Exxon Valdez Oil Spill
State/Federal Natural Resource Damage Assessment Annual Report

Sea Urchin Injury: Assessment of Impacts of Oil on Green Sea Urchins, *Strongylocentrotus droebachiensis*, in the Kodiak Island Area

Fish/Shellfish Study Number 26
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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211 Mission Road
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Study History: This project was initiated as part of the State of Alaska and U.S. Government Natural Resource Damage Assessment (NRDA) studies following the *Exxon Valdez* oil spill. Results from this study were planned to be used by Economic Study Numbers 1 and 3 to calculate damages to the sea urchin fishery resource. The project received funding for only one year and was terminated. No further analyses have been completed. The main body of the report was also prepared as Regional Information Report No. 4K90-20 for the Division of Commerical Fisheries of the Alaska Department of Fish and Game, dated January 1990.

Abstract: A total of 4,719 green sea urchins, *Strongylocentrotus droeachiensis*, were examined from 2 oiled and 4 non-oiled sites around Kodiak Island. Urchins ranged in size and estimated age from 8 mm (0.75 yrs) to 85 mm (6.0+ yrs). Histological and hydrocarbon tissue samples were collected at 6 sites for laboratory analysis. Divers did not observe any subtidal oil. In the contracted report attached as Appendix I, purple sea urchins were exposed to elutriates of sediments from areas oiled by the *Exxon Valdez* oil spill. Elutriates of samples collected from Bay of Isles in Fall 1990 were toxic to purple sea urchins. Purple and green sea urchins were exposed to water soