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School of Fisheries, WH-10
University of Washington
Seattle, WA 98195

COMPARISON OF CARBARYL PESTICIDE IMPACTS ON DUNGENESS CRAB (*CANCER MAGISTER*) VERSUS BENEFITS OF HABITAT DERIVED FROM OYSTER CULTURE IN WILLAPA BAY, WASHINGTON

Daniel C. Doty, David A. Armstrong and Brett R. Dumbauld

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1.0 INTRODUCTION

The Dungeness crab, *Cancer magister*, supports one of the most economically important crustacean fisheries in the state of Washington. Although the majority of Dungeness crab are harvested in the coastal nearshore waters of the state, a growing body of evidence strongly suggests that two large coastal estuaries in southwestern Washington, Willapa Bay and Grays Harbor, serve as important nursery areas for juvenile stages of crab (Stevens and Armstrong 1984; Armstrong and Gunderson 1985; Armstrong et al. 1986; Gunderson et al. 1989). Extensive sampling of Willapa Bay, Grays Harbor, and adjacent coastal nearshore habitats has revealed that large numbers of young-of-the-year (0+) and year-old (1+) juveniles make extensive use of the estuaries. Such habitat is viewed as important to small juvenile crab both for reasons of warmer temperature and faster growth (Armstrong and Gunderson 1985; Gutermuth and Armstrong 1989) and probably also greater food supply and production (Gunderson et al. 1989).

In recent years, attention has focused on projects and activities that can deleteriously affect estuarine juvenile crab populations through loss of habitat or direct mortality. Notable among several controversial issues concerning crab is the direct application of a carbamate insecticide, carbaryl, onto estuarine tideflats by the oyster industry to control two species of infaunal shrimp that reach high densities on prime oyster grounds. While the introduction of any chemical in estuarine waters raises questions regarding effects on non-target species and on the ecosystem as a whole, one central issue in the Willapa Bay and Grays Harbor estuaries is that concerning impacts of this pesticide on juvenile crab and perhaps, in turn, the coastal fishery.

1.1 Problem and Background

Commercially cultured oyster (*Crassostrea gigas*) are Washington State's most economically important molluscan resource. Over 50% of the state's annual oyster harvest is produced in the Willapa Bay and Grays Harbor estuaries along the southwest coast of state (Cheney et al. 1986) where juvenile crab are abundant. The vast majority of oyster are cultured directly on substrate in the intertidal region at depths ranging from +1.1 m (3.5 ft) above to -0.5 m (-1.5 ft) below MLLW. Efficient ground culture of oyster requires that the substrate be sufficiently firm to support growing animals (Washington Department of Fisheries 1970).

Two species of burrowing shrimp that adversely affect ground culture of oyster are the ghost shrimp (*Callianassa californiensis*) and the mud shrimp (*Upogebia pugettensis*), which are indigenous to Pacific northwest estuaries and which occupy the same intertidal zone as oyster. Burrowing and feeding activities of these shrimp can smother oysters by covering them with sediment, or soften the substrate and cause them to sink (Chambers 1970; Buchanan et al. 1985). Within the two estuaries, populations of these two shrimp have increased dramatically since the late 1950s, threatening some of the industry's prime oyster grounds (Washington Departments of Fisheries (WDF) and Washington Department of Ecology (WDOE) 1985). The oyster growers contend that periodic eradication of shrimp from grounds being prepared for culture is necessary in order to maintain oyster production at current levels (Buchanan et al. 1985).

A widely used carbamate pesticide, carbaryl (1-napthyl N-methylcarbamate sold under the tradename SEVIN), has been used to control burrowing shrimp populations on oyster grounds in Willapa Bay and Grays Harbor since 1963 (WDF and WDOE 1985). The pesticide is typically applied by either aerial or hand spray onto exposed intertidal oyster beds during minus tides in July and August. The annual application of carbaryl to the tideflats is regulated by the Washington Departments of Ecology, Fisheries, and Agriculture (WDA), and by the U.S. Environmental Protection Agency (EPA). Currently, the application rates of the pesticide are limited to either 5.6 or 8.4 kg/ha (5 or 7.5 lb/acre), and a maximum of 240 ha (600 acres) and 80 ha (200 acres) of tidelands can be treated per year in Willapa Bay and Grays Harbor, respectively (Creekman and Hurlburt 1987).

Of the various pesticides initially tested by WDF for controlling populations of burrowing shrimp, carbaryl was selected because it was highly effective on the targeted shrimp species, had the least effect on other associated organisms (WDF 1970), and because of its low relative toxicity to warm-blooded animals (Chambers 1970). In a review of the toxic effects of the pesticide, Buchanan et al. (1985) found that marine crustaceans are from 10 to 300 times more sensitive to the insecticide than fishes and molluscs. Studies of species mortality following field application of carbaryl have revealed, however, that non-target species of fishes and invertebrates are killed on site as well as intended ghost and mud shrimp.

Among non-target species killed, Dungeness crab are of particular significance because of their susceptibility to the pesticide (Buchanan et al. 1970), and because of extensive use of the estuaries during juvenile stages (Stevens and Armstrong 1984; Armstrong and Gunderson 1985; Armstrong et al. 1986). Reports of dead crab on sites after treatment led WDF to conclude that the timing and location of carbaryl application within the bay should be carefully regulated in order to avoid damage to crab (WDF 1970). The agency began to conduct systematic visual assessments of crab kill in 1976 and, during 1984, concern over large numbers of crab killed by carbaryl application prompted the agency to adopt a quota system which limits the number of crab killed (Hurlburt 1986; Creekman and Hurlburt 1987). The quota is presently set at 75 crab/ha (30 crab/acre) or 18,000 2-year-old (2+) sublegal adult equivalents where young-of-the-year (0+) crab are 10:1 and 1+ are 3:1. Active assessment of mortality during phases of treatment may lead to termination of further spray short of the 240 ha allotment if the crab quota is exceeded.

The most obvious and extensive loss of crab has been documented on intertidal sites which are directly treated with the pesticide (WDF and WDOE 1985). Intertidal regions have been identified as important for newly settled 0+ crab where epibenthic material presumably provides refuge from predation by fish and conspecifics (Stevens and Armstrong 1984; Armstrong and Gunderson 1985; Dumbauld and Armstrong 1987). The dominant forms of epibenthic refuge in the intertidal regions of Willapa Bay and Grays Harbor are eelgrass (*Zostera marina*) and shell, which includes deposits of the eastern softshell clam (*Mya arenaria*) and beds of commercially cultured oyster.

While the majority of crab kill has been documented on treated sites, potential direct and indirect off-site impacts have been identified. Carbaryl is transported off sites and onto adjacent areas during the first incoming flood tide after spray (Creekman and Hurlburt 1987), directly exposing crab for unknown distances beyond original treatment areas. Indirect exposure can occur if foraging crab ingest contaminated organisms transported off treated areas. Large numbers of mud shrimp (*U. pugettensis*) emerge from their burrows in response to the chemical and become potential prey for foraging crab. Crab die after eating contaminated clams (Buchanan et al. 1970) and there is concern that crab feeding on contaminated mud shrimp may be similarly affected (Creekman and Hurlburt 1987).

WDF has acknowledged that the visual assessment method currently being used is inadequate for estimating the number of 0+ crab actually killed, because of difficulty in counting small crab associated with epibenthic cover on the sites (Creekman and Hurlburt 1987). This problem, combined with uncertainty regarding the magnitude and extent of off-site impacts to crab, has raised concern among the agencies and crab fishermen that present assessment methods underestimate the actual incidental mortality of Dungeness crab associated with carbaryl treatment and, consequently, impacts to the fishery. The purpose of the present study was to address some of these questions regarding on- and off-site impacts of carbaryl to crab.

1.2 OBJECTIVES

During the period from 1986 through 1988, research sponsored by WDOE, the Washington State Conservation Commission, and the Willapa Bay/Grays Harbor Oyster Growers Association was conducted to monitor the effects of carbaryl on the Dungeness crab population in Willapa Bay, where the majority of the insecticide is sprayed. A major objective of this study was to estimate the magnitude and extent of crab mortality on and around the intertidal oyster grounds treated with the chemical. Intertidal quadrat sampling was conducted to determine the period of crab recruitment, estimate crab abundance, and determine direct impacts on crab recruited to oyster beds treated with the chemical. A series of crab feeding experiments, trawls, and cage experiments were conducted to document carbaryl-related mortality beyond the boundaries of treated areas.

Following carbaryl treatment, beds are used for several types of oyster culture ranging from seed beds to grow-out or fattening grounds. Results from studies in Grays Harbor suggest that shell generally supports higher long-term densities of 0+ crab than does eelgrass (Armstrong and Gunderson 1985; Dumbauld and Armstrong 1987). This has led to the hypothesis that short term impacts to crab populations caused by carbaryl may be offset by an increase in the amount of oyster shell cover on the beds, and a resultant increase in potential habitat for juvenile crab in the years subsequent to treatment (Buchanan et al. 1985). Another objective of the present study was to examine this hypothesis by sampling several sites over a 2- to 3-year period, monitoring both crab density in the various types of refuge and the relative changes in epibenthic refuge on the sites.

Specific Objectives were as follows:

1. Define the intertidal ecology of the 0+ crab with regards to timing of settlement, habitat use, and seasonal density patterns at several sites in Willapa Bay.
   a) Contrast the mean density of 0+ crab on three intertidal substrata (oyster shell, eelgrass, and open mud/sand) in order to compare the relative importance of commercial oyster shell as a refuge for 0+ crab.
   b) Determine the relative cover of each substrate type on sites sampled.
2. Estimate the magnitude of on-site 0+ crab mortality due to the application of carbaryl on selected treatment sites.
3. Examine carbaryl-related crab mortality caused by off-site transport of the pesticide by tidal currents, and by feeding on organisms exposed to the chemical.
   a) Estimate the spatial extent of carbaryl-induced mortality of 0+ crab due to transport of carbaryl onto adjacent intertidal areas.
   b) Determine off-site mortality to caged 1+ crab held in subtidal channels adjacent to selected treatment sites.
   c) Compare mortality of caged 1+ crab fed contaminated shrimp from a treated site to crab fed shrimp from a control site.
4. Estimate the net loss/gain of 0+ crab associated with increased habitat provided by commercial oyster on selected treatment sites.
   a) Monitor recruitment and use of sites by 0+ crab for 1-2 years after treatment.
   b) Determine changes in relative habitat cover on sites for 1-2 years after treatment.

2.0 METHODS AND MATERIALS

2.1 DESCRIPTION AND LOCATION OF SAMPLING SITES

2.1.1 Willapa Bay

All sampling was conducted in Willapa Bay, a shallow estuary located along the southwest coast of Washington (Fig. 1). The majority of the state's oyster production (Cheney et al. 1986) and most annual application of carbaryl (up to 240 ha/year) occurs in this estuary. Over 50% (or approximately 17,200 ha) of the total area of the bay is exposed at low tide (Hedgepeth and Obrebski 1981), and of this area about 10,500 ha (60%) of the intertidal region is privately owned and classified as oyster land (Shotwell 1977; Cheney et al. 1986), although not all classified land is used for culture. Presently, about 2,480 ha (6,200 acres) of the grounds are of moderate to high quality for oyster production and most of this area is being actively cultured (Cheney et al. 1986; WDF and WDOE 1989).

2.1.2 Locations of Treatment and Reference Sites

Intertidal 0+ crab were sampled at a total of 10 different sites from 3 general regions of the bay: Stony Point, Palix River, and northern Long Island (Figs. 2, 3, and 4; respectively), from 1986 to 1988. The sample sites were oyster beds ranging in area from 4.8 to 26.7 ha (12 to 66 acres) at tidal elevations of -0.3 (-1.0 ft) to +0.3 m (+1.0 ft) MLLW. Sites differed in the degree and type of epibenthic refuge material present, ranging from a predominantly open sand flat dominated by *Callianassa californiensis*, to areas covered with combinations of heavy shell and eelgrass.

Sites were selected to serve as either "treatment" or "reference" sites. The term "treatment site" refers to an oyster bed which was sprayed with carbaryl during a given year and "reference site" to a nearby oyster bed which was not treated during that same year. Treatment sites were periodically sampled to monitor the timing and magnitude of crab settlement, survival, and relative use of the various habitats, and to estimate the number of crab killed after the commercial application of carbaryl. Corresponding untreated reference sites were selected near treatment areas to monitor natural population fluctuations of crab throughout the season. A site treated one year could serve as a reference site during subsequent years under the assumption that there would be no long-term residual toxic effects of carbaryl to settling 0+ crab.

Frequency of sampling at each site was dependent both upon logistical considerations and the particular objectives being addressed. In addition to periodic intertidal sampling for 0+ crab at most sites, experiments were conducted at selected sites to investigate the off-site impacts of carbaryl. Brief descriptions of sites and the type of sampling conducted during this 3-year study are presented below. More specific site descriptions are summarized in Table 1.

<u>1986</u>: During 1986, two sites were sampled, beginning in June and extending through September. Site SP86 (total area = 13 ha) was located near Stony Point (Figs. 1 and 2) and station PR86 (total area = 26.7 ha) was located along the Palix River (Figs. 1 and 3). Logistical constraints prevented inclusion of reference sites during 1986.

Cage experiments and trawls were conducted in subtidal channels adjacent to these two sites during the time of treatment in July to examine off-site impacts (refer to section 2.4.2 for details).

<u>1987</u>: Intertidal sampling was conducted at six sites in 1987 from May through September. Three of the six were selected to serve as reference sites and three were commercially treated with carbaryl during July 1987. Two sites, one reference and one treatment site, were sampled in each of the three general regions. Sites designated for treatment were selected near Stony Point (Fig. 2;

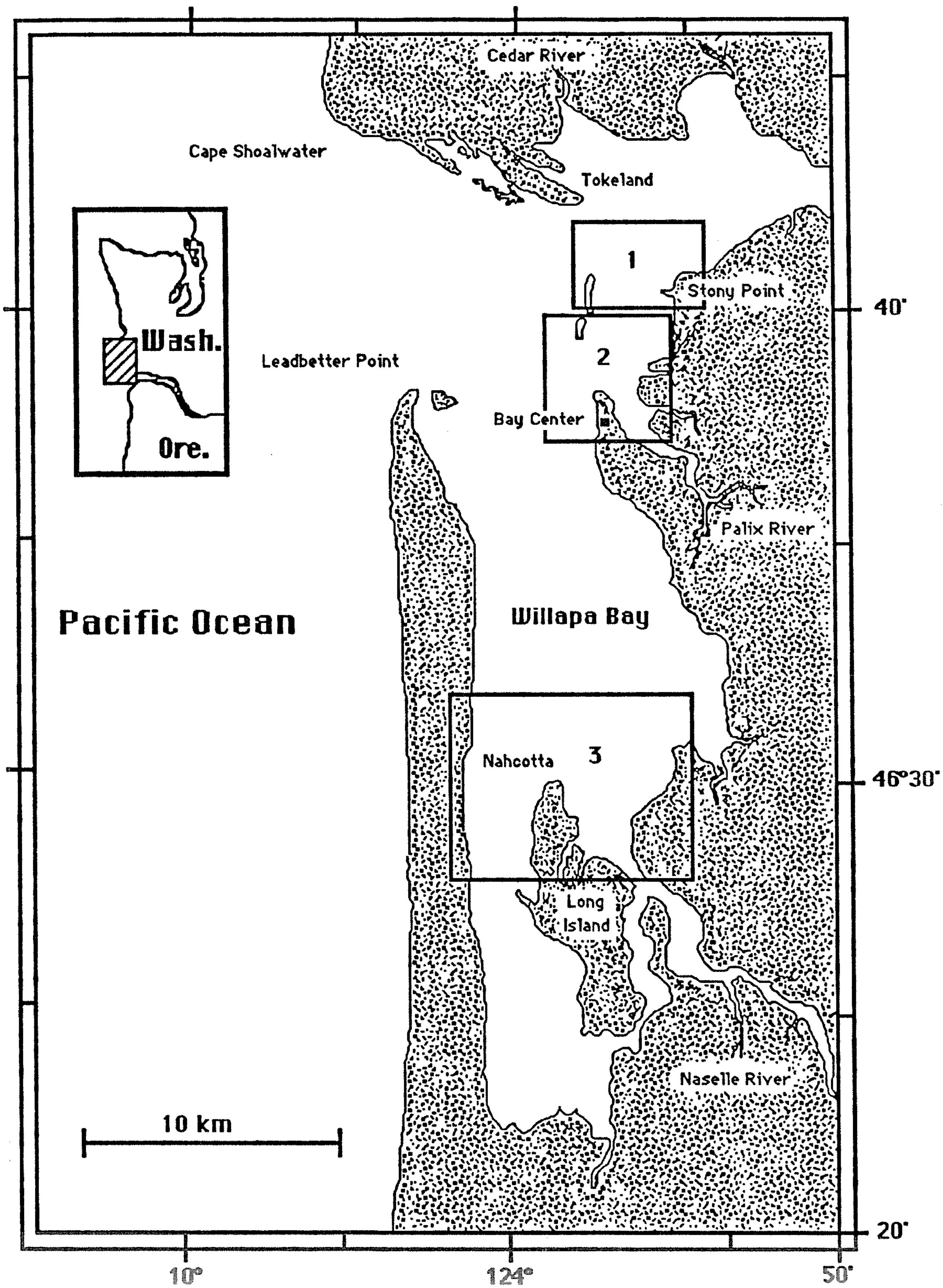

Figure 1. Map of Willapa Bay showing three general regions—(1) Stony Point, (2) Palix River, and (3) Long Island—sampled during 1986-1988. Inset shows location of Willapa Bay along the southwestern coast of Washington. See Figures 2-4 for locations of specific study sites within each region and Table 1 for site descriptions.

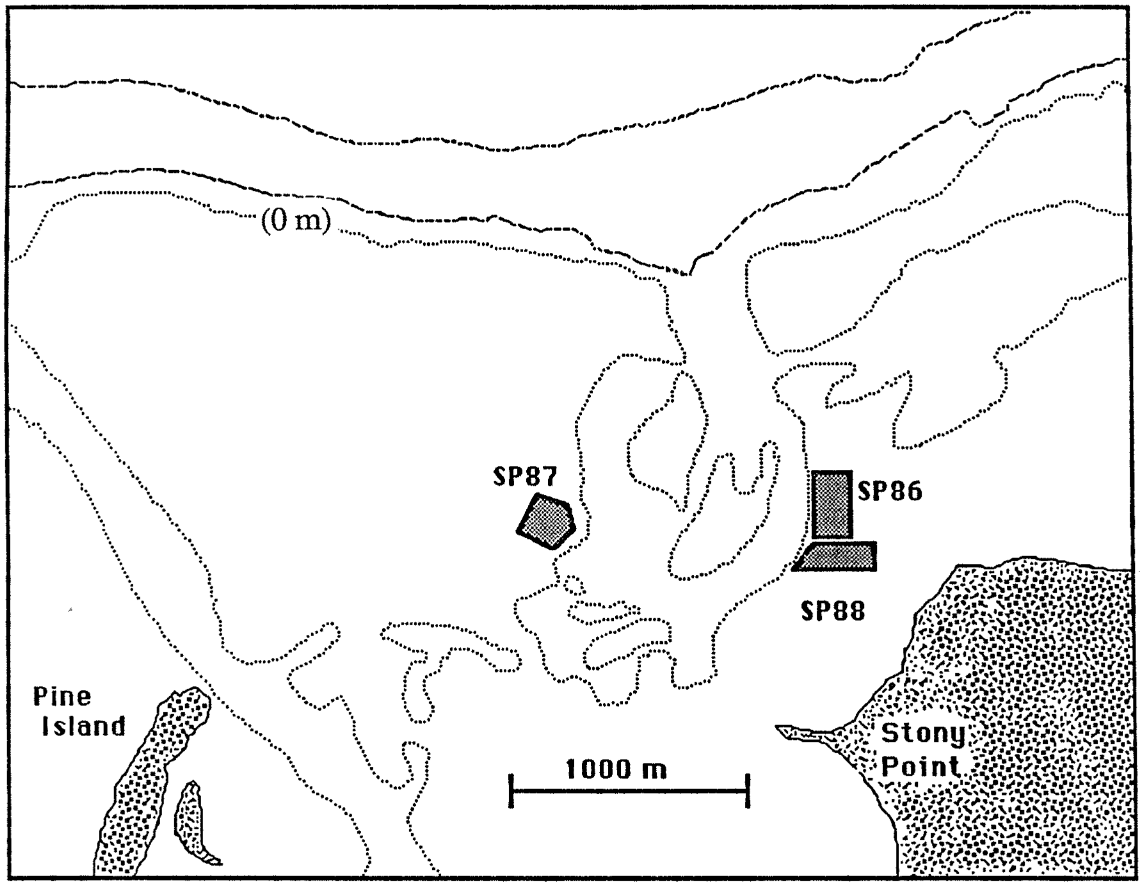

Figure 2. Locations of the three study sites at Stony Point region showing SP86, SP87, and SP88, which were sampled 1986-1988. See Figure 1 for general location within the bay and Table 1 for site descriptions.

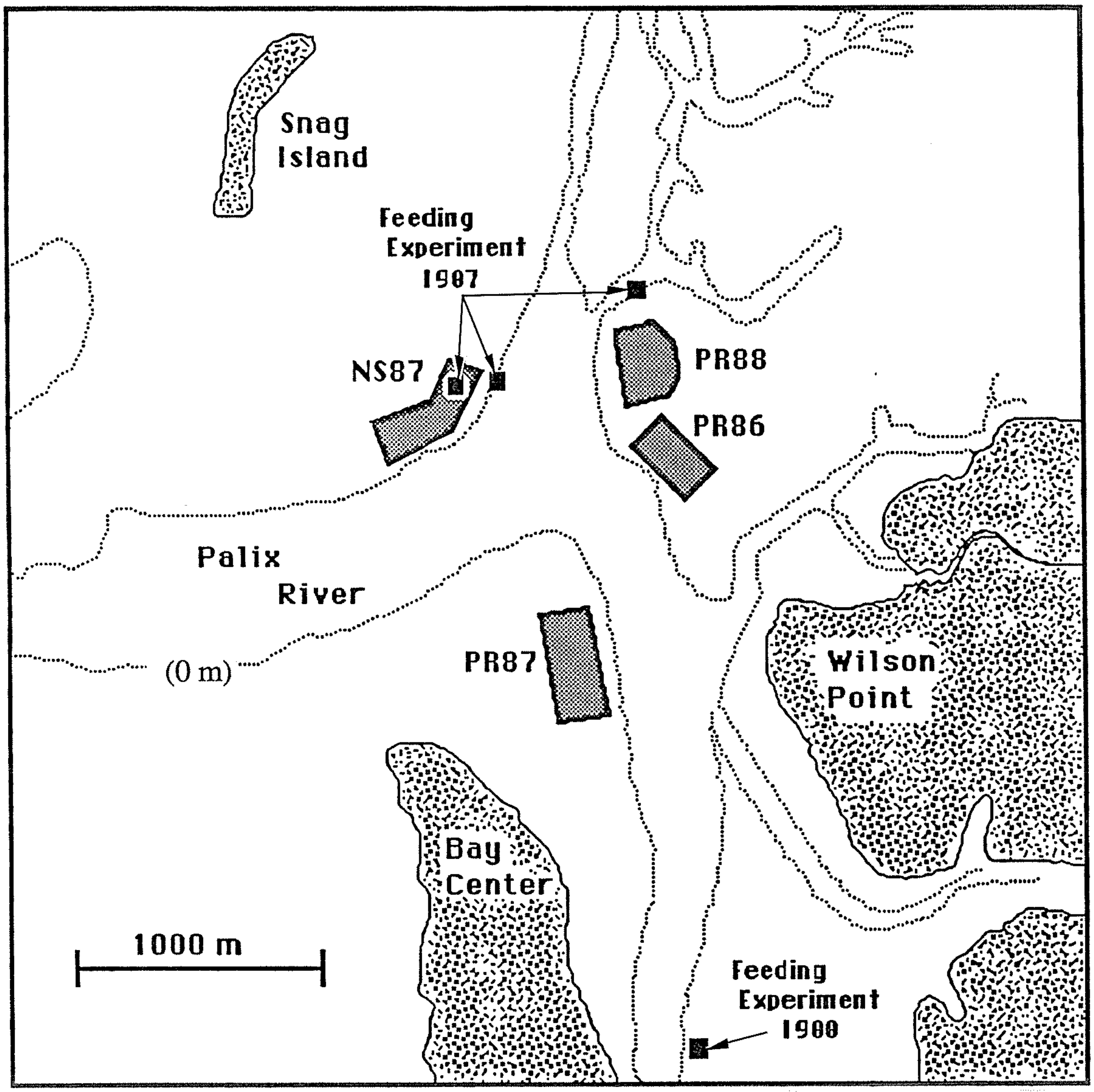

Figure 3. Location of four study sites sampled during 1986 through 1988 in the Palix River region. Also shown are the general locations of feeding experiments conducted during 1987-1988. See Figure 1 for the general location within the bay and Table 1 for specific site descriptions.

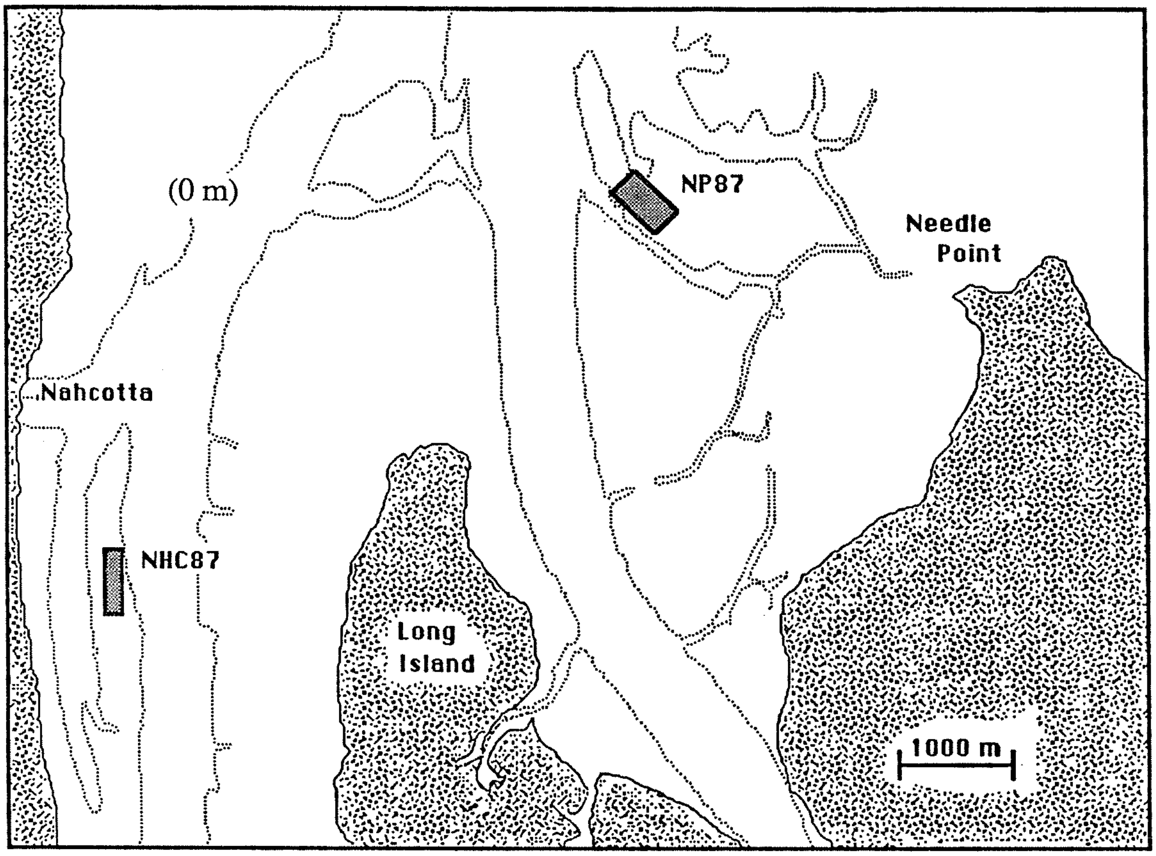

Figure 4. Map of northern Long Island region showing locations of study sites NP87 and NHC sampled during 1987-1988. See Figure 1 for the general location within the bay and Table 1 for specific site descriptions.

Table 1. Location and general description of sampling locations, bottom type at the time of treatment, and types of sampling conducted in Willapa Bay in the Stony Point (Fig. 2), Palix River (Fig. 3), and Long Island (Fig. 4) regions during 1986-1988.

Region Site	Area treated ha (acres)	Elevation m (ft) MLLW	Year treated	Initial bottom type	Typical culture method/Use of site[b]	Sampling method: Year(s)[a]
Stony Point						
SP86	13 (33)	+0.15 (+0.5)	1986	Mud Seed oyster	Growout Seed-harvest	IT: 1986-88 Cages: 1986 Trawls: 1986
SP87	8.1 (20)	-0.15 (-0.5)	1987	Mud Seed oyster Sparse eelgrass	Growout Seed-harvest	IT: 1987-88
SP88	4.8 (12)	0.0 (0.0)	1988	Mud Seed oyster Sparse eelgrass	Growout Seed-harvest	IT: 1988
Palix River						
PR86	26.7 (66)	-0.15 (-0.5)	1986	Mud Sparse shell Heavy eelgrass	Fattening 2-yr-harvest	IT: 1986-87 Cages: 1986 Trawls: 1986
PR87	8.1 (20)	+0.3 (+1.0)	1987	Sand Sparse eelgrass	Clam culture	IT: 1987
PR88	4.8 (12)	-0.15 (-0.5)	1988	Mud Heavy eelgrass Sparse shell	Growout Seed-harvest	IT: 1988 Bags: 1988
NS87	8.1 (20)	0.0 (0.0)	1987	Sand Moderate eelgrass	Longline oyster	FE: 1987
Long Island						
NP87	8.1 (20)	0.0 (0.0)	1987	Mud Heavy shell/seed Sparse eelgrass	Seed Seed - +2 yrs	IT: 1987-88
NHC[c]	8.1 (20)	+0.3 (+1.0)	Not Treated	Mud Seed oyster	Seed Seed - +2 yrs	IT: 1987-88

[a]Oyster culture methods include the following: (1) Growout = oyster seed is planted and remains on-site until harvest; (2) Seed = seed is planted and remains on-site until 2+ years, at which time oyster is transplanted; (3) Fattening ground where oyster remains for 1-2 years until harvest.
[b]IT = intertidal quadrat samples; Cages = 1+ crab held in cages subtidally; FE = feeding experiments (1+ crab); Bags = intertidal carbaryl transport experiment (0+ crab).
[c]NHC was not treated during the 1986-1988 study period.

SP87), Palix River (Fig. 3; PR87), and Needle Point (Fig. 4; NP87). The total area treated at each site was 8.1 ha (20 acres) and the sites were sprayed during July 1987. An additional site near the Palix River (Fig. 3; NS87) was used for a caging experiment to test effects of contaminated shrimp fed to crab (Section 2.4.2).

Two sites treated the previous year, SP86 (Fig. 2) and PR86 (Fig. 3), were selected to (1) serve as reference sites, and (2) determine changes in the extent and type of epibenthic cover on the sites resulting from the addition and/or growth of oysters. A third reference site was selected near Nahcotta (NHC; Fig. 4) to monitor crab recruitment in the southern portion of the estuary. NHC was not treated during the 3-year study.

<u>1988</u>: Two additional treatment sites were sampled in 1988 at Stony Point (SP88; Fig. 2) and Palix River (PR88; Fig. 3). Site PR88 was used primarily for an experiment to monitor off-site transport of carbaryl and its effects on 0+ crab held in bags on adjacent intertidal areas during the treatment period of June 27 to July 3, 1988 (Section 2.4.2). Intertidal samples were collected monthly at Site SP88 through August 1988 to monitor crab density and estimate the number of crab killed by the July 30, 1988 carbaryl treatment in that area.

Site SP86, which was immediately adjacent to SP88 (Fig. 2), served as a reference site and was sampled monthly throughout 1988. Stations SP87, PR86, NHC, NP87, were sampled intermittently during this period.

2.2 INTERTIDAL SAMPLING METHODOLOGY

Intertidal 0+ crab were collected along transects during periods of low tide. A 0.25 m^2 quadrat was used as the standard sample unit. After placement of the quadrat frame over the sample area, the relative cover of epibenthic material (shell and eelgrass) within the frame was visually estimated and recorded. The sample area was then excavated to a depth of 3-5 cm. All of the contents were placed into a 3 mm mesh dipnet or screen, rinsed, and remaining material was sorted. All crab found were identified to species and carapace width (CW) measured to the nearest 0.1 mm from points directly anterior to the 10th anterolateral spine. Sex was determined for all specimens greater than 20 mm CW.

2.2.1 <u>Habitat Categories and Percent Cover</u>

In order to minimize potential errors resulting from the visual estimation method, and for purposes of comparison and analysis, samples were grouped into five broad habitat categories (Table 2 and Fig. 5). All samples which had less than 10% of either shell or eelgrass cover were classified within the "open mud/sand" (OP) category. Samples which had oyster shell cover of 50% or greater were classified as "heavy shell" (HS) and any sample which had 10% to 49% shell cover as "light shell" (LS). Samples with eelgrass were grouped in a similar manner. Those samples which had eelgrass cover of 50% or greater were classified as "heavy eelgrass" (HE) and samples ranging from 10% to 49% were placed into the "light eelgrass" (LE) category. The average turion densities corresponding to these two eelgrass categories were: HE = 80 (±34; 1SD) turions/m^2 (n = 180) and LE = 21 (±18; 1SD) turions/m^2 (n = 23).

In occasional situations where both shell and eelgrass co-occurred within a quadrat, samples were assigned to the appropriate cover category of shell because of evidence presented by Armstrong and Gunderson (1985) and Dumbauld and Armstrong (1987) indicating that shell supports higher long-term densities of crab than does eelgrass. Specific balanced habitat comparisons between shell and eelgrass were conducted in 1987 to test the relative importance of each to 0+ crab, and gather further data on the validity of such habitat groupings (see Section 2.2.3).

Table 2. Habitat categories used for grouping intertidal crab samples and site transect survey estimates of cover during the 1986-1988 research period.

Habitat	Subdivision	Code	Characterization[a]
Oyster Shell	Heavy	HS	≥50% oyster shell cover
	Light	LS	≥10% and <50% oyster shell cover
Eelgrass	Heavy	HE	≥50% eelgrass cover and <10% oyster shell cover
	Light	LE	≥10% and <50% eelgrass cover, <10% oyster shell cover
Sand/Mud	Open	OP	<10% oyster shell and <10% eelgrass cover

[a]Characterizations based on visual estimates of relative percent cover within 0.25 m^2 quadrats.

These steps to verify sufficient accuracy of visual estimates of percent cover within broader habitat categories were taken to justify the procedure and thus allow us to collect the maximum number of samples possible during the short period of time when test sites were exposed during minus low tides. While not as precise as direct counts, visual estimates of relative cover have been widely used by other researchers (Dalby 1987). Zeh et al. (1981) report that relative cover can be efficiently estimated using a 0.25 m^2 quadrat; however, Dalby (1987) notes that one must accept a possible error of 10% when using visual estimation.

2.2.2 On-site Extrapolation of Percent Cover

During August of each year, a series of "ground truth" transect surveys were conducted over each site to (1) determine the proportion of ground covered by each habitat category, (2) determine changes in the proportion of ground covered by each habitat from one year to the next and, (3) use estimates of area covered and crab density/m^2 data by habitat category to calculate crab densities/ha.

Multiple transect lines were established in a random manner along the boundaries of each site as described in Section 2.2.3. Quadrats were systematically placed at 15-20 m (20 pace) intervals along each transect line and visual assessments of the relative percent cover of shell and eelgrass were recorded. This process was repeated along 3-6 transect lines per site. The total number of samples visually estimated at each site ranged from 109-529 (average # samples per site = 325).

These data were then grouped into one of the five broader habitat categories (HS, LS, HE, LE, or OP; see Section 2.2.1, Table 2) for specific comparisons and for use with crab density data corresponding to the same habitat categories.

2.2.3 Crab Density Samples

Sampling procedures to measure crab density/m^2 were modified slightly between years in accord with specific study objectives and as necessitated by major differences on specific sites. At

Figure 5. Examples of the various intertidal habitat categories (Table 2) sampled: (A) open mud (OP); (B) light shell (LS; upper right corner of photo) and light eelgrass (LE; lower left corner of photo); (C) heavy eelgrass (HE) with 0.25 m^2 quadrat used for sampling; and (D) heavy oyster shell (HS).

each site, transect lines were established and intertidal samples collected along these transects by the following procedure.

Property stakes, which marked the corners and perimeters of each oyster bed sampled, were used as reference markers from which transect lines were established. Prior to sampling each site, the starting point of the first transect (and the first set of samples) was determined by selecting two numbers (X and Y) between 1 and 100 at random and, starting from a preselected reference marker, walking X paces (about 1 m/pace) along one edge of the site and Y paces onto it. The first transect line was established parallel to the adjacent edge of the site, and quadrat samples were taken along the line until the edge opposite to X was reached. All subsequent transects were established parallel to the first by pacing out a randomly predetermined distance (20-50 m) perpendicular to the first and then proceeding back towards the original X edge of the site. The numbers of transects differed slightly between years and were dependent upon both time constraints and the total area of a particular site. In 1986, 1-2 transects were sampled across each site; in 1987 and 1988, sampling was conducted along 3-4 transects.

1986 Sampling: In order to acquire more specific information regarding the use of different habitats by juvenile crab, samples in 1986 were collected along the transect stratified by habitat category. Generally, a single sample was collected from each habitat category at each 50-100 m sample interval along the transect. For example, if the first quadrat sampled at a particular interval was HS, then corresponding samples would be collected from the nearest area of HE, LE, and LS within 1-2 m of the original sample, if that category was present. Open mud (OP) was generally assumed to not have any crab based on evidence presented by Armstrong and Gunderson (1985) and Dumbauld and Armstrong (1987) suggesting that juvenile crab are rarely found in open areas without refuge. However, OP was sampled periodically at each site to test the validity of this assumption. We generally attempted to collect a minimum of 3-5 samples per habitat category during 1986.

1987 Sampling: Analysis of 1986 data indicated high variability between samples and tests conducted to determine the minimum sample size required (Zar 1984) indicated that larger sample sizes were needed in order to detect differences in mean crab density between habitat categories. On the basis of this information, it was determined that a minimum of twelve samples from each category were needed for statistical comparisons and sampling during 1987 was modified accordingly. The short period of time in which sites were exposed during minus low tides limited the number of samples which could be taken. In order to obtain the desired sample size, we decided to focus primarily on the HS, HE and OP categories and, consequently, the LS and LE categories were not sampled on a regular basis during 1987. Samples from the three categories were collected along transect lines in a manner similar to that described for 1986.

In addition to long-term sampling of sites, a series of equally replicated random samples were collected (using procedures described above) specifically for two-way analysis of variance (ANOVA) to simultaneously compare crab densities between habitat type (HS, HE, and OP), and between sites from each of the three regions. Samples were collected from three sites (SP87, PR86, and NHC; Figs. 2, 3, and 4) during the last minus tide series in June 1987. Each had areas of eelgrass and shell present either on or immediately adjacent to the site. Twelve samples were collected from each of three habitat categories (HS, HE, and OP) at each site following procedures previously described. This comparison was repeated during the last minus tide series in July at SP86, PR86, and NHC (Note: SP87 was treated with carbaryl during early July; thus SP86, located nearby, was sampled instead).

1988 Sampling: Sampling was modified slightly during 1988 to incorporate a systematic quadrat sampling procedure and to increase the number of samples collected per site. The starting point for the transects and for the first sample were established using the same method as the previous 2 years. However, subsequent samples were collected at systematic intervals along each transect by dropping the quadrat every 15-20 m (20 paces). Once the quadrat was placed, the sample was collected using standard methods described previously. It was felt that the homogeneous nature of the oyster beds would (1) allow collection of a representative portion of samples from each habitat category in a fully randomized way as effectively as the stratified sampling approach used the previous 2 years, and (2) would allow direct estimates of density/ha and population abundance without the need to use stratified estimates based on each habitat category (Section 2.3.2).

2.3 INTERTIDAL DATA ANALYSIS

2.3.1 Intertidal Crab Density

Each sample was grouped into one of the five possible habitat categories by site and by trip, to calculate mean density for each habitat category.

Crab density data were highly variable, both spatially and temporally. Data did not show normal distribution patterns and variances were not homogeneous, violating the basic assumptions of parametric statistical tests (Conover 1980; Zar 1984). Subsequent transformations of the data such as $\log_{10}$ (abundance + 1) did not stabilize the variance, so statistical comparisons were restricted to Mann-Whitney and Kruskal-Wallis non-parametric rank tests. Two-way non-parametric analysis of variance procedures (Zar 1984) were used to test for significance of results from the balanced comparisons conducted during June and July 1987 (see Section 2.2.3). When non-parametric tests indicated significant differences between groups, Tukey-type multiple comparisons were applied to determine where the differences occurred (Conover 1980; Zar 1984).

2.3.2 Density/ha and Site Population Estimates

Crab density/m^2 was extrapolated to density/ha in two ways: either by a stratified approach from weighted sampling in the five habitat categories, or by the direct, fully systematic approach (used only in 1988). In either case, "site population" estimates were calculated based on the total area (hectares) of each site (Table 1). It should be noted that all estimates of density/ha or density/site were made under the assumption that the methods used for selecting intertidal crab samples resulted in the collection of quadrat samples which were representative of each habitat type.

Stratified Approach: Density of crab/ha was estimated for specific sites during July 1986 (SP86 and PR86; Table 1) and July 1988 (SP88 and PR88; Table 1) in order to estimate the number of crab/ha on these sites at the time of carbaryl treatment and, at long term reference Sites SP86 and PR86 in following years to estimate the number of crab/ha on site during the same period in July 1-2 years after treatment.

The average density/ha at each site was calculated utilizing the average density for each habitat category strata and accounting for the relative sizes of each stratum, by the method described by Pielou (1974) for estimating population size from a stratified sample.

$$\#\ \text{Crab/ha} = [(U_{HS} * P_{HS}) + (U_{LS} * P_{LS}) + (U_{HE} * P_{HE}) + (U_{LE} * P_{LE}) + (U_{OP} * P_{OP})] * 10{,}000\ \text{m}^2\text{/ha}$$

where U = the mean density of crab/m^2 within each specific habitat category (HS, LS, HE, LE, or OP; Table 1).

P = the relative proportion of ground on the site covered by each habitat category obtained from "ground truth" transect surveys of cover on site. (e.g., P_{HS} = number of quadrats designated as HS/total number of quadrats sampled).

In situations where a habitat category was not sampled for crab on a particular site, crab density values for the missing category were estimated by determining the average crab density for that habitat from other sites during the same sample period.

Direct Approach: Density/ha was estimated in two ways for data collected during 1988. For interannual comparisons, the samples were partitioned into the respective habitat categories (or strata) and density/ha calculated as described above. However, the systematic sampling approach also allowed us to estimate density/ha directly by multiplying the average crab density for all samples by the total number of m/ha.

(#crab/ha = Average #crab/m^2 * 10,000 m^2/ha).

Estimates of the population on the site at the time of treatment and during corresponding periods in subsequent years were made by multiplying the estimated density of #crab/ha by the size of the site:

#crab/ha * total number of ha = "Population" of crab on site.

2.4 CARBARYL IMPACT ASSESSMENTS

2.4.1 On-site (Intertidal) Crab Impacts

Intertidal sampling was conducted during periods immediately before and 24 h after treatment at selected sites in order to assess the on-site impacts to juvenile crab (primarily 0+ age class) directly exposed to carbaryl. Procedures for sampling the sites and for estimating the number of crab killed are described above in Sections 2.2.2, 2.2.3 and 2.3.2.

2.4.2 Off-site Crab Impacts

Several methods were used to ascertain the magnitude and extent of off-site impacts to crab in the areas adjacent to the treatment sites:

1. Trawling was conducted around the treatment sites to determine if large numbers of dead older crab were evident in subtidal channels 24 h after treatment and over sites at high tide.
2. Cages, holding live 1+ juvenile crab, were placed in channels around treatment sites.
3. Feeding experiments were conducted to determine if 1+ crab fed shrimp killed by the pesticide themselves die after ingesting the poisoned shrimp.
4. An intertidal bag-cage experiment was designed to determine off-site toxicity to 0+ crab held in the path of carbaryl transported by the first flood tide after spray.

Subtidal and Intertidal Trawls: In 1985 and 1986, a series of trawls were made in subtidal channels adjacent to treatment sites near Palix River and Stony Point during periods before, and 24 h after, spray in order to determine if large numbers of dead crab were evident in channels after use of carbaryl. Trawling was conducted from a 7 m Boston Whaler using a 3 m beam trawl developed for sampling demersal fish and invertebrates by Gunderson and Ellis (1986), following protocols developed for sampling Dungeness crab (Armstrong and Gunderson 1985; Armstrong et al. 1986; Dinnel et al. 1986). Locations of trawl samples for 1986 are depicted for SP86 and PR86 in Figs. 6 and 7, respectively. Crab caught in trawls were counted, CW measured to the nearest 1.0 mm, and classified as either alive or dead (including moribund animals).

