposure concentration		->	11.2%	1.1%

Discussion

Experiment A: Effects on Phytoplankton

Phytoplankton abundance and the growth rate at the net-pens varied significantly with the stage of the tidal cycle or the time of day. The most striking feature of this analysis was the very similar pattern of primary productivity seen before and after fish were release from the pens (Fig. 5). There was a general trend toward increased phytoplankton density (chlorophyll a) and C-14 productivity at the net-pens during the morning ebb tide, with no significant difference during the afternoon flood tide, regardless of the sampling date and amount of fish held in the net-pens (compare figs. 5a and 5b).

Total nitrogen to phosphorus ratios (table 4) indicated that growth was not limited due to nutrient depletion during the period before fish release, when greater phytoplankton density and significantly greater primary productivity was observed at the net-pens. Thus, the observed increased chlorophyll a during one tidal period of maximum fish biomass was not the result of

nutrient production from the net-pens, but may be related to the natural hydrodynamics of Peale Passage.

The reference areas used in experiment A were selected to be within the same passage, but as remote as possible from the net-pens. These areas were near the source waters of Pickering and Dana Passages, which are both subject to fairly intense horizontal and vertical mixing (URS 1986b) compared to the shallow Peale Passage area. It is likely that vertical mixing, and accordingly, light limitation for phytoplankton cells in both of the source areas is much greater than in Peale Passage. By the time source waters bearing nutrients and phytoplankton seed stock reach the middle portions of Peale Passage, a day or longer has passed, allowing for significant growth to occur in the water column, which is entirely within the euphotic zone.

Although similar trends in C-14 primary productivity were apparent on both sampling dates, there was no statistical difference among sites on the later sampling date, due to one possible outlier, a replicate sample that was not discarded. Further, the absolute values were different between sampling dates due to methodological differences and thus no comparison of primary production rates is possible between sampling dates.

Dissolved nutrient concentrations appeared to be roughly correlated with chlorophyll *a* values and were lower at all stations after most of the salmon were released. As discussed previously, Pease (1977) found an inverse relationship between the two throughout his study of Henderson Inlet. No causal relationship between these factors at the Squaxin Island net-pens is likely, due to the remoteness of the unperturbed reference areas, the relatively small amount of dissolved nitrogen contributed by the net-pens (theoretically about 33 kg/day total dissolved nitrogen) and results of nutrient sampling over the tidal cycle discussed below.

Some of the dissolved nitrogen concentrations during this study were below the threshold of limitation for diatom growth (0.04 mg/l; URS 1986). To show maximum effects this study should have been conducted when surface waters were nearly depleted of all forms of nitrogen (dissolved and total), if it ever occurs, to test the possibility that the pens could cause or sustain a phytoplankton bloom. These results and pre-experiment nutrient sampling indicated that dissolved nitrogen values at the pens fluctuated regularly, above and below the 0.04 mg/l growth limitation threshold. This value obtained for nutrient limitation of diatoms is not absolute and should be used with caution because growth limitation also depends upon the concentration of phytoplankton cells and organic matter in the water, rates of remineralization of the organic matter to dissolved inorganic nitrogen (Harris 1986), and the type and size of phytoplankton (Redfield 1958, Ryther and Dunstad 1971, Eppley 1972, McCarthy 1980, URS 1986a).