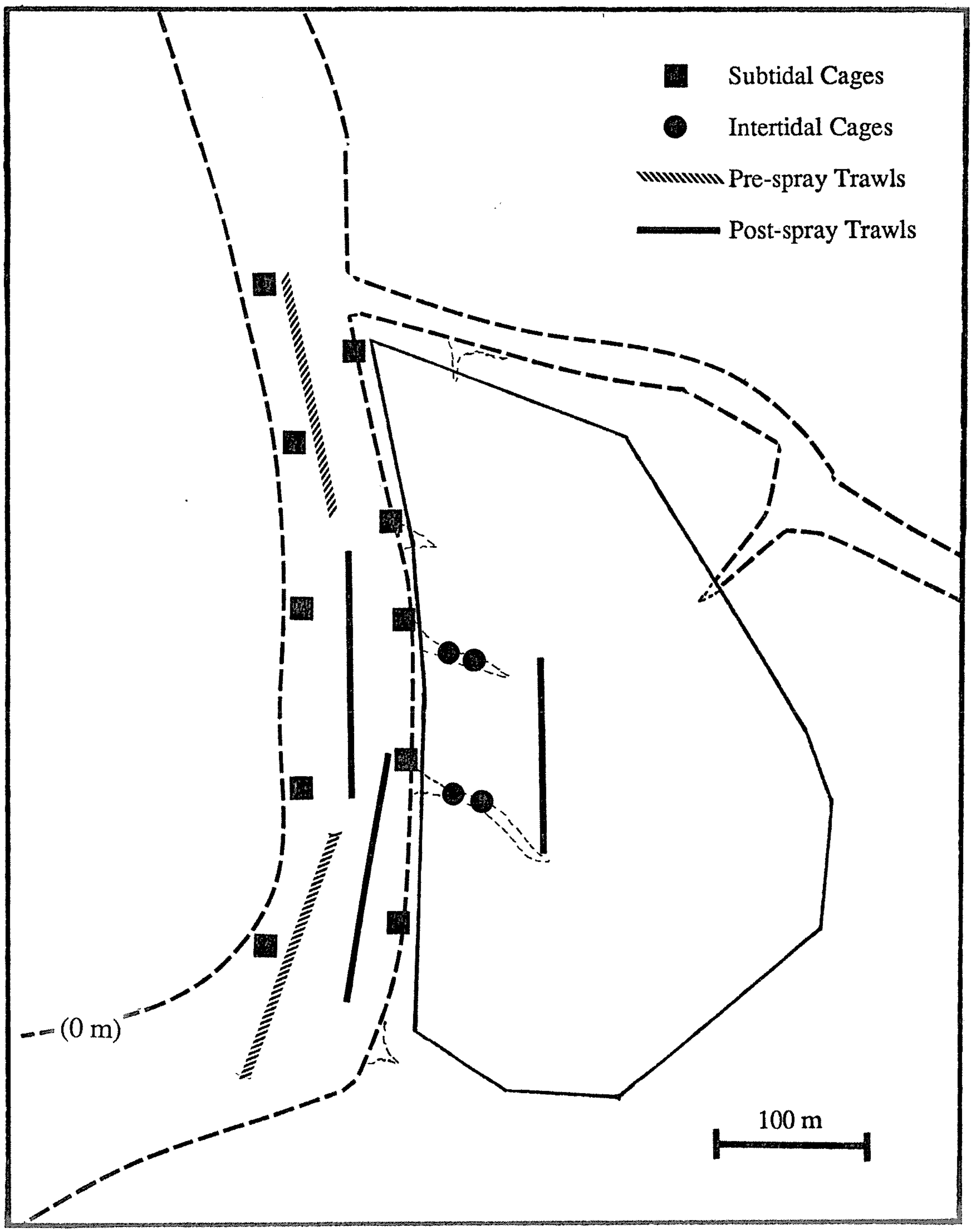

Figure 6. Stony Point treatment site (SP86), showing approximate locations of subtidal and intertidal trawls and locations of cages holding 1+ juvenile crab during treatment in July 1986. Solid line indicates boundary of 13 ha sprayed with carbaryl.

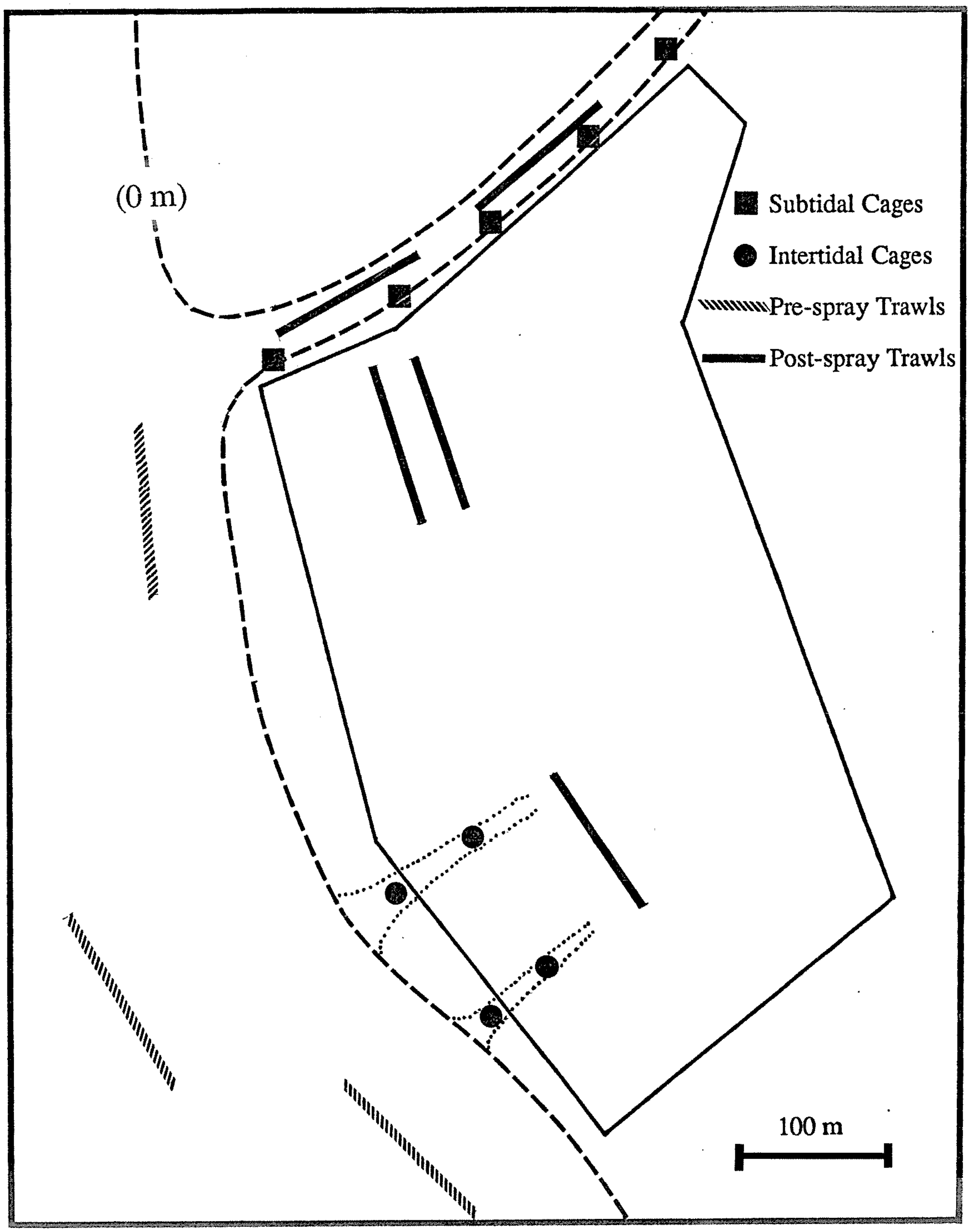

Figure 7. Palix River treatment site (PR86), showing approximate locations of intertidal and subtidal trawls and locations of cages holding 1+ crab during treatment period in July 1986. Solid line indicates boundary of 26.7 ha sprayed with carbaryl.

Caged Crab 1986: Preliminary experiments were conducted in 1986 with caged 1+ crab to monitor the effects of carbaryl in channels adjacent to treated areas. Carbaryl can be transported from treated sites in several ways and affect crab residing in adjacent areas. Many sites continue to drain following spray at low tide carrying dissolved and particulate carbaryl into the adjacent subtidal channels or the next incoming flood tide can transport the pesticide onto adjacent intertidal areas and into subtidal channels. Juvenile 1+ crab caught in trawls before treatment were placed into commercial shrimp pots which were lined with plastic mesh (10 mm stretch) to prevent escape. Prior to treatment, several cages with 15-20 crab each (mean size = 72 mm CW) were placed in subtidal channels adjacent to SP86 and PR86 treatment sites (Figs. 6 and 7, respectively). Additional cages were placed in tidal creeks (4-5 cm flowing water) on each site. Cages were retrieved 24 h after treatment, crab measured and deaths recorded.

Feeding Experiment 1987: Mud shrimp, *Upogebia pugettensis*, emerge from burrows in response to carbaryl spray and become potential prey for animals which forage on the sites during flood tides after treatment. During 1987, some preliminary cage experiments were initiated to determine if older 1+ crab feed on contaminated shrimp and subsequently die, or are able to do so and survive. Twenty 1+ crab (mean size = 55 mm CW) were placed into each of 22 cages (modified pandalid shrimp traps) and put at three locations: on the NS87 treatment site (n = 8 cages), in a subtidal channel along the perimeter of the NS87 site (n = 8 cages), and in an area (n = 6 cages) several hundred meters away (Fig. 3). Mud shrimp killed by carbaryl on the nearby PR87 site were placed into half of each set of cages (10 shrimp per cage) as shown in Figure 8a. Eight cages (4 with contaminated shrimp, 4 without shrimp) were interspersed and placed in a row directly on the site after treatment as the tide began to flood the area. Eight more cages with and without contaminated shrimp were placed in a channel about 50 m from the perimeter of the treatment site. Six cages (3 with shrimp and 3 without shrimp) were placed about 500 m away from the site and at the same tidal elevation as the on-site cages. Cages were inspected 24 h later and data on the number of dead crab and the number of shrimp eaten (defined as the number of shrimp missing or exoskeletons remaining) were recorded.

Feeding Experiments 1988: Two crab feeding experiments were conducted in 1988 along the Palix River channel to examine the mortality of 1+ crab which fed on mud shrimp contaminated with carbaryl (treatment) compared to mortality of crab fed uncontaminated shrimp (control). For each experiment, 1+ crab (mean size = 60 mm CW) were collected from trawls conducted in subtidal channels prior to carbaryl treatment and 10 crab were placed into each of 20 cages (modified pandalid shrimp traps). Cages were placed in a single row along a subtidal channel over 1 km away from the nearest treatment site, and randomly assigned to either treatment or control groups (Fig. 8b). Cages were placed at a level below the morning minus tide to ensure continuous submersion so that crab were not weakened by exposure to air and higher temperatures.

In the first experiment (Exp. 1), conducted on July 1 and 2, 1988, dead shrimp were removed from a bed treated with 8.4 kg carbaryl/ha (7.5 lb/acre) and 10 each were placed into 10 of the cages. Shrimp collected from an untreated site were placed into the other 10 cages (10 shrimp per cage) to serve as controls (Fig. 8b). In the second experiment (Exp. 2), conducted July 12 and 13, 1988, mortality of crab fed shrimp removed from experimental plots treated with carbaryl at rates of 3.4 , 5.6, and 8.4 kg/ha (3, 5, or 7.5 lb/acre) was compared to that of crab fed shrimp from a control site (Fig. 8b). In both cases, cages were placed a sufficient distance from treated beds to ensure that water-borne carbaryl did not affect animals and that ingestion was the only route of exposure.

Crab were held in the cages for 24 h after which time the cages were removed, dead crab counted and measured, and the percentage of dead crab in each cage calculated. Since percentage data show a binomial distribution, mortality data from cage experiments were transformed using the arcsine transformation, as recommended by Zar (1984), prior to conducting statistical

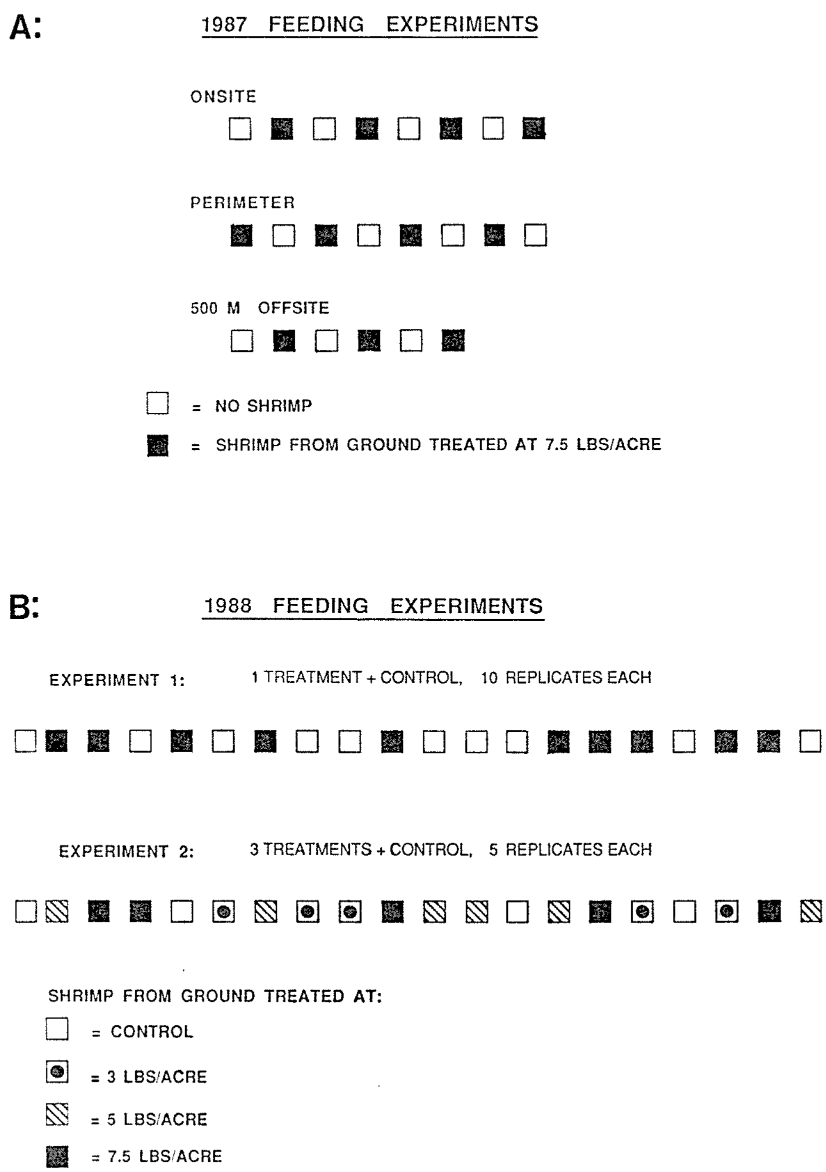

Figure 8. Design of crab feeding experiments conducted during 1987 (A) and 1988 (B). (A) In the 1987 experiment, caged 1+ crab (20 crab per cage) were placed at three Palix River locations (Fig. 3). Contaminated shrimp (10 shrimp per cage) were placed in half of the cages at each of the three locations as shown. (B) In 1988, experiment 1 involved 10 cages with shrimp treated at 7.5 and 0 lb/acre. Experiment 2 involved 5 cages each with shrimp taken from ground treated at 7.5, 5, 3, and 0 lb/acre. Ten crab and 10 dead shrimp (*Upogebia pugettensis*) were placed in each cage in both experiments at a location along the Palix River, well away from treated areas (Fig. 3).

comparisons. Statistical comparisons between control and treatment groups were conducted using t-test or one-way ANOVA on transformed data.

Carbaryl Transport Off-site: Evidence from previous WDF studies has shown that after intertidal sites are treated some of the carbaryl is transported into adjacent intertidal areas by the next incoming flood tide (Creekman and Hurlburt 1987). During 1988, an experiment was conducted to examine the off-site impacts to intertidal 0+ juvenile crab. The experiment was conducted at Site PR88 (Figs. 3 and 9) which was sprayed with carbaryl at a rate of 5.6 kg/ha (5 lb/acre) over 4.8 ha (12 acres) on June 30, 1988. This site was selected for study because preliminary investigations indicated that the incoming tide moved inland over the site and onto an adjacent intertidal area in a clearly defined, unidirectional path. Prior to treatment, the direction and path of the incoming tide was determined and marked with stakes at intervals of 0 (on-site), 25, 50, 75, 100, and 200 m from the edge of the site. An area located about 800-1,000 m from the treatment site was selected and marked to serve as a control (Fig. 9).

Juvenile 0+ crab used in the experiment were collected by taking advantage of their affinity for shell habitat. Plastic mesh bags filled with oyster shell were placed on the tideflats during the period of settlement in May. These bags were retrieved several weeks later, rinsed with salt water, and the young crab collected as they emerged from the shell matrix. A standard volume of oyster shell (6 liters or 1.5 gal) was placed into each of 35 experimental bags (30 x 50 cm; Norplex™ plastic mesh; 2 x 3 mm mesh) and 10 crab (mean size = 16.3 mm CW) were placed in each. Five of the bags were placed at each of the seven marked stations (0, 25, 50, 75, 100, 200 m, and control) immediately after the PR88 site was treated on June 30, 1988. Bags were placed in a row, perpendicular to the predetermined tidal flow and approximately 0.5-1 m apart, at each location (Fig. 9).

Concentrations of carbaryl and 1-napthol were measured from water samples taken by a person at each station as the incoming tide moved over bags holding the 0+ crab. Water samples were taken from both the surface and 2.5 cm off bottom as flood tide reached total depths of 2.5, 5, 10, 20, and 40 cm water (using marked stakes to indicate depth) at each station (to an extent, tidal depth was equivalent to elapsed time from first intrusion of water over the flats). At each depth/ time interval, four samples were taken, two from the surface and two from the bottom. Samples were collected in 100 ml plastic bottles into which 0.5 ml of 4% acetic acid had been added, and then refrigerated following protocols outlined in Creekman and Hurlburt (1987). Half of the samples were analyzed by WDF using colorimetric procedures of Karinen et al. (1967) and the other half analyzed by Washington Department of Agriculture's Dairy and Food Laboratory using high pressure liquid chromatography (HPLC).

Bags were left at each location for 24 h and then processed on July 1, 1988. The contents of each bag were removed, sorted over 3 mm mesh screens, dead crab counted, and percent mortality determined. Since results are expressed as proportions, and since values ranged from 0 to 100%, data were transformed using the arcsine transformation equation: $p' = 0.5 * [\text{arcsine} (X/n+1)^{-0.5} + \text{arcsine} (X+1/n+1)^{-0.5}]$ (Zar 1984) prior to statistical analysis. A one-way ANOVA was used to test for differences in mortality between stations.

Post-Spray Effects: After carbaryl is sprayed on sites, the sediments may remain toxic to crustaceans for extended periods of time (Buchanan et al. 1985). Crab which settle or migrate onto sites after treatment may be affected by the residual carbaryl associated with the sediments. During 1988, Site PR88 (Figs 3 and 9) and its control site from the study above were used for a preliminary experiment to examine survival of 0+ crab which were placed at the two locations 24 h after carbaryl spray. Twelve plastic mesh bags were prepared with a matrix of oyster shell and 10 crab per bag as previously described. Six bags were placed on Site PR88 treatment site 24 h after it was treated with 5.6 kg/ha (5 lb/acre) and six on the control site (Fig. 9) on July 1, 1988, at the same tidal elevation (0.0 m). Bags were left on the site for 14 days and then retrieved, sorted, and

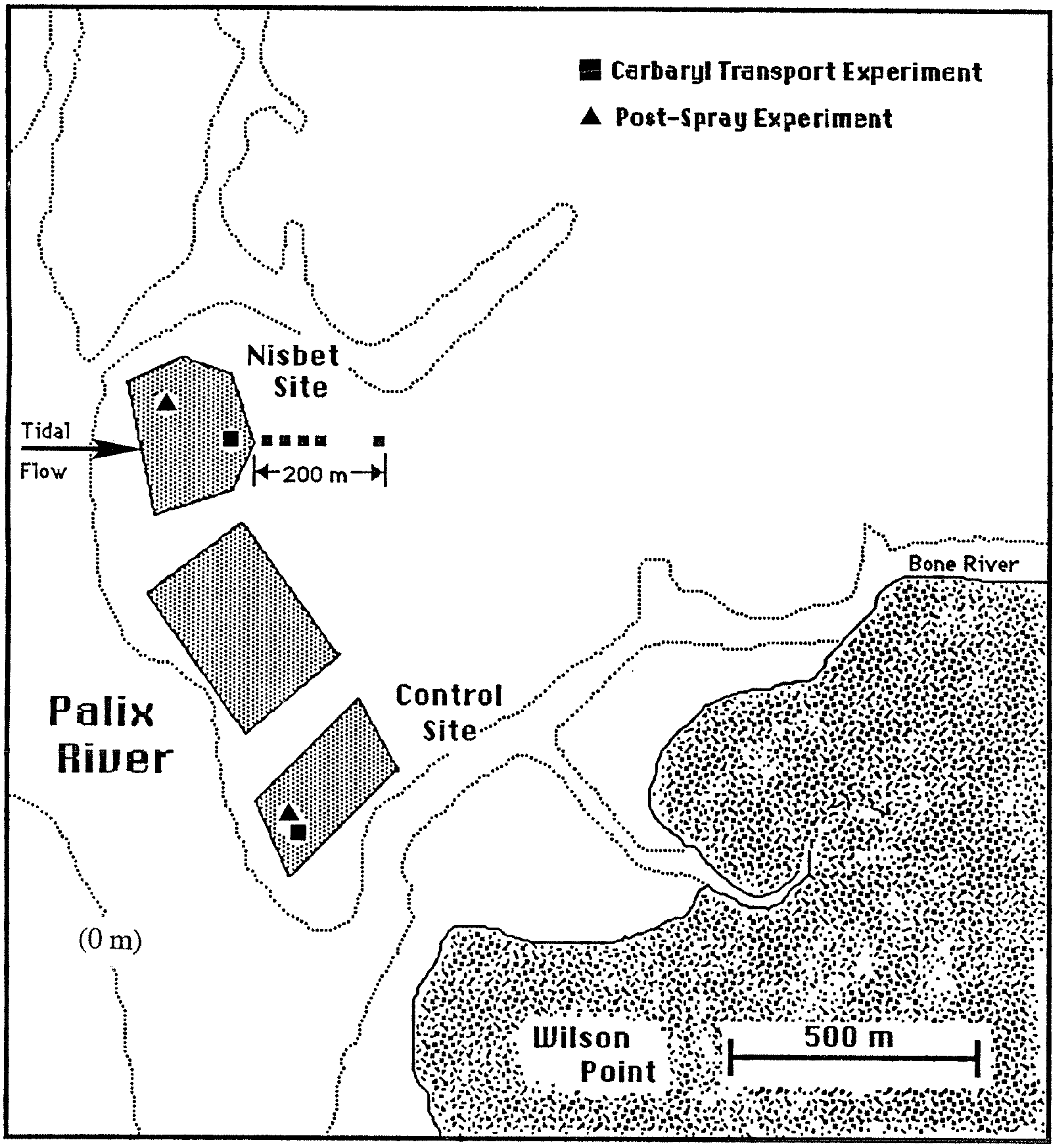

Figure 9. Study site PR88 (Nisbet site) in the Palix River region showing locations of the carbaryl transport and post-spray experiments conducted during 1988.

processed as described above. Results were expressed as percent survival and data transformed before use in t-tests.

3.0 RESULTS

3.1 INTERTIDAL CRAB ECOLOGY

3.1.1 Timing of Recruitment and Seasonal Size Composition

The seasonal size composition of intertidal crab (all sites combined) for each year is presented in Figure 10. Seasonal size frequency patterns were similar each of the 3 years and indicate that intertidal areas support primarily 0+ juvenile crab from 6-30 mm CW. Peak settlement of crab occurred during May and June of each year, as indicated by the large proportion of newly metamorphosed 1st instar (I1; 6-8 mm CW) crab in samples (Fig. 10) and by high densities corresponding with this period (Fig. 11). Some recruitment occurred throughout the summer since first instar crab were found in intertidal samples through August and September. By June of each year, three to four size modes were evident at 6-8 mm, 9-11 mm, 12-15 mm, and 17-20 mm CW, corresponding to instars 1-4 (I1 to I4). In July, size groups became less distinct and crab were predominantly between 10 and 25 mm CW. Only a small portion of crab found in intertidal samples during the 3 years sampled were over 30 mm CW.

3.1.2 Seasonal Crab Density at Reference Sites and the Relative Importance of Habitat

Density data from each site sampled during the 3 years are summarized by habitat, sample period, and year in Appendix Tables 1-8. Data from all reference sites were combined and mean densities by habitat category were calculated to highlight seasonal trends and differences between habitat categories in 1987 and 1988 (Fig. 11; Note: No reference sites were sampled in 1986. Density data from sites which were treated with carbaryl during 1986-1988 are presented in subsequent sections). Crab density at reference sites varied seasonally and by habitat category throughout the summers of 1987 and 1988. Crab densities were low in April but increased considerably during May and June corresponding to settlement of the year class. Mean densities in all habitat categories peaked during this period and were as high as 12 and 25 crab/m^2 in 1987 and 1988 respectively. Beginning in late June, crab density declined to more stable levels of 2 to 4 crab/m^2 during July and August of both years.

Nearly all crab found intertidally were associated with some form of epibenthic habitat (refuge), although levels of abundance differed by habitat type. Crab were rarely found in OP (open mud or sand) except during the period of initial settlement in May and early June of 1988 (Fig. 11). Initial settlement densities were always highest in HS and LS, but very low in HE and LE. Later in the summer, however, densities in eelgrass sometimes approximated those in LS at about 1-2 crab/m^2. During June and July of both years, crab densities in both "heavy" and "light" oyster shell were from two to five times greater than in either category of eelgrass. Highest average densities observed in HE were 3-4 crab/m^2 compared to 8-12/m^2 in LS and 12-16/m^2 in HS collected during the same mid-June sample periods. In July, the trend of higher densities in shell continued, although the magnitude of the differences between habitat categories was not as great.

Differences in relative crab density between shell and eelgrass were not caused by disparate settlement of megalopae over broad spatial scales in the estuary since both habitats occurred intermixed on some sites. A comparison in 1987 showed that crab density on one site in the Palix

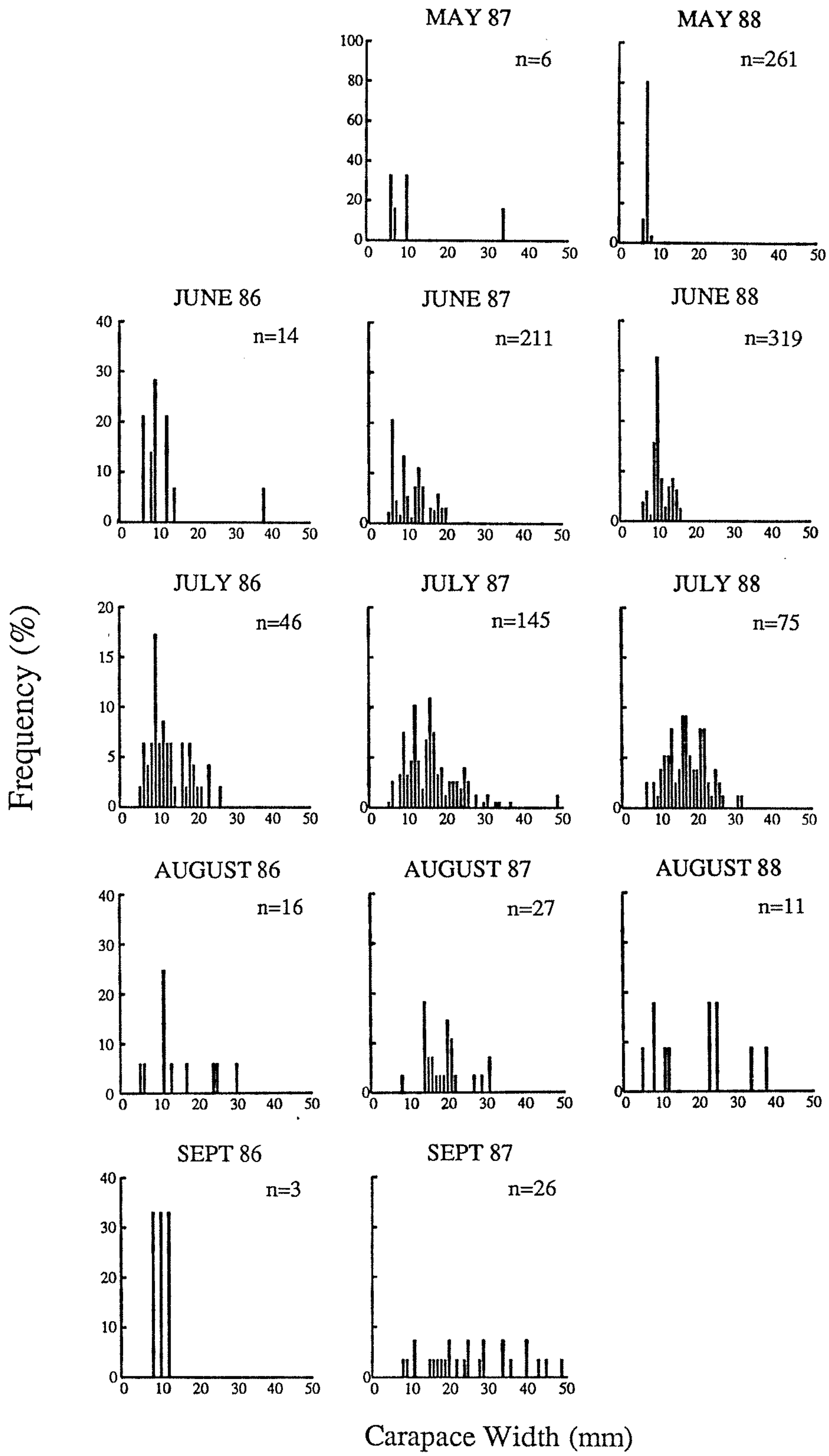

Figure 10. Size frequency distributions (CW in mm) of Dungeness crab sampled at all intertidal sites combined in Willapa Bay by month during 1986-1988.

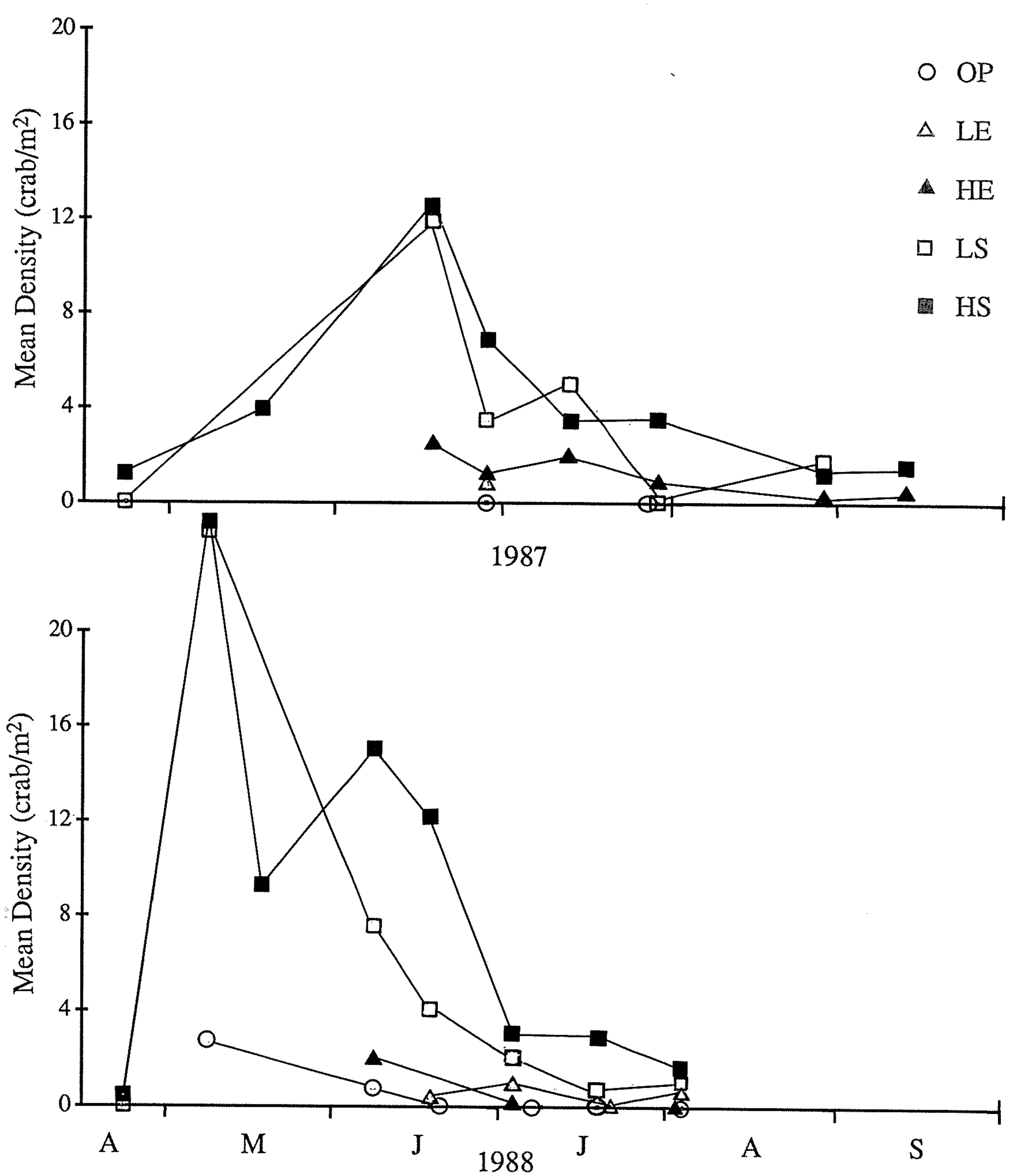

Figure 11. Average intertidal crab density on reference sites (all sites combined; refer to Section 2.4.2 for definition of reference sites) by habitat category (see Table 2 for category descriptions) throughout the summer of 1987 and 1988.

River region (PR86) had densities 4 to 6 times higher in shell than in eelgrass (Fig. 12; Appendix Table 4; see Fig. 3 for map); a difference that was also measured 18 km to the south at Nahcotta (NHC; Fig. 12; Appendix Table 7; see Fig. 4 for map). Crab density was significantly higher in HS compared to HE during each period sampled throughout the summer at NHC ($p < .05$; Mann-Whitney; $n = 12$ per habitat type, each sample period). At Site PR86, significantly higher densities were detected in HS compared to HE during three sample periods in June and July ($p < .05$; Mann-Whitney; $n = 12$ per habitat type each sample period) but no differences were detected in August or September ($p > .10$).

Statistically significant differences were detected when average crab densities were compared between HS, HE, and OP at three specific sites during June and July 1987. Average crab densities were higher in HS than in HE in all cases and no crab were found in OP during either sample period (Fig. 13; OP not shown). When crab density within each habitat was tested at each site, average density ranked significantly higher in HS at two out of three sites (PR86 and NHC) during June and at all three sites in July (Fig. 13; Kruskal-Wallis; $P < .01$; $n = 12$ per habitat category), but no significant differences were detected between HE and OP at any of these sites ($p > .10$).

Samples from sites SP87, PR86, and NHC were pooled by habitat and tested using a two-way nonparametric ANOVA for June 1987. Significantly higher densities were detected in HS ($p < .0001$; $n = 36$ per habitat category) and no differences were evident between HE and OP (Table 3). Average crab densities also differed significantly between the three sites sampled during June when samples from all three habitat categories (OP, HE, and HS) were pooled by site. In June, crab density was significantly lower at SP87 ($p = .004$; $n = 36$ per site) than PR86 and NHC (Table 3).

Similar differences in crab density were detected between the three habitat categories when samples collected from SP86, PR86, and NHC during July 1987 were compared. Crab density in HS was significantly higher than in HE and OP ($p < .0001$; 2-way non-parametric ANOVA; Table 3). Densities also differed significantly between Sites SP86 and NHC, and density at Site PR86 was intermediate between the two ($p = .015$; 2-way nonparametric ANOVA; Table 3).

In general, HS tended to support higher seasonal densities of crab than LS although significant differences were not consistently detected between the two. Densities between HE and LE were similar in most cases with no consistent trend toward higher densities evident in either category.

3.2 Proportion of Ground Covered by Habitat Category

3.2.1 Percent Cover During Year of Treatment

Sites differed in the type and extent of habitat present at the time of carbaryl treatment. The results of transect surveys to determine relative cover by habitat category (see Table 2 and Section 2.2.1) at each of the sites sampled during the 3-year study are presented in Figure 14. Shown are the percent of ground covered by each habitat both at the time of treatment, and in subsequent years at several of the sites. The amount of oyster shell on sites at the time of treatment was dependent upon the culture method used. Each of the three sites sampled near Stony Point (SP86, SP87, and SP88; Fig. 2 and Table 1) were "growout" beds, on which seed is planted and left to grow on site for 3 years until harvest. These three beds were covered with newly planted oyster seed 3-4 months prior to treatment, which is reflected by the high portion of ground covered with oyster shell (LS = 40-60%) on each site (Fig. 14A, B, and C). HE, LE, and HS covered only a small portion of these sites and the remainder of ground was open mud.

About 95% of site NP87, in the Long Island region (Fig. 4), was covered with shell at the time of treatment (65% HS and 30% LS; Fig. 14G). Heavy cover on this site was due to a thick base layer of shell that had been placed over the area to firm the substrate, over which a layer of oyster seed had been placed during the spring prior to spray in July.

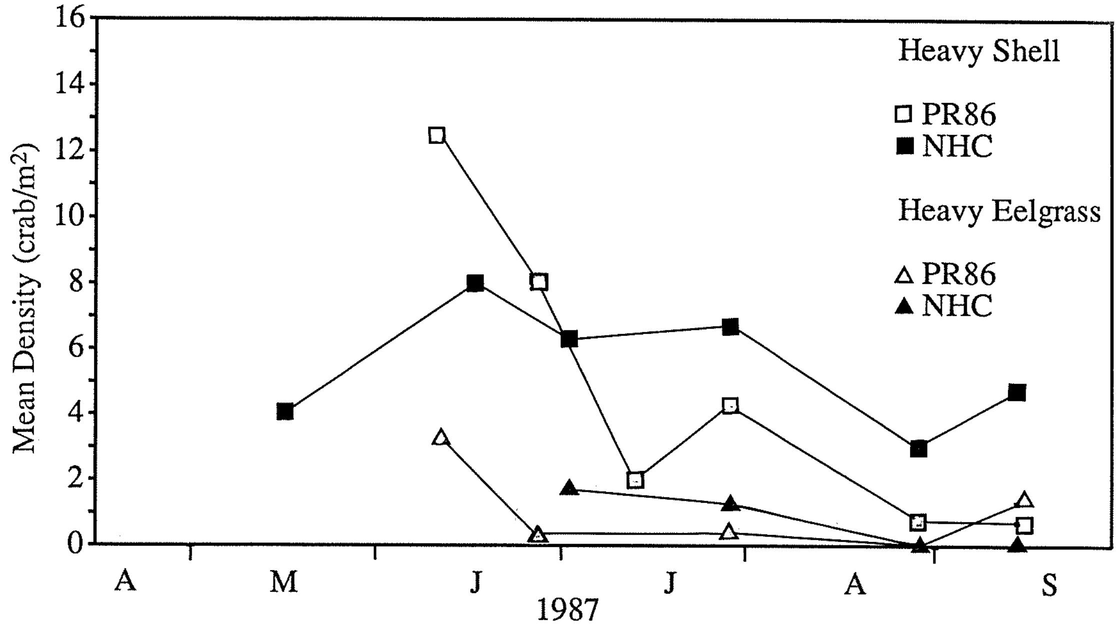

Figure 12. Comparison of crab density patterns in HS and HE at two sites (PR86 and NHC) sampled during 1987 (see Figs. 3 and 4 for site locations).

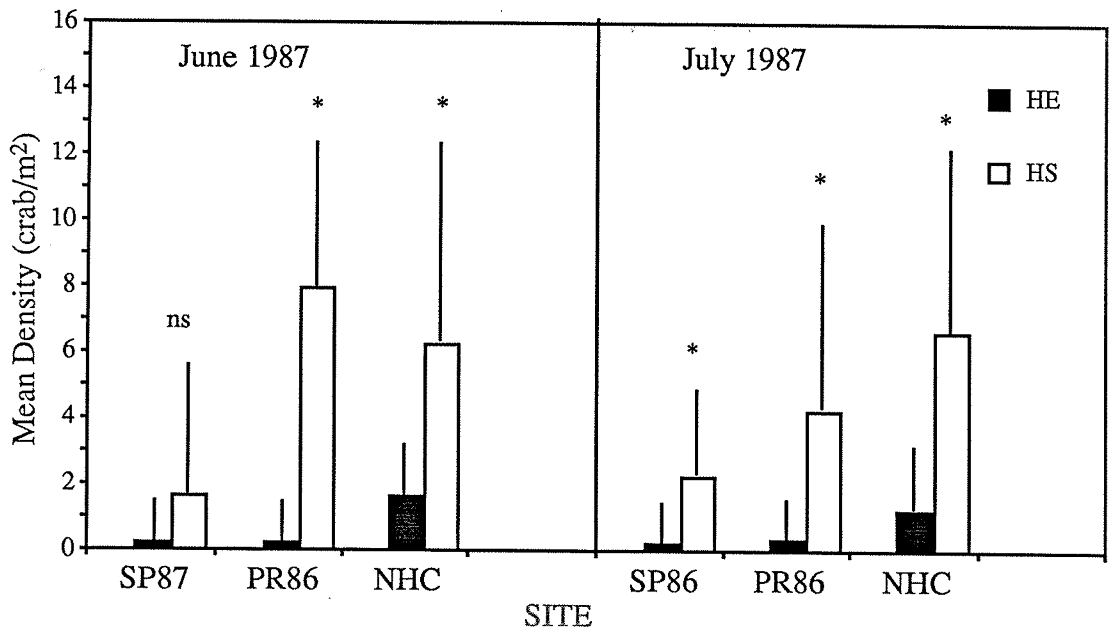

Figure 13. Average crab densities in HS and HE from balanced habitat comparisons at several sites (see Figs. 2, 3, and 4 for locations) sampled during June and July 1987 ($n = 12$ per habitat type per site). OP was zero at all sites and is not presented in the figure. Bars represent 1 SD above the mean; * indicates crab density in HS was significantly higher than in HE and OP at that site ($p < 0.01$; Kruskal-Wallis non-parametric ANOVA).

Table 3. Results of balanced non-parametric two-way analysis of variance to test for differences between crab density and habitat category, and between crab density and location for sample periods during June and July 1987. Results of Tukey-type multiple comparisons are presented with bars below factors with no detectable difference between mean ranks. Refer to Table 2 for definitions of habitat categories and Figs. 1-4 for locations of sites.

H_0: Crab densities are the same in all three habitat categories (OP, HE, HS).
H_A: Crab densities are different between the three habitat categories.

n = 36 per habitat category (for each sample period, 12 samples from each site were combined by habitat category to test for differences in density by habitat).

H_0: Crab densities, from all habitats combined, are the same at all three locations.
H_A: Crab densities, from all habitats combined, are not the same at the three locations.

n = 36 per location (for each sample period, 12 samples from each habitat category were combined by site to test for differences in density by location).

Criteria: Reject null if probability (p) $<.05$.

Date Factor	Result	Probability (p)	Multiple comparisons Rank: (Low - High)		
June 26-28, 1987					
Habitat Category (OP, HE, HS)	Reject H_0	$p < .0001$	OP	HE	HS
			________	________	____
Location (SP87, PR86, NHC)	Reject H_0	$p = .004$	SP87	PR86	NHC
			____	________	________
July 25-26, 1987					
Habitat Category (OP, HE, HS)	Reject H_0	$p < .0001$	OP	HE	HS
			________	________	____
Location[a] (SP86, PR86, NHC)	Reject H_0	$p = .015$	SP86	PR86	NHC
			________	________	
				________	________

[a]SP87 was treated with carbaryl on 7/9/87. An alternate site within the same region, SP86, was sampled during July 25-26.

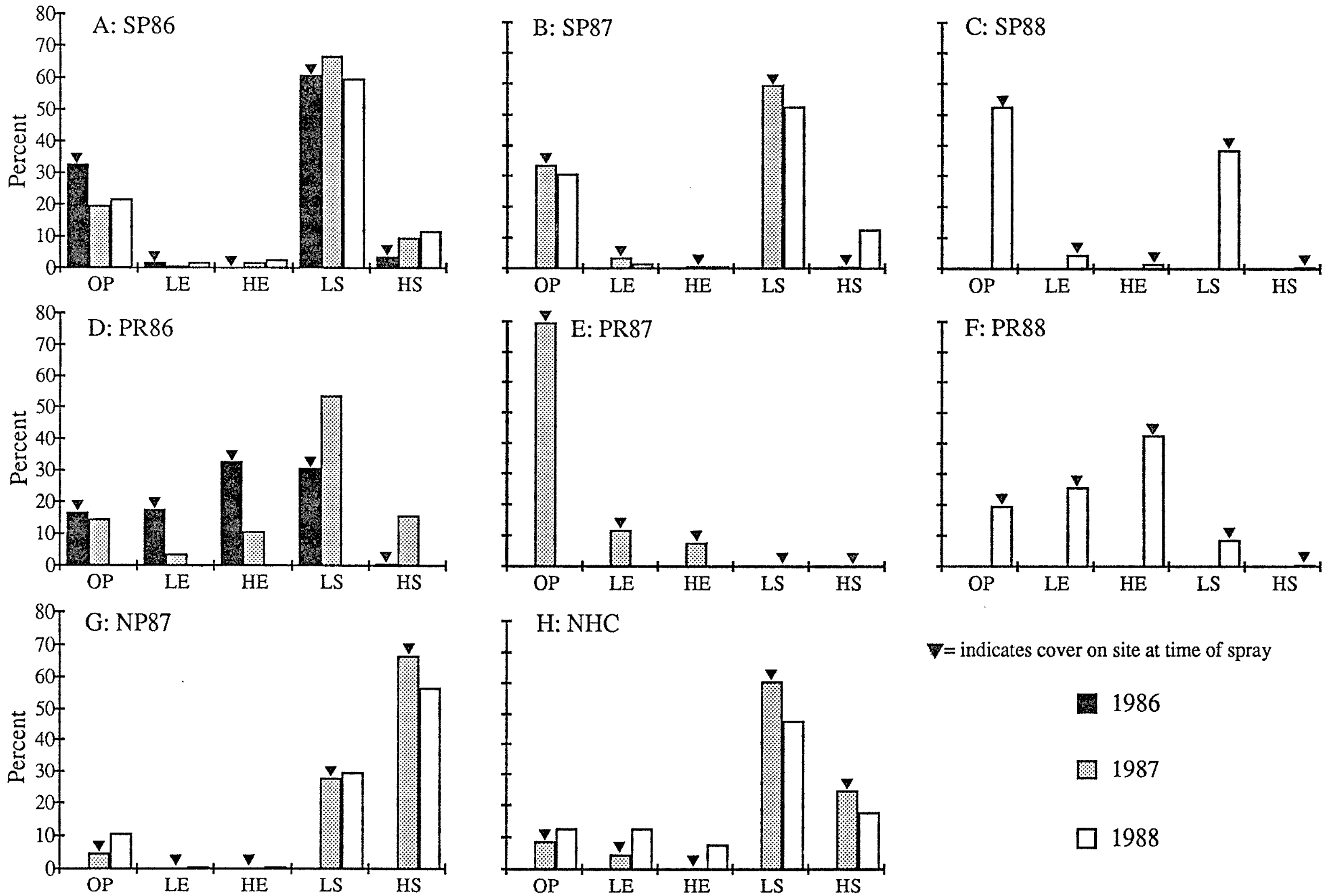

Figure 14. Percent of ground covered by each habitat category at each site sampled from 1986 through 1988. Transect surveys were done on each site during the year of spray, and in subsequent years at several of the sites to monitor changes in habitat coverage. The arrows indicate the amount of cover within each category at the time of treatment on each of the sites.

Two of the Palix River sites, PR86 and PR88 (Fig. 2), were covered primarily with eelgrass (50 and 70%, respectively) and some remnant shell (LS = 30% at PR86 and 10% at PR88; Fig. 14D and F). Oyster were not being actively cultured on these two sites at the time of spray (shell was put on sites after spray). The other Palix River site, PR87, was predominantly open sand with a sparse covering of eelgrass and no oyster shell at the time of treatment (Fig. 14E).

3.2.2 Annual Changes in Relative Cover on Sites

The degree of annual changes in relative cover differed by site. Slight increases in overall shell cover were observed at the two growout beds, SP86 and SP87, near Stony Point (Fig. 14A and B) as the seed oyster grew. At these two sites, most of the increase was noted in the HS category since the cultch method (many spat per shell) tends to produce thick clumps of larger oyster.. There was little overall change in LS at SP86 (Fig. 14A) and a slight decrease in LS at SP87 (Fig. 14B).

A much larger increase of shell cover was measured at Site PR86, where the proportion of LS cover nearly doubled from 30% to 55% and HS increased from 1% to 16% (Fig. 14D) between 1986 and 1987. The increase in shell cover by 1987 was due to addition of 2-year-old oyster, which were transplanted onto the site after treatment (in 1986) for a 2-year period of "fattening" prior to harvest. The relative amount of ground covered by HE and LE declined as a result of the additional oyster. However, it should be noted that the relative amount of ground covered by eelgrass decreased primarily because LS and HS superseded eelgrass by definition (see section 2.2.1). Actual declines in the amount of eelgrass cover were not as extreme.

The extent of shell cover generally declined at both sites near Long Island. The coverage of HS declined 10% between 1987 and 1988 at treatment site NP87 (Fig. 14G), and at reference Site NHC (which was not treated during the 3-year study) both LS and HS declined between 1987 and 1988 (Fig. 14H).

3.3 ON-SITE (INTERTIDAL) IMPACTS TO CRAB DUE TO CARBARYL TREATMENT

3.3.1 Change in Density/m^2 and Size Classes Affected

Carbaryl was applied to sites during July of each of the 3 years, after recruitment of 0+ crab had occurred. While 0+ crab densities varied by site and habitat type (see Section 3.1), levels associated with epibenthic cover typically ranged from 0.3-6 crab/m^2 at the time of treatment during July of each year (Fig. 15, see SP86 and PR86 in 1986; Fig. 16; Table 4; and Appendix Tables 1-8). Crab densities on treatment sites during July were generally highest within HS (4-6 crab/m^2) followed by LS (1-4 crab/m^2) and HE and LE (0-3 crab/m^2).

On the basis of the timing of carbaryl application in July of each year and the size frequency distribution (Fig. 10), most 0+ crab directly exposed were instars I2-4 (10-25 mm CW).

While crab were found on sites, no living crab were found in any quadrat samples collected from treatment sites 24 h after carbaryl application or in bags held directly on site (see Section 3.4.4). Consequently, density of live crabs on all treatment sites dropped to zero in all habitat categories after spray (Fig. 15, see SP86 and PR86 in 1986; Fig. 16A, B, and C). Some recovery did occur on treatment sites in subsequent months (see Section 3.5.1).

3.3.2 Density/ha—Stratified Estimates

Estimates of 0+ crab/m^2 were extrapolated to total number of crab/ha based on average crab densities by habitat type at the time of spray, and the estimated coverage of habitats on several

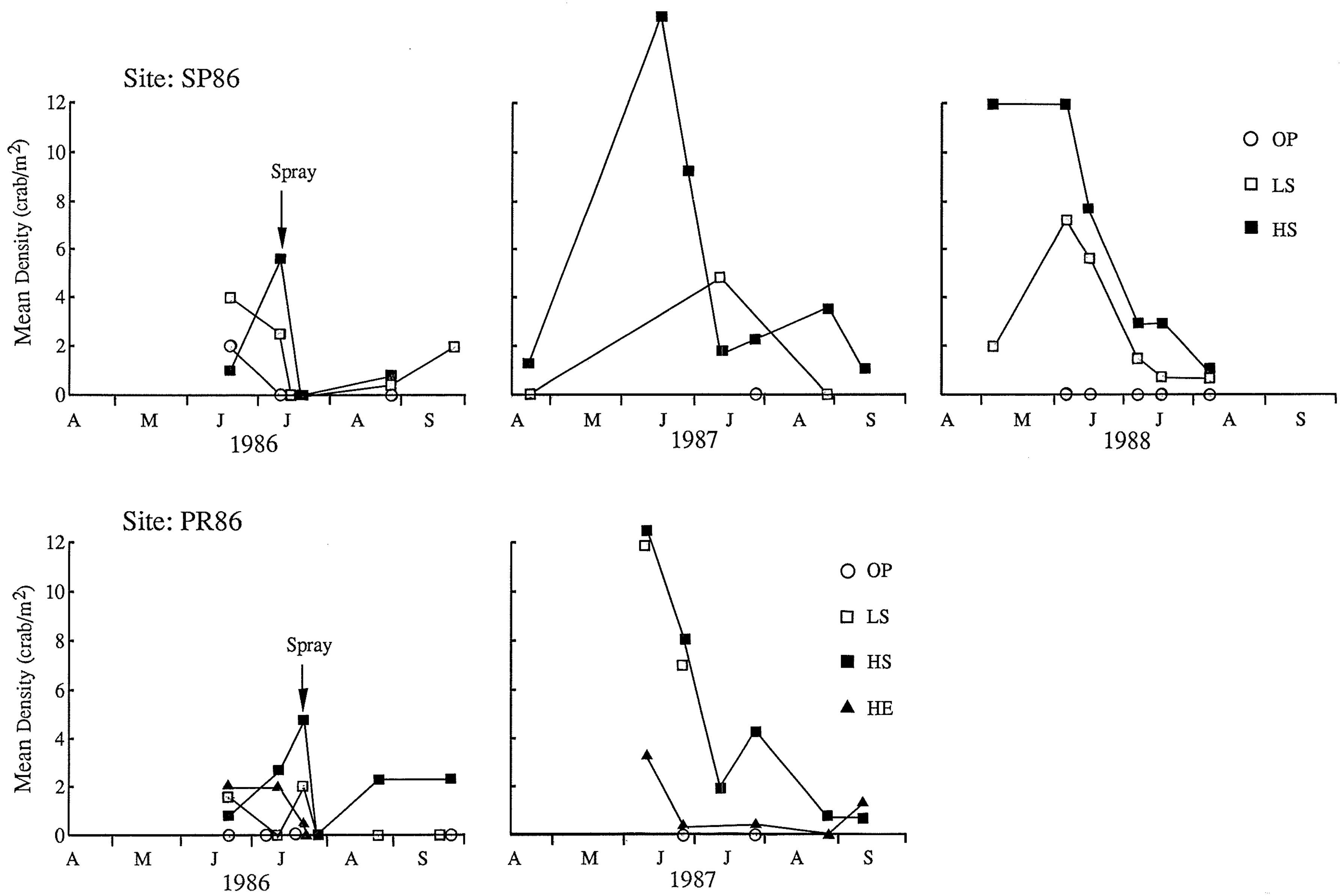

Figure 15. Average crab density within each habitat category sampled at Sites SP86 and PR86 during the year of spray (1986) and for 1-2 years following treatment. Arrows indicate time of carbaryl application to each site during 1986.

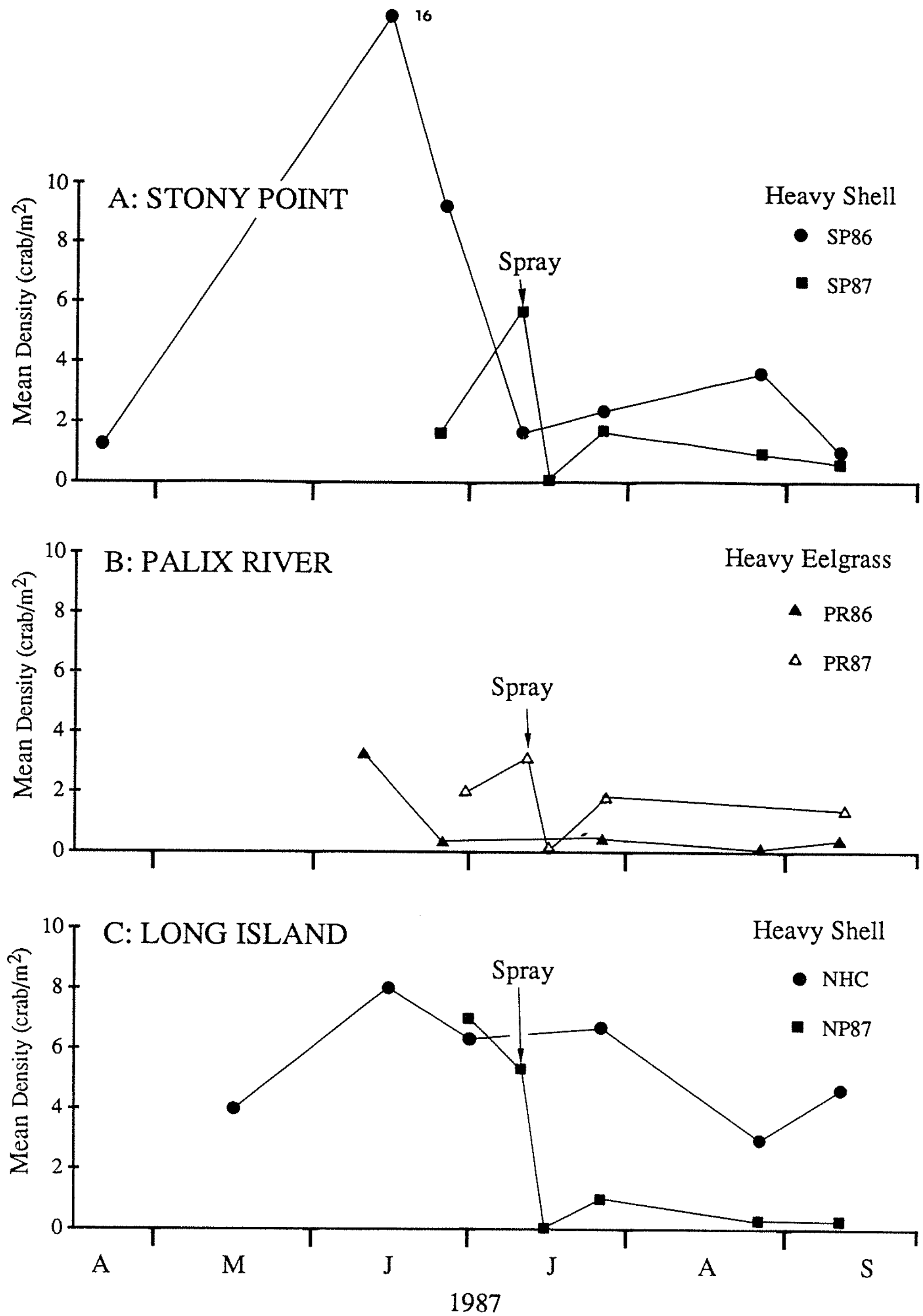

Figure 16. Comparison of crab density during 1987 in two habitats (HS and HE) in three regions of Willapa Bay on treatment sites SP87, PR87, and NP87, and within corresponding habitat categories at reference sites SP86, PR86, and NHC sampled within each of the three general regions: Stony Point, Palix River, and Long Island. (Note: Virtually no shell was present on PR87; see Fig. 14). The arrow indicates the time at which carbaryl was applied to the treatment sites.

Table 4. Estimated number of 0+ crab killed/ha by carbaryl treatment during July in 1986 and 1988 by habitat category (stratum) at treatment sites SP86, PR86, SP88, and PR88. Estimates were derived from average crab densities from quadrat samples at the time of carbaryl application (see Appendix Tables 1, 3, 4, and 6) and relative cover estimates from transect surveys conducted during the year of treatment (Figure 14A, C, D, and F).

Site (Date)	Habitat category[a]	Ground cover (m^2/ha)	Crab density (crab/m^2)	Crab killed/ha
SP86	OP	3,300	0.0	0
(7/8/86)	LE	200	0.4[b]	80[b]
	HE	0	0	0
	LS	6,100	2.5	15,250
	HS	400	5.5	2,200
				Total kill/ha = 17,530
PR86	OP	1,700	0.0	0
(7/22/86)	LE	1,800	0.4[b]	720[b]
	HE	3,400	0.4	1,360
	LS	3,000	2.0	6,000
	HS	100	4.8	480
				Total kill/ha = 8,560
SP88	OP	5,300	0.0	0
(7/30/88)	LE	500	0.8	400
	HE	200	0.8[b]	160[b]
	LS	3,900	1.5	5,850
	HS	100	1.6[b]	160[b]
				Total kill/ha = 6,570
PR88	OP	2,000	0.0	0
(6/30/88)	LE	2,600	0.9	2,340
	HE	4,400	0.3	1,320
	LS	900	0.0	0
	HS	100	3.0[b]	300[b]
				Total kill/ha = 3,960

[a]Refer to Table 2 for definitions of habitat categories.
[b]Indicates habitat categories which were not sampled for crab at the time of treatment. Crab density values used to derive estimates of kill/ha for each missing habitat category were computing by using the average density of the habitat from other sites during the same period.

sites in 1986 and 1988 (Table 4). Estimates of average crab density/ha at two sites, PR86 and SP86, treated in 1986 were 8,560/ha and 17,530/ha, respectively, and in 1988 were 3,960/ha and 6,570/ha at PR88 and SP88, respectively.

The total number of 0+ crab killed by carbaryl treatment at each site was dependent upon the type and extent of each habitat category present as well as the average crab density within each habitat. At Site PR86, for example, the area covered by HE and LS were similar (Table 4; 3,400 and 3,000 m^2/ha, respectively) but average crab density within LS (2.0/m^2) was five times higher than that of HE (0.4/m^2). Consequently, the number of crab in LS (6,000 crab/ha) was over 4 times higher than in HE (1,360 crab/ha). The area covered by HE (3,400 m^2/ha) was 34 times higher than that of HS (100 m^2/ha), but owing to the high crab density in HS (4.8 crab/m^2), the number of crab/ha associated with HE was only 2.8 times higher than in HS (Table 4).

Higher crab kills were estimated on sites which had larger areas of shell cover. The largest crab kill was measured on Site SP86 which also had the largest amount of shell cover on site (65%) at the time of treatment compared to the other three sites (Table 4). Crab densities/m^2 within HS and LS were similar at both SP86 and PR86 at the time of spray but SP86 had over twice the total area of shell which resulted in a much higher kill (over 2 times higher) on this site compared to PR86 (Table 4). Similarly, in 1988 the higher kill at SP88 was due primarily to the high portion of LS (seed oyster) on the site compared to that on PR88 which was covered primarily with eelgrass (Table 4). Note also that SP88 was treated on July 30, a month after PR88 was treated. The magnitude of kill on SP88, and consequently, the differences between it and PR88, would have been much higher had the site been sprayed during late June when average crab densities in shell were higher (Appendix Table 3).

3.3.3 Populations on Sites at Time of Treatment

When stratified estimates of crab density (and mortality)/ha for each habitat type were extrapolated to the total area at each site (see Tables 1 and 4), estimated numbers of 0+ crab killed were 227,890, 228,552; 31,536; and 19,008 at SP86, PR86, SP88, and PR88, respectively. Even though estimated kill/ha was lower at PR86 than at SP86 (Table 4), the total area sprayed at PR86 was over twice as large (26.7 ha; Table 1). Consequently the total number of crab killed on the two sites was similar.

3.4 OFF-SITE CRAB IMPACTS

3.4.1 Subtidal and Intertidal Trawls

Subtidal Trawls: Data from 6 beam trawls taken during the 1985 Sevin treatment period and 13 trawls taken during the 1986 Sevin treatment period are presented in Table 5. Results from trawls taken in channels adjacent to sites sprayed at the Palix River in 1985 showed no mortalities in 12 or 24 h post-spray samples, while in a single trawl at Stony Point, 2% of the crab caught were dead. The number of crab caught was too variable to detect a before-after trend.

The average number of crab caught by trawl in the subtidal channels adjacent to the Palix River (PR86) and Stony Point (SP86) sites just prior to the application of Sevin in 1986 were 2,064 crab/ha and 3,883 crab/ha respectively (Table 5). These numbers are comparable to individual values measured at other locations in the bay during the same time period as part of a more extensive survey funded by Washington Sea Grant, but are higher than the average number of crab caught at 5 stations in the Palix/Stony Point geographical area (984 crab/ha, Sea Grant Stratum 1).

Post-treatment trawls, taken in the same subtidal channels 24 h after spray in 1986 yielded few dead or moribund crab. The average crab catches were slightly higher than pre-spray values (3,610 and 4,173 crab/ha for the Palix and Stony Point sites respectively). Only one or two dead

Table 5. Crab catches and mortality estimates (#crab/ha) from beam trawls taken in subtidal channels next to Palix River and Stony Point treatment sites in 1985 and 1986. The actual number of crab caught, live and dead, are given in parentheses below the density and mortality estimates.

Location: Site Time	Treatment date	Mean density # crab/ha (total # crab in trawl)	Mortalities # crab/ha (# dead crab in trawl)	Trawls N
Palix River	7-1-85			
Pre-spray		12,953 (991)	0	2
12 hour post-spray		914 (88)	0	2
24 hour post-spray		6,593 (232)	0	1
Stony Point	7-3-85			
24 hour post-spray		4,640 (111)	93 (2)	1
Palix River: PR86	7-22-86			
Pre-spray		2,064 (155)	0	3
24 hour post-spray		3,610 (135)	39 (1)	2
Stony Point: SP86	7-8-86			
Pre-spray		3,883 (312)	0	2
6 hour post-spray		1,665 (113)	0	1
24 hour post-spray		4,173 (177)	47 (2)	1

crab were found in each trawl which resulted in 24 hour post-spray mortality estimates of approximately 1% in both cases.

Most of the crab taken by trawling in the adjacent channels were 1+ juveniles in both years (66 and 88% in 1985 and 1986, respectively), the remainder being 2+ based on size-at-age calculated by Stevens and Armstrong (1984) and Armstrong et al. (1987).

Intertidal Trawls Over Spray Sites at Flood Tide: Trawls made over Palix River and Stony Point intertidal treatment sites during the first and second high tides after spray in 1985 and 1986 showed very low densities of juvenile crab present and few mortalities (Table 6). Catches from a single trawl made over the intertidal treatment site at PR88 during high tide before spray and another trawl taken during the first high tide 6 hr after spray in 1988 were similar, but 9 out of 10 crab taken after spray were dead or moribund (90% mortality). Crab taken in trawls over the intertidal were a combination of 1+ juveniles (35-80 mm CW) and smaller 0+ crab (6-35 mm CW).

Other species were also caught in these post-treatment intertidal trawl samples including several types of fish as well as mud shrimp which were abundant at the Palix River location in 1986. Mud shrimp and small Dungeness crab were found in the gut contents of Pacific staghorn sculpin (*Leptocottus armatus*) and cabezon (*Scorpaenichthys marmoratus*) captured on-site. A more detailed analysis of the stomach contents of fish taken in 1988 is given by Hueckel et al. (1989).

3.4.2 Caged 1+ Crab Experiments

Caged juvenile 1+ crab (mean = 72 mm CW) placed in subtidal channels at shallow depth (-1 m at MLLW) along the perimeter of two treatment sites in 1986 exhibited little or no mortality when examined 24 h after spray (Table 7). One crab was found dead among 71 crab placed in five cages at the Palix River site (PR86). No crab died in cages placed in slightly deeper water (-2 to -3 m at MLLW) at Stony Point (SP86).

Variable results were obtained from cages of 1+ crab placed in intertidal drainage creeks directly on the treatment areas. At the Palix River site (PR86), crab mortalities were 40% and 45% in two cages placed in one drainage creek, while no mortality was observed in 2 cages placed in another drainage creek (mean of all four observations = 21%, Table 7).

All crab placed in creeks on the Stony Point site were healthy and active when checked 24 h after spray.

3.4.3 Crab Feeding Experiments

1987: Results of a feeding experiment carried out in 1987 (Section 2.4.2) were mixed (Table 8). The experiment was designed to determine if older 1+ crab feed on contaminated shrimp and subsequently die, or if they are able to consume the contaminated shrimp and survive. Mortality was significantly higher (2-way ANOVA, location effect, $p < .0001$) in cages placed directly on the treatment site (means of 40% and 50% for cages with and without shrimp, respectively) than in cages placed along the perimeter and 500 m off-site (average mortalities of 3% to 24%). Even though most of the shrimp were eaten, no consistent contaminated shrimp treatment effect could be detected ($p = .0918$, Table 8).

1988: Two caged crab feeding experiments in 1988 were carried out in a location not affected by spray in order to remove site treatment effects. Uncontaminated shrimp were used as control food, and contaminated shrimp killed at several rates of spray were the treatments. Results of the first experiment showed a significant difference in crab mortality between cages with shrimp treated at 8.4 kg/ha (38% mortality) and cages with uncontaminated controls (2% mortality; Students t test, $p < .001$, Table 9). A significant difference in mortality was found between crab fed shrimp that were sprayed with carbaryl at 8.4, 5.6, or 3.4 kg/ha (7.5, 5 or 3 lb/acre) and non contaminated controls in the second experiment (ANOVA, $p = .036$), but results of the Tukey multiple comparisons test were inconclusive for discerning a dose effect between the shrimp food

Table 6. Crab catches and mortality estimates (#crab/ha) from intertidal beam trawls taken over the Palix River and Stony Point treatment sites at high tide in 1985, 1986, and 1988. The actual number of crabs caught, live and dead, are given in parentheses below the density and mortality estimates.

Location: Site Time	Treatment date	Mean density # crab/ha (total # crab in trawl)	Mortalities # crab/ha (# dead crab in trawl)	Trawls N
Palix River	7-1-85			
18 hour post-spray		170 (6)	0	1
Palix River: PR86	7-22-86			
18 hour post-spray		353 (56)	0	3
Stony Point: SP86	7-8-86			
6 hour post-spray		47 (2)	24 (1)	1
Palix River: PR88	6-30-88			
24 hour pre-spray		183 (8)	0	1
6 hour post-spray		229 (10)	206 (9)	1

Table 7. Results of 1986 caged crab experiments showing average mortality of 1+ crab held in cages for 24 h. Cages were placed in subtidal channels around the perimeter of the treatment areas (-0.5 to -1 m MLLW), in deeper water at mid-channel (-2 to -3 m MLLW) and directly in intertidal drainage creeks on-site (see Figures 6 and 7 for cage locations). Each cage held 15-20 animals (see Section 2.4.2).

Site Cage location	Treatment date	Average mortality (%) (SD)	# cages (N)
Palix River: PR86	7-22-86		
Perimeter		1 (3)	5
Intertidal drainage creek		21 (25)	4
Stony Point: SP86	7-8-86		
Perimeter		0	5
Mid-channel		0	5
Intertidal drainage creek		0	2

Table 8. Results of crab feeding experiments carried out in 1987. Average mortality of crab in cages (20 crab per cage) at three different locations: directly on treatment site, along the perimeter in 2-3 m of water, and at a control site away from the treatment area (NS87, Fig 3). Crab in one set of cages at each location were fed with contaminated mud shrimp, *Upogebia pugettensis*, from a nearby spray site. Means (X), standard deviations (SD), and the number of cages (N) are given for untransformed data.

	Average crab mortality (%)					
	With shrimp			Without shrimp		
Cage location	X	SD	N	X	SD	N
500 m Off-site	24	13	3	5	1	3
Perimeter	3	3	4	8	9	4
On-site	40	14	4	50	22	4

Table 9. Results of crab feeding experiments carried out on 7/2/88 and 7/13/88 along the Palix River channel. Ten crab per cage were fed *Upogebia pugettensis* in both experiments. Mean percent kill and standard deviation (SD) are shown for untransformed data. Refer also to Figs. 3 and 8 for location and design of experiments.

Date	Treatment at which shrimp killed kg/ha (lb/acre)	# replicate cages	Mean % crab kill (SD)	Significance leve
7/2	0 (0)	9	2 (6)	p = 0.001
	8.4 (7.5)	10	38 (20)	
7/13	0	4	0 (0)	p = 0.036
	3.4 (3)	4	10 (8)	
	5.6 (5)	5	2 (4)	
	8.4 (7.5)	4	14 (14)	

treatments. Mortality was variable in this experiment and the mean proportion of crab killed ranged from 2% to 14% among contaminated food treatments (Table 9).

3.4.4 Carbaryl Transport Off-site and Impacts on Crab

Concentrations of Carbaryl Off-site: Site PR88 was sprayed with carbaryl on June 30, 1988 at a rate of 5.6 kg/ha (5 lb/acre) and water samples were taken at each of seven marked stations (on-site, 25, 50, 75, 100, 200 m, and control) during the next incoming flood tide (Fig. 9). Concentrations of carbaryl in water samples (ppm) taken near the bottom (2.5 cm above bottom) at each of these stations are presented for each depth/time interval in Figure 17 (note logarithmic scale), Figure 18, and Appendix Table 9. For clarity, only water samples which were taken near the bottom (where bags holding 0+ crab would be exposed) and which were analyzed using high pressure liquid chromatography (HPLC) are presented (carbaryl concentrations from all surface and bottom water samples analyzed using HPLC and colorimetric procedures are presented in Appendix Table 9). Concentrations are presented for each station until water depth reached 40 cm (approximately 30 min).

Carbaryl was detected in all water samples, including those taken at the control site (0.005 to 0.063 ppm) approximately 800 m away from the treatment site (Figs. 9 and 17). Concentrations fluctuated widely at all stations within 100 m of the site as water depth increased. On-site, concentrations declined from a high of 16.7 ppm (depth = 2.5 cm) to 0.25 ppm within the first 30 min (40 cm; Fig. 17) as the chemical was both moved off-site and diluted by increasing water column depth. At the 100 m station, concentrations increased from 0.018 ppm as the flood tide first reached this point (2.5 cm depth) to 11.6 ppm 30 min later (40 cm) (Figs. 17 and 18). At 200 m off-site, carbaryl concentrations increased from initial levels of 0.003 ppm to 0.92 ppm 30 min later when 40 cm water overlaid this station.

Although concentrations generally declined on-site with time (and depth) after initial flood tide, carbaryl sometimes increased at intermediate distances (25 to 100 m) and ranged between 3-10 ppm 10 min after flood tide first reached these stations (Figs. 17 and 18).

Mortality to 0+ Crab Held in Bags: Nearly all of the 0+ crab held in bags at stations within 100 m of the site were dead when bags were sampled 24 h after treatment, with average mortalities of 100, 83, 98, 100 and 100%, respectively at the on-site, 25, 50, 75, and 100 m stations (Fig. 19). Average mortality was significantly lower ($p < .0001$; 1-way ANOVA) at the 200 m and control sites, which had 8.5 and 4.3% average crab mortality, respectively.

3.5 RECOLONIZATION OF CRAB ON SITES AFTER CARBARYL TREATMENT

3.5.1 Recolonization and Post-spray Effects

Post-Spray Cage Experiment: Bags holding 0+ crab were placed on site PR88 24 h after treatment and at an untreated control station (Fig. 9) and retrieved 14 days later. Average survival was 77% in bags which were held on the treatment site compared to 90% survival in bags held on the control site (Fig. 20). While the number of crab surviving in bags on PR88 was lower than those found on the control, differences in average survival between the two were not significant ($p > .05$; two-sample t-test).

Crab Density on Sites After Treatment: There was some evidence of recolonization on sites during the months following treatment. Live 0+ crab were found on treatment sites during sample periods 2, 4, and 6 weeks after treatment in 1986 and 1987 (Fig. 15, see SP86 and PR86 in 1986; Fig. 16A, B, C). The level of recolonization differed by site: Site PR87 had post-spray densities in HE of 1-2 crab/m^2, which were comparable to pre-spray levels (Fig. 16B), while average post-spray density at treatment site NP87 (which had densities within HS that were similar to reference

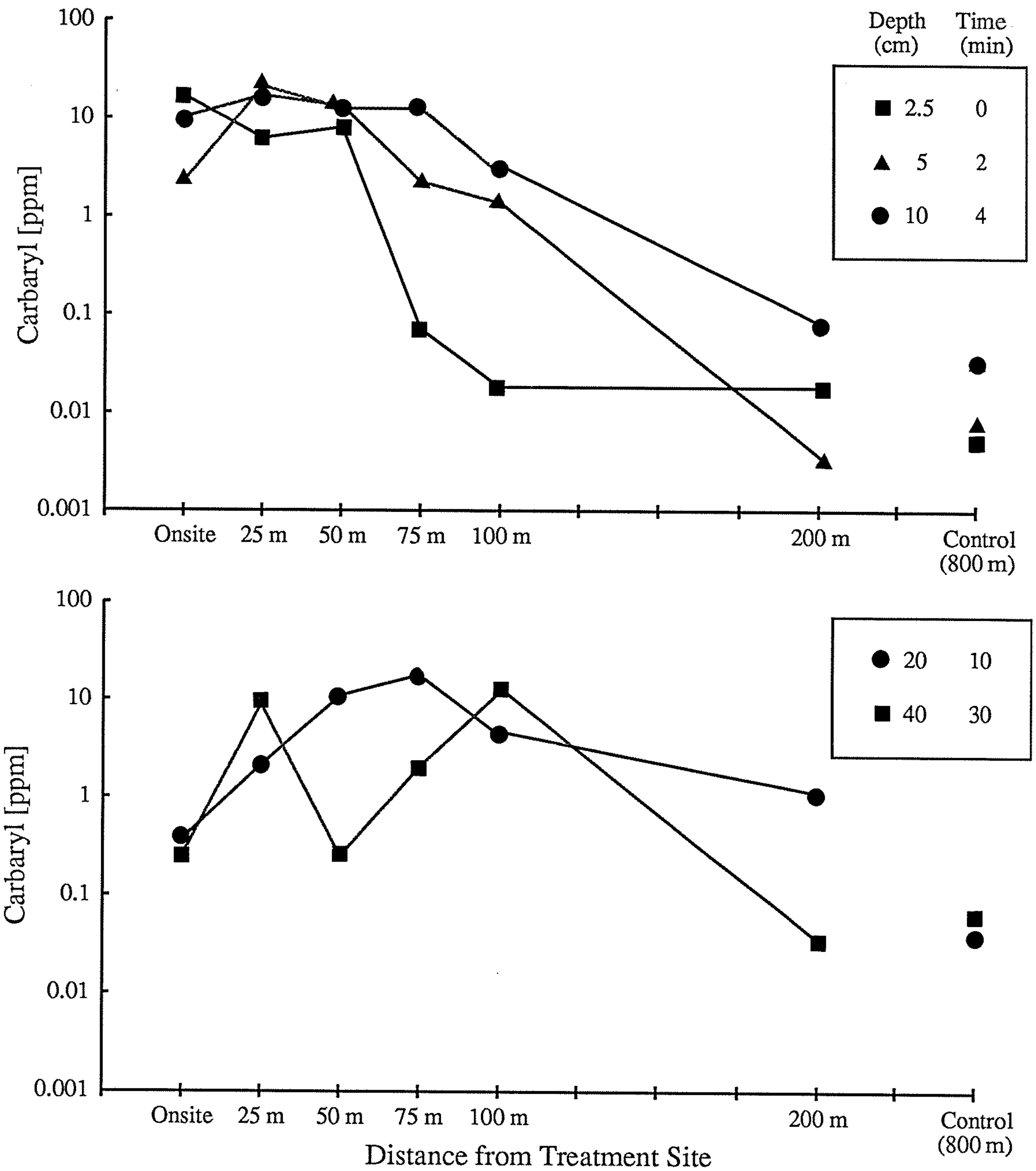

Figure 17. Carbaryl concentrations (ppm) by distance from treatment site taken from water samples 2.5 cm from the substrate next to bags holding 0+ crab at treatment site PR88. Symbols are as follows: depth—cumulative water column depth over each station (distance from site) as sequential water samples were taken during the flood tide; time—approximate elapsed time required for a given water depth at each station (e.g., bottom water samples were taken when 20 cm of water overlay each station in sequence, which took 10 min after flood tide first reached that point).

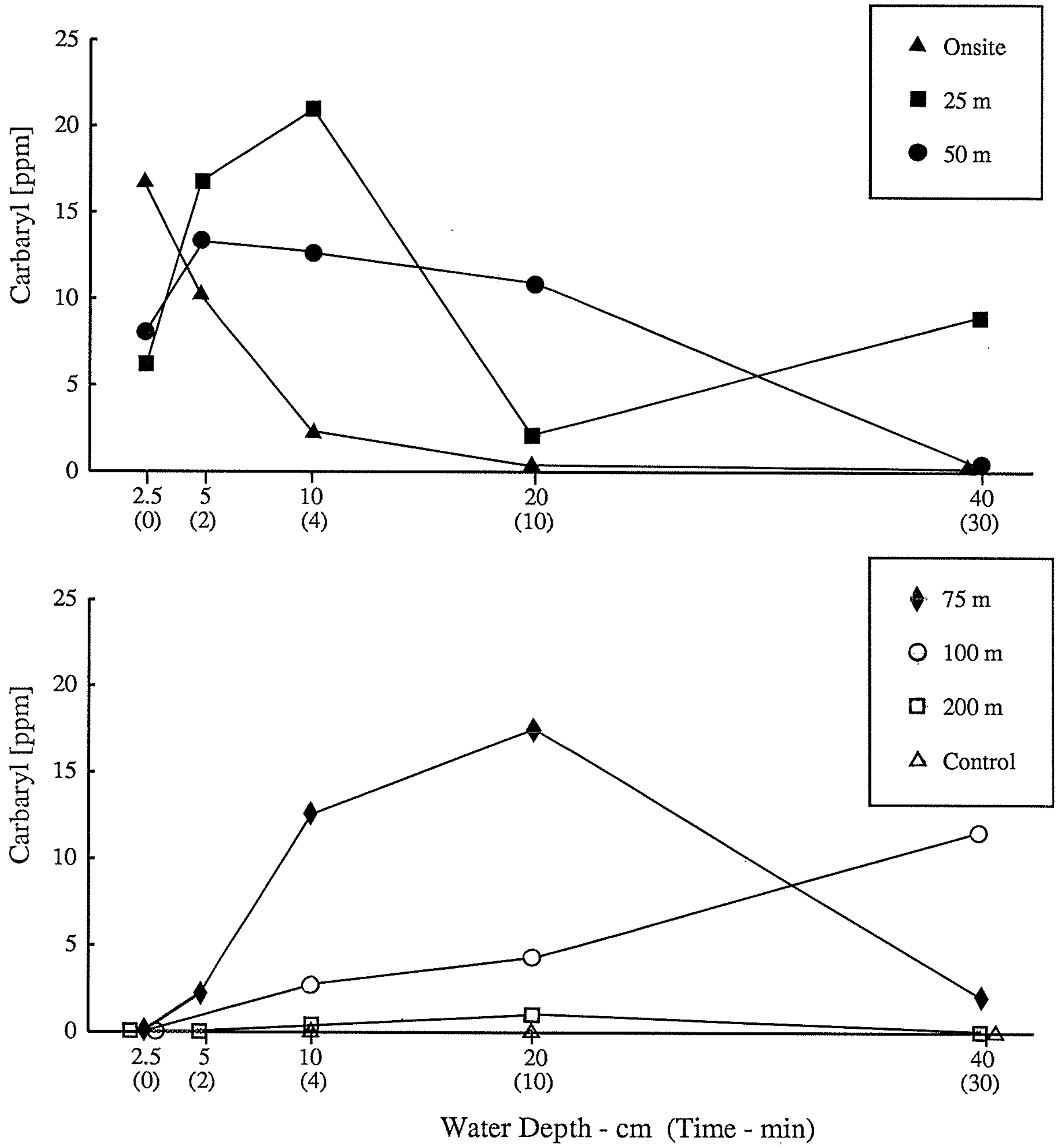

Figure 18. Changes in carbaryl levels (ppm) by water depth during flood tide taken from samples 2.5 cm from the substrate next to bags holding 0+ crab at treatment site PR88.

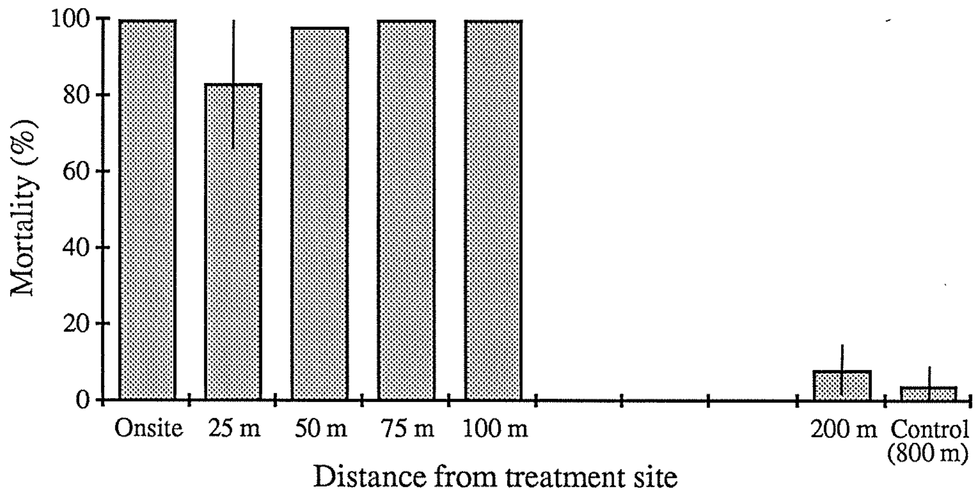

Figure 19. Average mortality (±1 SD) of 0+ crab held in bags on treatment site PR88 and at stations located 25, 50, 75, 100, and 200 m from the site in the path of the incoming flood tide, and at a control site 800 m away from the treatment site (n = 5 bags per station and 10 crab per bag).

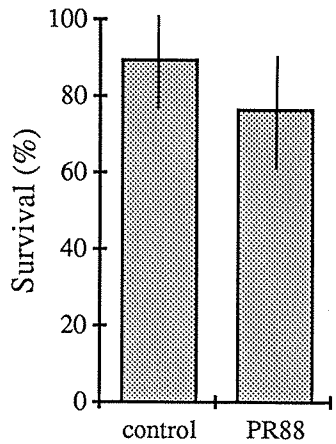

Figure 20. Average survival (±1 SD) at the end of 14 days of 0+ crab placed on treatment site PR88, and at a control site 24 h after site PR88 was treated with carbaryl (n = 6 bags per location and 10 crab per bag).

site NHC prior to spray) remained near zero in subsequent months (Fig. 16C). Crab found on these sites after treatment were generally larger 0+ ranging from 25-35 mm CW, although some newly settled crab were occasionally found in samples.

3.5.2 Utilization of Treatment Sites by Crab in Subsequent Years

Recruitment and Crab Density on Long Term Study Sites: There was strong evidence of 0+ crab recruitment and utilization of treatment sites in years following treatment. Crab populations were periodically monitored at Sites SP86 and PR86 throughout the summer of 1987 (1 year after treatment). 0+ Crab recruited onto both sites during May and June and were found associated with epibenthic material throughout the summer (Fig. 15); similar trends were observed at SP86 during 1988 as well (Fig. 15).

Stratified estimates of the number of crab/ha on sites PR86 and SP86, both at the time of treatment during July 1986 and during corresponding periods 1 to 2 years following treatment, are presented in Tables 10 and 11, respectively. Estimates of crab abundance on both sites were higher in the year following treatment, although the reasons for higher abundance differed by site. Differences in crab abundance between 1986 and 1987 at site PR86 were due primarily to changes in the amount of epibenthic oyster shell covering the site (2-year-old oyster were transplanted onto the site after treatment; see Section 3.2.2, Figure 14D, and Table 10). Average crab densities within the habitat categories were similar both years; however, increases in both LS and HS cover between 1986 to 1987 resulted in estimates of crab abundance/ha (12,960/ha) 1.5 times higher than the previous year (8,560 crab/ha; Table 10).

At site SP86, interannual differences in crab abundance were due primarily to differences in crab density within the predominant habitat (LS). The estimated number of crab at SP86 at the time of treatment on July 8, 1986 was 17,530 crab/ha (Table 11), but the following year, on July 10, 1987, the estimated number of crab on the site was nearly twice as high (34,070 crab/ha). While the amount of epibenthic refuge (primarily oyster shell) on the site increased slightly from original 1986 levels, higher estimates of crab on SP86 during 1987 were due primarily to the high average density observed in LS ($4.8/m^2$) compared to the previous year ($2.5/m^2$). Relatively low crab densities were measured in LS during July 15, 1988 resulting in estimates of abundance which were 2-4 times lower than observed the previous 2 years.

4.0 DISCUSSION

4.1 INTERTIDAL ECOLOGY OF JUVENILE DUNGENESS CRAB AND THE IMPORTANCE OF COMMERCIALLY CULTURED OYSTER AS REFUGE

This study showed that intertidal habitats of Willapa Bay are used by large numbers of Dungeness crab ranging in size from 6-35 mm CW, which suggests that these areas are important as nurseries for newly settled crab. The spatial and temporal patterns of 0+ crab on the intertidal are very similar to those observed in Grays Harbor by Armstrong and Gunderson (1985) and Dumbauld and Armstrong (1987). Recruitment of the new year class occurred primarily during May and June of each year and abundance in all habitat categories peaked during this period. Recruitment was followed by high natural mortality and a rapid decline in abundance, probably due to intense predation of the early benthic stages by fish and conspecifics (Stevens 1982; Armstrong and Gunderson 1985; Dinnel et al. 1986; Dumbauld and Armstrong 1987). Densities stabilized during July and August and size frequency patterns indicate that many of the surviving crab had attained sizes of 20-30 mm CW (I4-6), suggesting that growth was rapid. The absence of crab

Table 10. Estimated number of 0+ crab killed/ha by carbaryl treatment during July, 1986 by habitat category (stratum) at site PR86 and estimated number of new recruits during the same period in July 1987 1 year after treatment. Estimates were derived from average crab densities from quadrat samples at the time of carbaryl application and during the corresponding period 1 year after treatment (see Appendix Table 1) and, from relative cover estimates for each year from annual transect surveys (Fig. 14D).

Date	Habitat category[a]	Ground cover # m^2/ha	Crab density # crab/m^2	Estimated # crab/ha
7/22/86	OP	1,700	0.0	0
	LE	1,800	0.4[b]	720[b]
	HE	3,400	0.4	1,360
	LS	3,000	2.0	6,000
	HS	100	4.8	480
Total estimated number of crab/ha killed by carbaryl treatment =				8,560 (all killed)
7/26/87	OP	1,500	0.0	0
	LE	400	0.4[b]	160[b]
	HE	1,100	0.4	440
	LS	5,400	1.2[b]	5,480[b]
	HS	1,600	4.3	6,880
Total estimated number of crab/ha =				12,960 (new recruits)

[a]Refer to Table 2 for definitions of habitat categories.
[b]Indicates habitat categories which were not sampled for crab at the time of treatment. Crab density values used to derive estimates of kill/ha for each missing habitat category were computed by using the average density of the habitat from other sites during the same period.

Table 11. Estimated number of 0+ crab killed/ha by carbaryl treatment during July 1986 by habitat category (stratum) at site SP86 and estimated number of new recruits during the same period in July 1 and 2 years after treatment (1987 and 1988). Estimates were derived from average crab densities from quadrat samples at the time of carbaryl application and during corresponding periods 1 and 2 years after treatment (see Appendix Table 1) and, from relative cover estimates for each year from annual transect surveys (Figure 14A).

Date	Habitat category[a]	Ground cover # m^2/ha	Crab density # crab/m^2	Estimated # crab/ha
7/8/86	OP	3,300	0.0	0
	LE	200	0.4[b]	80[b]
	HE	0	0	0
	LS	6,100	2.5	15,250
	HS	400	5.5	2,200
Total estimated number of crab/ha killed by carbaryl treatment =				17,530 (all killed)
7/10/87	OP	2,000	0.0	0
	LE	100	0.7[b]	70[b]
	HE	200	0.7[b]	140[b]
	LS	6,700	4.8	32,160
	HS	1,000	1.7	1,700
Total estimated number of crab/ha =				34,070 (new recruits)
7/15/88	OP	2,200	0.0	0
	LE	200	0.0	0
	HE	300	0.7[b]	210[b]
	LS	6,000	0.7	4,200
	HS	1,200	3.0	3,600
Total estimated number of crab/ha =				8,010 (new recruits)

[a]Refer to Table 2 for definitions of habitat categories.
[b]Indicates habitat categories which were not sampled for crab at the time of treatment. Crab density values used to derive estimates of kill/ha for each missing habitat category were computed by using the average density of the habitat from other sites during the same period.

greater than 25-30 mm CW through the summer in the intertidal suggests that small juveniles are either not surviving beyond this size range or are moving into subtidal areas. The vulnerability to predation by demersal fish rapidly declines as crab exceed this size range (Reilly 1983), and evidence from Grays Harbor suggests that crab greater than 25 mm CW leave intertidal habitats and migrate into adjacent subtidal channels (Dumbauld and Armstrong 1987). Consequently, low numbers of larger 0+ crab in intertidal samples may reflect movement into channels as they grow to sizes which provide some protection from predation.

Abundance and early survival of small crab following settlement was dependent on the presence of suitable intertidal habitat. Nearly all 0+ juvenile crab found after initial settlement were associated with either oyster shell or eelgrass, but rarely were found in open, unprotected, areas of mud or sand. The relative importance of epibenthic cover within shallow subtidal and intertidal regions for the survival of newly settled juvenile crustaceans has been widely reported for Dungeness crab (Stevens and Armstrong 1984; Armstrong and Gunderson 1985; Dinnel et al. 1986; Dinnel et al. 1987; Dumbauld and Armstrong 1987) as well as for other decapods, such as blue crab, *Callinectes sapidus* (Heck and Orth 1980a; Orth et al. 1984; Zimmerman and Minello 1984; Orth and van Montfrons 1987) and brown shrimp, *Penaeus aztecus* (Zimmerman and Minello 1984). It is widely believed that the abundance and distribution of these juvenile crustaceans is largely determined by predation, and that epibenthic cover enhances survival by providing these animals with a structurally complex shelter which mediates this intense predation (e.g., Heck and Orth 1980b; Orth et al. 1984; Armstrong and Gunderson 1985; Knieb 1987). Recent experimental studies support this belief. In tethering experiments, predation on juvenile spiny lobster (*Panulirus argus*) was highest on open sand and substantially reduced in algal clumps, seagrass, and dense algal meadows (Herrnkind and Butler 1986). Similar experiments with juvenile blue crab indicate significantly lower predation in vegetated areas compared to open sand (Wilson et al. 1987).

Although it was evident that both eelgrass and shell serve as habitat for early juvenile stages of *C. magister*, studies in Grays Harbor have shown that shell is more important for the survival of 0+ crab than eelgrass (Armstrong and Gunderson 1985; Dumbauld and Armstrong 1987), and results from Willapa Bay further substantiate these observations. In general, "heavy" and "light" oyster shell categories supported consistently higher numbers of crab throughout the summer than either category of eelgrass. This pattern was evident on sites where both habitats occurred interspersed, and over widely separated locations within the estuary, suggesting that differences in relative density were due to the nature of habitat cover rather than differential settlement of megalopae over broader spatial scales within the estuary. To some extent these results suggest that crab may prefer oyster shell over eelgrass since density at earliest settlement was usually higher in the former. While the reasons for this apparent preference are not known, oyster shell may afford a greater degree of protection from predation at both high tide and low tide, and also reduce exposure to air and temperature at low tide by providing a more structurally complex habitat for the small crab to hide within than do the turions of *Z. marina*. Regardless of the reasons for the differences in density between shell and eelgrass, the consistent pattern of higher densities in oyster shell underscores the importance of this mollusc for the survival of newly settled 0+ crab.

Transect surveys indicate that oyster shell distributed over littoral flats at commercial levels can provide a substantial amount of prime habitat for 0+ crab. The combined area of "heavy" and "light" shell categories on study sites under active culture ranged from 40 to 95%, with an average of 74% of ground covered with shell (n = 11). Light shell was the predominant category of cover on nearly all of the beds, and accounted for most of the crab present. Heavy shell generally supported somewhat higher levels of crab, but the relative proportion of ground covered was much lower, particularly on first year seed beds. The extent and relative importance of heavy shell was greater on fattening beds of 2- to 3-year-old oyster and increased on seed beds in subsequent years as oysters grew, thereby accounting for a greater portion of crab on these sites. Stratified estimates of crab abundance on several commercial sites suggest that oyster beds will support higher numbers of crab than sites covered with comparable amounts of eelgrass.

Dumbauld and Armstrong (1987) reported that intertidal shell supported a greater portion of the 0+ age class through most of the summer compared to subtidal regions of Grays Harbor. Similarly, comparisons in Willapa Bay suggest that commercial oyster beds can support more 0+ crab than adjacent subtidal channels. This pattern was evident, for example, when direct estimates of 0+ crab density/ha at Site SP86 were compared with average crab densities in subtidal trawls taken from the same region as a part of another study (Armstrong unpublished data for Palix River/Stony Point geographical area, Sea Grant Stratum 1) through the summer of 1988 (Fig. 21). At the time of settlement in May, average densities in the intertidal were 100,000 crab/ha, almost fiftyfold higher than densities measured in adjacent subtidal channels. The trend of higher intertidal density continued through late July and, although differences decreased, were still nearly an order of magnitude greater. While differences in abundance between the oyster bed and the adjacent channels may be due, in part, to inefficiencies of the trawl gear used in subtidal samples (e.g., Dinnel et al. 1986), the presence of large numbers of older crab and fish in subtidal channels suggests that the risks of cannibalism (Stevens et al. 1982) and predation (Gunderson et al. 1989) are high in these areas. Consequently, higher 0+ crab abundance in the oyster bed may reflect benefits of enhanced survival afforded by intertidal shell cover. While the preceding example was for just one site, similar high intertidal crab abundance at other oyster beds suggests that this pattern is consistent at other areas of the bay as well.

It is apparent from this study that shell is of primary importance for survival of newly settled crab, and that oyster planted in commercial quantities provides a substantial amount of cover for newly recruited crab. In Willapa Bay, estimates of the total area of ground covered with oyster range between 2,400 and 4,000 ha (6,000-10,000 acres; Cheney et al. 1986; WDF and WDOE 1989). Given the total area of ground under commercial culture, the oyster industry indirectly provides substantial benefits to juvenile crab through placement and maintenance of shell habitat in the intertidal region.

4.2 IMPACTS OF CARBARYL ON CRAB

4.2.1 On-site Crab Mortality

A major objective of this study was to examine the magnitude and extent of impacts to crab on and around intertidal areas treated with carbaryl. The most notable of these impacts was observed directly on treated sites where large numbers of 0+ crab were found associated with epibenthic refuge. Mortality of crab on-site and directly exposed to carbaryl appears to be virtually 100% based on several lines of evidence. No living crab were ever found in samples collected from treatment sites 24 h after application (only dead animals). In addition, experiments conducted in 1988 (Site PR88) indicate that all 0+ crab held in bags were dead when examined 24 h after treatment, while nearly all crab held at a control site were alive and active. Carbaryl concentrations in water samples collected near the bags as the tide flooded over the site indicate that crab were exposed to levels as high as 16.7 ppm (WDA, Appendix Table 9), a concentration more than an order of magnitude higher than the 24 h EC_{50} of 0.35-0.70 ppm reported for 2nd instar (10-12 mm CW) crab by Buchanan et al. (1970).

The magnitude of crab killed on-site differed according to both the type and extent of refuge present and average crab densities within each habitat type at the time of treatment. All of the sites examined had some form of epibenthic refuge present, but varied in the extent of each type. Consequently, estimated kills of 0+ crab on sites ranged widely from 4,000 to 18,000 crab/ha, with

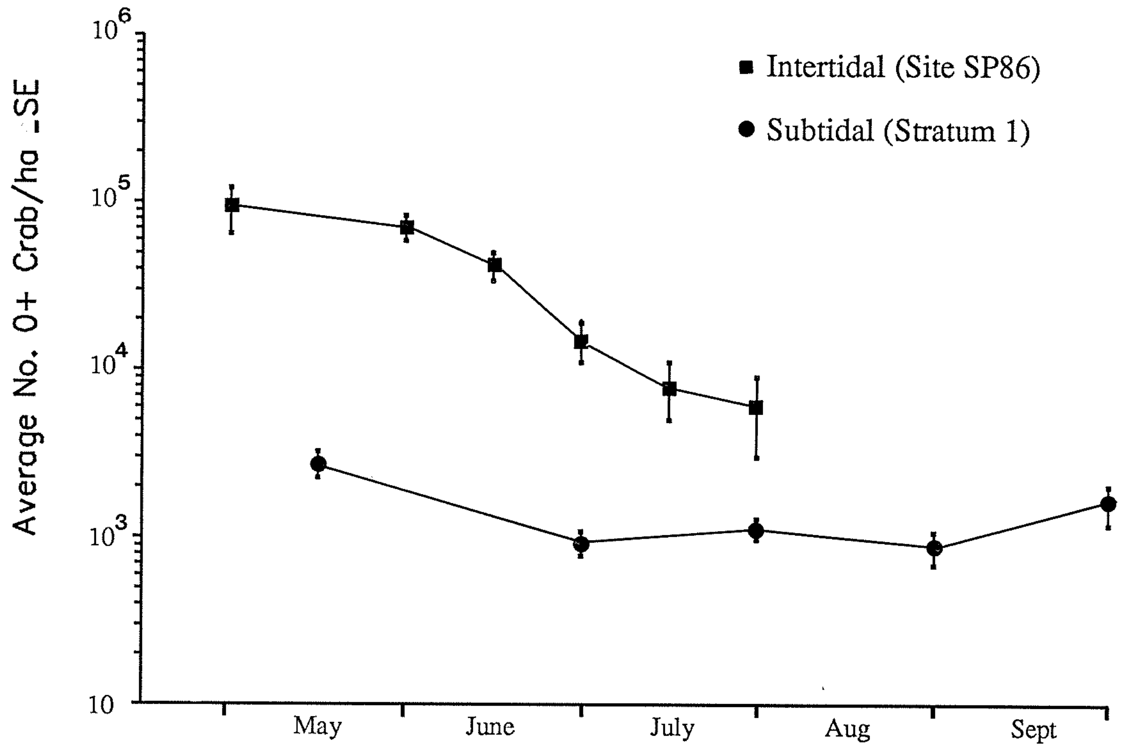

Figure 21. Comparison of mean densities of 0+ crab (crab/ha) during the summer of 1988 on intertidal study site SP86 (based on direct estimates of abundance from this study) and in subtidal channels taken from the same area near Stony Point during 1988 as part of another study (Armstrong and Gunderson, Univ. Washington unpublished data for Sea Grant Stratum 1).

highest crab kills observed on beds which had a high percentage of coverage as shell (beds covered with seed oyster). Although several sites were covered with substantial amounts of eelgrass at the time of spray, estimated kills were lower on these areas owing to low average crab densities found in eelgrass.

In addition to 0+ crab killed on the sites at the time of treatment, some dead 1+ and older juveniles were evident in limited intertidal trawls conducted over the sites during flood tide, and mortalities of 1+ crab held on several sites for 24 h after treatment ranged from 0 to 50%. These results suggest that older crab which move onto contaminated sites to forage during the flood tide (a behavior described by Stevens et al. 1982) after treatment can be killed, probably from direct exposure to water and/or sediments, and ingestion of contaminated food. Similar conclusions have been previously reported by WDF based on presence of dead 1+ and 2+ crab on sites in 24 h post-spray assessments (Creekman and Hurlburt 1987; Tufts 1989).

4.2.2 Off-site Impacts to Crab

Results of subtidal trawls and experiments with caged 1+ crab suggest that the off-site mortality to older 1+ crab, which reside in subtidal channels, is low. Very few dead 1+ crab were found in subtidal trawls conducted adjacent to sites during the period between 6 and 24 h after treatment, or in cages placed along perimeters and channels immediately adjacent to treatment sites. Low mortalities suggest that any carbaryl carried off of treatment sites may have been diluted enough in channels to minimize toxic exposure to larger crab, which are not as sensitive as to carbaryl as are 0+ stages (Buchanan et al. 1985). It is also possible that direction of incoming tide prevented exposure to crab in channels by keeping the animals upstream of any pesticide carried off-site.

While there was little evidence of mortality of older crab in the subtidal channels, the carbaryl transport experiment conducted at Site PR88 during 1988 (Section 3.4.4) indicates that carbaryl can be carried substantial distances from treated sites and adversely impact 0+ crab in adjacent intertidal areas. Most 0+ crab held in bags within 100 m of the treatment site down current of flood tide were dead when examined 24 h after treatment. During the first 30 min, these crab were exposed to concentrations of carbaryl which were over an order of magnitude higher than reported EC_{50} values (Buchanan et al. 1970). Crab mortalities at 200 m and control (800 m) locations were significantly lower than those within 100 m of the site indicating that carbaryl from the site had been diluted to sublethal concentrations.

Although this experiment confirms that crab will be killed off-site, quantitative estimates of the magnitude and extent of kill are difficult because the full two-dimensional spread of pesticide by flood tide cannot be calculated from these data. Furthermore, the distance at which impacts were observed at test site PR88 should not be considered indicative of what will happen at other sites. The area impacted off-site will depend upon the path of transport and how rapidly the pesticide is diluted by the incoming tide. Similar transport studies conducted by WDF (Creekman and Hurlburt 1986; Tufts 1989) indicate that patterns of transport and dilution differ by site and initial dosage, suggesting that areal impact off-site is site-specific and dependent on local tidal patterns. Since the relative abundance of crab in the intertidal region is dependent upon the type and extent of refuge present, the magnitude of off-site crab kill will depend not only upon how rapidly the pesticide is diluted, but also on the type of habitat over which the chemical is transported.

4.2.3 Feeding Impacts

Although the results of feeding experiments were highly variable, significant differences in crab mortality were observed between animals fed contaminated shrimp or uncontaminated shrimp, indicating that crab can be killed by ingestion of contaminated shrimp. Similar results have been observed in laboratory experiments conducted by Creekman and Hurlburt (1986) who concluded that older crab found dead on sites by WDF 24 h after treatment may have been poisoned by foraging on contaminated shrimp. While mortality of crab off-site may be caused by contaminated shrimp, there are currently no methods for determining how many shrimp are carried off sites after exposure to treatment.

4.2.4 Post-spray Effects

Preliminary evidence suggests that carbaryl impacts are short term after spray. Over 75% of 0+ crab placed in bags on site PR88 24 h after treatment were alive when recovered 2 weeks later. Although survival was slightly higher at the control site, differences were not significant and suggest that some crab may be able to survive on treatment sites as early as 24 h after spray. These results should be viewed as tentative since bags may have allowed only partial contact with contaminated sediments. However, evidence of recolonization on some sites provides further evidence that crab may survive when they recruit or migrate onto sites soon after treatment.

4.3 Relative Impacts of Carbaryl Treatment on the Estuarine Crab Population

On the basis of available evidence, it appears that the vast majority of crab killed on intertidal sites sprayed with carbaryl are 0+ juveniles associated with epibenthic refuge, and to a lesser extent, older 1+ and 2+ crab which move onto these sites to forage during flood tides immediately following treatment. While there was some evidence of off-site impacts on crab, particularly to 0+ crab in intertidal areas adjacent to sites, these effects are very difficult to quantify because of the site-specific differences in habitat, current direction and amplitude.

It is apparent that substantial numbers of crab are killed on treatment sites. A central question surrounding the annual application of carbaryl in Willapa Bay concerns the relative importance of cumulative mortality on the total intertidal area treated annually (up to 240 ha) compared to the total crab population residing within the estuary. As noted above, impacts appear to be confined primarily to 0+ crab both on-site and for some distance off-site at the time of treatment, and to 1+ and older juvenile crab which move onto sites during flood tides after treatment. These two general age groups (0+; 1+ and older) are considered separately in the following discussion.

4.3.1 Impacts on 0+ Crab Population

In order to quantify impacts on the 0+ crab population, it is necessary to estimate the total intertidal population of 0+ crab on oyster ground throughout the estuary at the time of spray, as well as estimate the total number of crab killed by treatment. Since intertidal crab abundance is directly related to the type and extent of available habitat, estimates of the total areal extent of intertidal habitat are required in order to extrapolate abundance values to the entire estuary. Currently, there are no reliable estimates of total area covered with eelgrass or shell within the estuary. However, the total area of ground under commercial culture can be estimated based on industry records, and the actual area of ground treated annually with carbaryl is also known from WDF/WDOE permits and monitoring activities. This information, along with measures of crab density within oyster shell, patterns of oyster coverage from transect surveys, and information regarding commercial growing practices can be used to derive estimates of the population within oyster beds as compared to losses associated with treatment of a portion of this ground.

Of particular interest are estimates of intertidal 0+ crab abundance in commercial oyster beds at the time of treatment which typically occurs in July. Data from 1986-88 are used in the following discussion as examples. In order to calculate the abundance of crab within oyster beds under active culture in Willapa Bay during the July 1988 treatment period, the following steps were taken:

The first step was to estimate the amount of ground under active culture. About 4,000 ha (10,000 acres) of ground are currently used by the oyster industry and around 2,480 ha (6,200 acres) of this area are classified as prime grounds used for the most intensive oyster production, while remaining ground is only partially utilized (Cheney et al. 1986; WDF and WDOE 1989; interviews with oyster growers). Assuming a conservative estimate of 2,400 ha under cultivation and an average harvest cycle of 3 years, then approximately 1/3 (800 ha) of the beds are first year seed and the remaining 2/3 (1,600 ha) are beds covered with 2- and 3-year-old oyster.

The second step was to estimate cover correction factors for typical oyster beds, differentiating between beds covered with first year seed and with 2-3 year oyster. Transect surveys of 3 oyster beds covered with 1 year seed (SP86, SP87 and SP88) resulted in average cover estimates of 54% as light shell (LS) and 2% as heavy shell (HS; see Section 3.2.1). Average cover from transect surveys on beds of 2-3 year oyster were 57% for LS and 17% for HS (n = 9). Although the number of sites sampled represent only a fraction of the oyster beds in the bay, interviews with oyster growers indicate that most growers plant their beds at very similar levels. Transect surveys show that coverage is very similar at most sites, so for this calculation we are assuming that average values from transect surveys are representative of all other sites.

Finally, average crab densities were determined for HS and LS from all sites for the period in early July 1988 when the majority of bed were treated. These densities were then multiplied with correction factors for area and cover for first year seed and 2-3 year oyster from steps 1 and 2 above to derive estimates of the crab population associated with oyster.

The number of 0+ crab killed by treatment were estimated using the total amount of ground actually sprayed during 1986, 1987, and 1988 (144, 145, and 111 ha, respectively; WDF and WDOE 1989). Recall from Section 1.1 that, while up to 240 ha of ground may be treated, spraying may be terminated short of this amount if the crab kill quota is exceeded on less ground. In order to calculate the total number of 0+ crab killed by treatment during each year losses were calculated following procedures similar to those described above with the following additional assumptions:

Firstly, 0+ crab is highest on areas covered with seed oyster as suggested by estimates of abundance on-site, and that all beds treated were seed. Although only a portion of sites annually treated were covered with seed at the time of spray (average 44% for period from 1984-88; WDF and WDOE 1989), nearly all "open" sites examined had some degree of refuge present at the time of treatment. This assumption (using cover corrections of 54% as LS and 2% as HS for seed as described above) should overestimate crab abundance on-site and compensate for variations in cover between sites and in part for impacts to 0+ crab killed in areas adjacent to treatment sites by off-site transport.

Secondly, the entire portion of ground was assumed to be treated during early July, although portions were actually treated later in the month. Average densities were higher in both HS and LS during early July, so this assumption should somewhat overestimate actual numbers killed and, like the preceding assumption, partially offset any crab killed off-site.

Using the preceding assumptions and the total area treated during each of the 3 years, the total crab killed was then calculated using cover correction factors for seed and average densities for HS and LS as described above.

<u>0+ Crab Population on Commercial Oyster Beds and Losses Incurred by Carbaryl Treatment</u>: An estimated population of 40.9, 75.7, and 35.5 million 0+ crab were present on 2,400 ha of commercial oyster beds at the time of treatment in early July 1986, 1987 and 1988, respectively (Table 12). The treatment of 144, 145, and 111 ha of this ground killed an estimated 1.7, 3.7, and 1.3 million 0+ crab during 1986, 1987, and 1988; respectively (Table 13). Comparisons of

Table 12. Estimates of 0+ crab population in Willapa Bay on 2,400 ha (6,000 acres) of oyster ground under active culture during early July 1986, 1987 and 1988.

Oyster bed area (ha)[a]	Shell habitat category[b]	Cover correction factor[c]	Corrected area (ha)	Corrected area (m^2)	Average density (crab/m^2)[d] 1986	1987	1988	Estimated 0+ crab population (millions) 1986	1987	1988
First Year Seed										
800	LS	0.54	432	4,320,000	2.0	4.8	2.0	8.6	20.7	8.6
	HS	0.02	16	160,000	4.9	3.9	3.0	0.8	0.6	0.5
2-3 Year Oyster										
1600	LS	0.57	912	9,120,000	2.0	4.8	2.0	18.2	43.8	18.2
	HS	0.17	272	2,720,000	4.9	3.9	3.0	13.3	10.6	8.2
Total estimated intertidal 0+ crab population on 2400 ha of oyster beds (1986-88)								40.9	75.7	35.5

[a]Area based on estimated total area of 2400 ha of which 1/3 is first year seed and the remaining 2/3 is 2-3 year oyster.
[b]Oyster cover categories where LS = shell cover from 10-49% and HS = shell cover ≥50%.
[c]Correction factors derived from average cover from transect surveys on seed beds (n = 3 sites) and beds covered with 2-3 year oyster (n = 9 sites).
[d]Average crab densities for each category from all sites sampled during early July 1986, 1987 and 1988.

Table 13. Estimates of 0+ crab killed on oyster ground treated with carbaryl in Willapa Bay during early July 1986, 1987 and 1988.

Area of ground sprayed (ha)[a]	Shell habitat category[b]	Cover correction factor[c]	Corrected area (ha)	Corrected area (m^2)	Average density (crab/m^2)[d]	Estimated 0+ crab population (millions)
1986						
144	LS	0.54	77.8	778,000	2.0	1.6
	HS	0.02	2.9	29,000	4.9	0.1
Total estimated intertidal 0+ crab population killed on treated oyster beds during 1986 =						1.7
1987						
145	LS	0.52	75.4	754,000	4.8	3.6
	HS	0.02	2.9	29,000	3.9	0.1
Total estimated intertidal 0+ crab population killed on treated oyster beds during 1987 =						3.7
1988						
111	LS	0.54	59.9	599,000	2.0	1.2
	HS	0.02	2.2	22,000	3.0	0.1
Total estimated intertidal 0+ crab population killed on treated oyster beds during 1988 =						1.3

[a]Total area of ground actually treated during 1986-1988 from WDF and WDOE (1989).
[b]Oyster cover categories where LS = shell cover from 10-49% and HS = shell cover ≥50%.
[c]Correction factors derived from average cover from transect surveys on seed beds (n = 3 sites) and beds covered with 2-3 year oyster (n = 9 sites).
[d]Average crab densities for each category from all sites sampled during early July 1986, 1987 and 1988.
[e]Assumes that all ground treated was covered with seed oyster at the time of treatment.

0+ crab killed on sprayed ground with total population associated with commercial oyster during 1986-88 (Fig. 22) indicates that relative losses were 4 to 5% annually. If the maximum allotment of ground (240 ha) had been sprayed during these years, then around 8% of the resident 0+ population would have been killed annually. It is apparent that, given these levels of kill, the impact to the 0+ crab population may be significant. However, it should be noted that estimates of total abundance are just for 2,400 ha of commercial oyster under active ground culture and, in terms of relative kill, these values probably represent a "worst case" loss to the population. The 2,400 ha of actively cultured ground in Willapa Bay represents only a fraction (15%) of the total intertidal area of the estuary (16,000 ha). While there are currently no reliable estimates of the total area of suitable refuge available, substantial amounts of eelgrass and additional intertidal shell are present on the remaining 13,600 ha of intertidal flats. In addition, a substantial number of 0+ crab reside in subtidal channels even though such channels do not support as many crab as the intertidal region (Section 4.1; Dumbauld and Armstrong 1987). At the time of treatment in July 1988, for example, the estimated subtidal population of 0+ crab, determined from subtidal trawls taken as a part of an extensive population survey of the estuary, was over 3.7 million crab (Armstrong and Gunderson; University of Washington unpublished data). Consequently, if the additional 0+ crab residing in remaining intertidal and subtidal areas are also considered, then relative crab kill would be substantially lower.

Patterns of abundance on reference sites indicate that natural mortality of newly settled crab was extremely high. Studies in Grays Harbor suggest that only about 3.3% of the 0+ crab will survive the first year, and expected survival to reach the fishery (at 3-4 years of age) is about 0.4% (Armstrong et al. 1987). At these rates of survival, it is apparent that the majority of 0+ crab killed by treatment would not have survived to reach the fishery anyway. For example, of the estimated 1.3 million 0+ crab killed in 1988, fewer than 6,000 crab would have survived to reach the fishery. Assuming that 50% of these crab were males, then expected losses to the future commercial fishery will be about 3,000 crab.

4.3.2 Impacts to 1+ and Older Juvenile Crab Population

In terms of overall impacts to the fishery, loss of older 1+ crab may be more important because, having survived beyond the first year of life, their prospects for surviving to fishery age/size are much better. Currently, the best available estimates of the total number of 1+ and older crab killed by treatment are from 24 h post-spray transects conducted by WDF on sites following treatment. Relative impacts of carbaryl application on the population of older juveniles were determined using WDF mortality estimates for the years 1986-1988 (from Tufts 1989 and WDF unpublished data), and July estimates of estuarine abundance for 1+ and older crab from subtidal trawls taken as a part of extensive population surveys of the estuary (from Armstrong and Gunderson; University of Washington unpublished data) corresponding to the periods of treatment. The results of these comparisons suggest that less than 0.3% of the older crab population was killed by treatment during each of the 3 years from 1986-1988 (Table 14). Such a small loss from the 1+ population probably does not represent significant losses to the fishery.

4.4 OVERALL PERSPECTIVE OF CARBARYL IMPACT ON ESTUARINE CRAB POPULATIONS

At issue in this research is whether or not the use of carbaryl in Washington State estuaries significantly impacts the crab resource and, in turn, the fishery. Results of this study have shown the importance of habitat, primarily shell, for newly settled crab. In Willapa Bay, a major source of epibenthic shell is provided by the commercial oyster industry. The majority of crab killed by

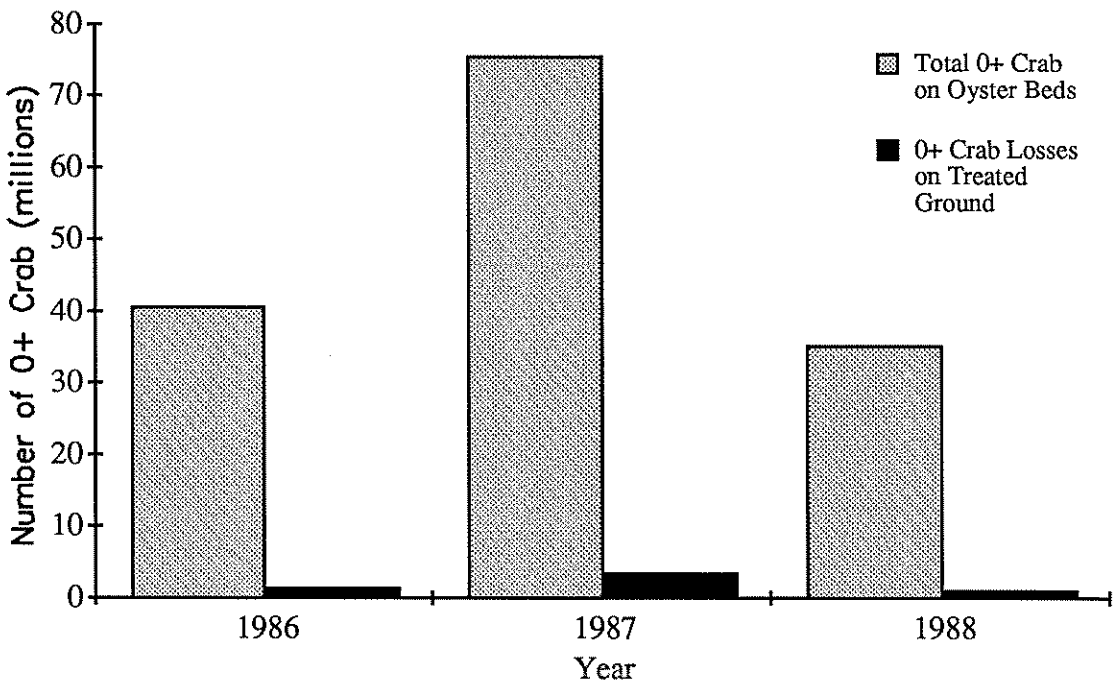

Figure 22. Comparison of estimated total 0+ crab abundance on 2,400 ha of oyster ground under active culture in Willapa Bay and estimates of 0+ crab killed on oyster ground treated with carbaryl during early July 1986, 1987, and 1988. Refer also to Tables 12 and 13.

Table 14. Percentage of total Willapa Bay crab population (≥1+ juveniles) killed by carbaryl, using WDF 24-h on-site transect estimates of ≥1+ crab killed (Tufts 1989; WDF unpublished data) and University of Washington trawl estimates of the total population of ≥1+ crab present (Armstrong and Gunderson unpublished data) during July treatment period of each year.

Date	Total # ha (acres) treated	WDF crab kill estimates ≥1+ total #[a]	UW population estimates ≥1+ mean (± 95% CI)[b]	% of total ≥1 + crab population killed: mean (±95% CI range)
July 1986	144 (355)	13,600	5,088,000 (±3,083,000)	0.27% (0.17-0.68%)
July 1987	145 (359)	13,900	6,542,000 (±3,164,000)	0.21% (0.14-0.43%)
July 1988	111 (275)	16,500	7,648,000 (±3,925,000)	0.22% (0.14-0.44%)

[a]Tufts (1989) and WDF unpublished data

[b]Unpublished data from Armstrong and Gunderson, UW/Washington Sea Grant.

carbaryl treatment were associated with oyster shell on sites and consequently, the oyster industry is held responsible for crab losses which, in a large part, were in habitat that it provided. In this sense, the oyster industry perpetuates the impression of impact since the very nature of oyster culture itself provides ideal habitat for 0+ crab. However, since only a fraction of actively cultured ground is treated (less than 10% annually), the percentage killed appears to be low.

In the absence of oyster provided through culture activities, refuge would be restricted primarily to eelgrass. While not downplaying the overall importance of eelgrass within the estuary, data from habitat comparisons indicate that crab densities were typically 4 to 6 times lower in eelgrass than in shell. Consequently, the number of 0+ crab expected to recruit and survive in eelgrass is substantially lower and further underscores the value of shell.

In conclusion, data from this study indicate that shell is of primary importance for newly settled crab and that oyster planted on sites at commercial levels provides benefits to crab in the form of substantial amounts of cover on approximately 2,400 ha within the estuary. Such benefits are evident in the preceding section, for example, in which estimates of 35 to 76 million crab can be attributed solely to shell habitat on the most intensely cultured ground during early July 1986-88 (Tables 12 and 13; Fig. 22). If oyster grounds need to be treated in order to maintain production at current levels, as the industry contends, then the cost of enhancement in terms of crab losses during this period was 1-4 million crab (or 4-5%); which conceptually provided net benefits of over 34-72 million crab through these years. It seems clear that enhancement of crab survival in untreated commercial oyster habitat substantially mitigates losses incurred elsewhere on treated ground. Therefore, it is likely that the industry is providing overall net benefits to estuarine populations of juvenile crab through the addition of prime shell habitat to the intertidal region in spite of the impacts incurred by carbaryl.

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APPENDIX TABLES

SP86

Appendix Table 1. Average intertidal crab density (#/m^2) by habitat category at Stony Point site SP86 for sample periods in 1986, 1987, and 1988.

Site: SP86	Habitat Category[1]				
	OP	LS	HS	LE	HE
Date	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n
6/21/86	2.0 (2.8), 2	4.0 (2.5), 6	1.0 (2.0), 4	-----[3]	-----
7/8/86	0.0 (0.0), 10	2.5 (2.9), 16	5.5 (6.8), 11	-----	-----
-------Site treated with carbaryl on 7/8/86------					
7/9/86 [2]	0.0 (0.0), 3	0.0 (0.0), 5	0.0 (0.0), 5	-----	-----
8/21/86	0.0 (0.0), 3	0.4 (1.3), 9	0.7 (1.6), 6	0.0 (0.0), 1	1.0 (2.0), 4
9/17/86	-----	2.0 (3.3), 6	-----	-----	0.8 (1.8), 5
4/18/87	-----	0.0 (0.0), 1	1.3 (2.3), 3	-----	-----
6/16/87	-----	-----	15.6 (11.4), 10	-----	1.6 (2.2), 5
6/26/87	-----	-----	9.3 (5.2), 12	-----	-----
7/10/87	-----	4.8 (5.2), 5	1.7 (3.1), 7	-----	-----
7/25/87	0.0 (0.0), 12	-----	2.3 (2.7), 12	-----	0.3 (1.2), 12
8/24/87	-----	0.0 (0.0), 1	3.6 (2.8), 11	-----	-----
9/6/87	-----	-----	1.0 (1.8), 12	-----	-----
5/4/88	-----	2.0 (2.3), 4	12.0 (12.0), 11	-----	-----
6/2/88	0.0 (0.0), 3	7.2 (6.5), 21	12.0 (6.3), 5	-----	0.0 (0.0), 1
6/13/88	0.0 (0.0), 13	5.6 (5.5), 18	7.6 (4.2), 9	-----	-----
7/2/88	0.0 (0.0), 10	1.5 (2.4), 19	2.9 (3.6), 11	-----	-----
7/15/88	0.0 (0.0), 4	0.7 (1.5), 18	3.0 (3.8), 4	0.0 (0.0), 4	-----
8/1/88	0.0 (0.0), 4	0.6 (1.5), 19	0.7 (1.6), 6	2.0 (2.8), 2	0.0 (0.0), 1

[1] Refer to Table 2 for definitions of habitat categories.
[2] ----- indicates that no samples were collected from that particular habitat category.
[3] While crab were found onsite, no living crab were found in samples taken 24 h after treatment.

SP87

Appendix Table 2. Average intertidal crab density (#/m^2) by habitat category at Stony Point site SP87 for sample periods in 1987 and 1988.

Site: SP87	Habitat Category[1] OP	LS	HS	LE	HE
Date	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n
6/26/87	0.0 (0.0), 12	-----[2]	1.7 (4.0), 12	-----	0.3 (1.2), 12
7/09/87	-----	-----	5.7 (6.0), 12	-----	-----
----------Site treated with carbaryl on 7/09/87----------					
7/10/87 [3]	-----	-----	0.0 (0.0), 12	-----	-----
7/25/87	-----	-----	1.7 (2.7), 12	-----	-----
8/24/87	-----	-----	1.0 (1.8), 12	-----	-----
9/06/87	-----	-----	0.7 (1.6), 12	-----	-----
6/03/88	0.0 (0.0), 5	10.7 (6.6), 9	21.0 (11.1), 16	-----	-----
6/15/88	0.7 (1.6), 12	4.6 (3.5), 20	17.7 (23.1), 7	0.0 (0.0), 1	-----

[1] Refer to Table 2 for definitions of habitat categories.
[2] ----- indicates that no samples were collected from that particular habitat category.
[3] While crab were found onsite, no living crab were found in samples taken 24 h after treatment.

SP88

Appendix Table 3. Average intertidal crab density (#/m^2) by habitat category at Stony Point site SP88 for sample periods in 1988.

Site: SP88	Habitat Category[1]				
	OP	LS	HS	LE	HE
Date	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n
6/14/88	0.2 (0.9), 19	2.5 (3.9), 21	12.0 (0.0), 1	-----[2]	-----
7/03/88	0.0 (0.0.), 14	2.4 (5.2), 25	4.0 (0.0), 1	-----	-----
7/30/88	0.0 (0.0.), 11	1.5 (2.4), 1), 19	-----	0.8 (1.8), 5	-----
----------Site treated with carbaryl on 7/30/88---------					

[1] Refer to Table 2 for definitions of habitat categories.
[2] ----- indicates that no samples were collected from that particular habitat category.

PR86

Appendix Table 4. Average intertidal crab density (#/m^2) by habitat category at Palix River site PR86 for sample periods in 1986, 1987, and 1988.

Site: PR86	Habitat Category[1] OP	LS	HS	LE	HE
Date	**#/m^2 (SD), n**	**#/m^2 (SD), n**	**#/m^2 (SD), n**	**#/m^2 (SD), n**	**#/m^2 (SD), n**
6/21/86	0.0 (0.0), 5	1.6 (2.2), 5	0.8 (1.8), 5	2.7 (2.3), 3	2.0 (2.8), 2
7/10/86	0.0 (0.0), 3	0.0 (0.0), 4	2.7 (2.3), 3	-----[2]	2.0 (2.8), 2
7/22/86	0.0 (0.0), 3	2.0 (4.9), 6	4.8 (4.4), 5	-----	0.4 (1.3), 9
-------Site treated with carbaryl on 7/22/86------					
7/23/86 [3]	-----	0.0 (0.0), 5	0.0 (0.0), 5	-----	0.0 (0.0), 3
8/20/86	0.0 (0.0), 5	0.0 (0.0), 6	2.3 (3.1), 7	-----	0.0 (0.0), 4
9/17/86	-----	0.0 (0.0), 6	2.4 (3.6), 5	-----	0.0 (0.0), 4
6/11/87	-----	12.0 (0.0), 1	12.4 (7.3), 9	-----	3.2 (5.2), 5
6/25/87	0.0 (0.0), 12	7.0 (2.0), 4	8.0 (4.5), 12	-----	0.3 (1.2), 12
7/11/87	-----	-----	2.0 (3.2), 12	-----	-----
7/26/87	0.0 (0.0), 12	-----	4.3 (5.8), 12	-----	0.4 (1.2), 12
8/23/87	-----	-----	0.7 (1.6), 12	-----	0.0 (0.0), 12
9/05/87	-----	-----	0.7 (1.6), 12	-----	1.3 (2.0), 12
4/18/88	-----	-----	0.0 (0.0), 12	-----	-----
5/05/88	2.7 (6.4),15	37.7 (14.8), 7	71.5 (24.8), 8	-----	-----
----------oysters were harvested off of this site during May, 1988----------					

[1] Refer to Table 2 for definitions of habitat categories.

[2] ----- indicates that no samples were collected from that particular habitat category.

[3] While crab were found onsite, no living crab were found in samples taken 24 h after treatment.

PR87

Appendix Table 5. Average intertidal crab density (#/m^2) by habitat category at Palix River site PR87 for sample periods during 1987.

Site: PR87	Habitat Category[1]				
	OP	LS	HS	LE	HE
Date	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n
6/27/87	0.0 (0.0), 12	-----[2]	-----	-----	2.0 (3.6), 12
7/10/87	-----	-----	-----	-----	3.0 (3.9), 12
----------Site treated with carbaryl on 7/10/87---------					
7/11/87 [3]	-----	-----	-----	-----	0.0 (0.0), 12
7/26/87	-----	-----	-----	-----	1.7 (2.1), 12
8/23/87	-----	-----	-----	-----	0.0 (0.0), 12
9/5/87	-----	-----	-----	-----	0.3 (1.1), 12

[1] Refer to Table 2 for definitions of habitat categories.
[2] ----- indicates that no samples were collected from that particular habitat category.
[3] While crab were found onsite, no living crab were found in samples taken 24 h after treatment.

PR88

Appendix Table 6. Average intertidal crab density (#/m^2) by habitat category at Palix River site PR88 at the time of carbaryl treatment on June 30, 1988.

Site: PR88	Habitat Category[1]				
	OP	**LS**	**HS**	**LE**	**HE**
Date	**#/m^2 (SD), n**	**#/m^2 (SD), n**	**#/m^2 (SD), n**	**#/m^2 (SD), n**	**#/m^2 (SD), n**
6/30/88	0.0 (0.0), 11	0.0 (0.0), 1	-----[2]	0.9 (1.7), 14	0.3 (1.1), 14
----------Site treated with carbaryl on 6/30/88---------					

[1] Refer to Table 2 for definitions of habitat categories.
[2] ----- indicates that no samples were collected from that particular habitat category.

NHC

Appendix Table 7. Average intertidal crab density (#/m^2) by habitat category at Nahcotta reference site NHC fo sample periods in 1987 and 1988. This site was not treated during the course of the study.

Site: NHC	Habitat Category[1]				
	OP	LS	HS	LE	HE
Date	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n
5/15/87	-----[2]	-----	4.0 (2.5), 6	-----	-----
6/15/87	-----	-----	8.0 (9.5), 6	-----	-----
6/28/87	0.0 (0.0), 12	-----	6.3 (6.0), 12	-----	1.7 (2.7), 12
7/24/87	0.0 (0.0), 12	-----	6.7 (5.7), 12	-----	1.3 (2.0), 12
8/25/87	-----	-----	3.0 (3.0), 12	-----	0.7 (1.6), 12
9/08/87	-----	-----	4.7 (4.1), 12	-----	0.0 (0.0), 12
7/29/88	0.0 (0.0), 3	0.3 (1.1), 25	2.7 (3.1), 12	-----	-----

[1] Refer to Table 2 for definitions of habitat categories.
[2] ----- indicates that no samples were collected from that particular habitat category.

NP87

Appendix Table 8. Average intertidal crab density (#/m^2) by habitat category at Needle Point site NP87 for sample periods in 1987 and 1988.

Site: NP87	Habitat Category[1]				
	OP	LS	HS	LE	HE
Date	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n	#/m^2 (SD), n
6/28/87	0.0 (0.0), 12	-----[2]	7.0 (5.4), 12	-----	-----
7/11/87	-----	-----	5.3 (5.5),12	-----	-----
----------area treated with carbaryl on 7/11/87----------					
7/12/87 [3]	-----	-----	0.0 (0.0), 12	-----	-----
7/24/87	-----	-----	1.0 (2.5), 12	-----	-----
8/25/87	-----	-----	0.3 (1.2), 12	-----	-----
9/08/87	-----	-----	0.3 (1.2), 12	-----	-----
5/03/88	-----	-----	0.4 (1.3), 10	-----	-----
5/15-88	4.0 (5.7), 2	6.4 (5.7), 18	8.3 (5.2), 12	-----	2.7 (4.6), 3
7/29/88	0.0 (0.0), 4	0.0 (0.0), 8	0.8 (2.0), 21	-----	-----

[1] Refer to Table 2 for definitions of habitat categories.
[2] ----- indicates that no samples were collected from that particular habitat category.
[3] While crab were found onsite, no living crab were found in samples taken 24 h after treatment.

Appendix Table 9. Concentrations of carbaryl and 1-napthol in water samples taken at Palix River treatment site PR88 which was treated at 5.6 kg/ha (5 lbs/acre) on July 30, 1988. Water samples were taken at stations located onsite, 25, 50, 75, 100 and 200 m from the site in the predetermined path of the incoming flood tide and at a control site 800 meters away from the site. (See Figs. 3 and 9 for location of site PR88).

Distance from Treated Site (m)	Water Depth (cm)	Depth of Sampling	Time of Sample	Concentration [ppm] carbaryl WDF[1]	WDA[2]	1-napthol WDF[1]
Onsite	2.5	Surface	1101	13.7	16.7	0.3
	5	Surface	1103	3.1	4.0	0.2
	5	Bottom	1103	8.3	10.1	0.3
	10	Surface	1105	1.5	1.5	0.1
	10	Bottom	1105	2.2	2.3	0.1
	20	Surface	1111	0.7	1.7	<0.1
	20	Bottom	1111	0.7	0.37	<0.1
	40	Surface	1130	0.3	0.19	<0.1
	40	Bottom	1130	<0.1	0.25	<0.1
25	2.5	Surface	1102	6.3	6.2	0.2
	5	Surface	1104	9.7	17.6	0.3
	5	Bottom	1104	15.7	16.8	0.3
	10	Surface	1109	19.0	17.3	0.2
	10	Bottom	1109	16.0	21.0	1.7
	20	Surface	1121	2.5	1.7	<0.1
	20	Bottom	1121	2.8	2.1	<0.1
	40	Surface	1138	5.2	6.8	0.2
	40	Bottom	1138	5.2	8.9	0.2
50	2.5	Surface	1106	12.6	8.0	0.1
	5	Surface	1107	13.4	15.2	0.3
	5	Bottom	1107	13.2	13.2	0.3
	10	Surface	1109	11.5	14.9	0.1
	10	Bottom	1109	12.4	12.6	0.1
	20	Surface	1123	6.3	4.5	<0.1
	20	Bottom	1123	12.4	10.8	0.1
	40	Surface	1140	0.8	0.67	<0.1
	40	Bottom	1140	0.8	0.25	<0.1

Table Continued on next page

Appendix Table 9. (Continued from previous page)

Distance from Treated Site (m)	Water Depth (cm)	Depth of Sampling	Time of Sample	Concentration [ppm] carbaryl WDF[1]	Concentration [ppm] carbaryl WDA[2]	1-napthol WDF[1]
75	2.5	Surface	1114	0.7	0.071	0.1
	5	Surface	1115	<0.1	0.12	<0.1
	5	Bottom	1115	1.9	2.24	0.2
	10	Surface	1118	13.0	13.7	0.2
	10	Bottom	1118	9.9	12.6	0.1
	20	Surface	1121	17.3	13.4	0.1
	20	Bottom	1121	16.2	17.5	0.2
	40	Surface	1140	2.8	NA	<0.1
	40	Bottom	1140	NA	2.0	NA
100	2.5	Surface	1117	1.0	0.018	0.2
	5	Surface	NA	NA	NA	NA
	5	Bottom	NA	NA	NA	NA
	10	Surface	1118	0.2	0.18	0.1
	10	Bottom	1118	4.4	2.7	0.2
	20	Surface	1122	5.1	4.1	0.1
	20	Bottom	1122	4.6	4.3	0.2
	40	Surface	1141	4.9	4.6	0.1
	40	Bottom	1141	5.1	11.6	<0.1
200	2.5	Surface	1124	1.3	0.016	0.1
	5	Surface	NA	NA	NA	NA
	5	Bottom	1124	0.6	0.003	0.2
	10	Surface	1125	0.6	0.034	0.1
	10	Bottom	1125	2.4	0.07	0.1
	20	Surface	1128	2.3	0.55	<0.1
	20	Bottom	1128	1.5	0.92	0.1
	40	Surface	1145	0.3	0.48	<0.1
	40	Bottom	1145	0.3	0.03	<0.1
Control (800 m)	2.5	Surface	1058	<0.1	0.005	0.1
	5	Surface	1103	<0.1	0.007	<0.1
	5	Bottom	1103	0.1	0.008	<0.1
	10	Surface	1108	<0.1	0.059	<0.1
	10	Bottom	1108	<0.1	0.031	<0.1
	20	Surface	NA	NA	0.005	NA
	20	Bottom	NA	NA	0.038	NA
	40	Surface	NA	NA	0.044	NA
	40	Bottom	NA	NA	0.063	NA

[1] Concentrations of carbaryl and 1-napthol in water samples analyzed by Washington Department of Fisheries using colorimetric method.

[2] Concentrations of carbaryl in water samples analyzed using high pressure liquid chromatography by Washington Department of Agriculture.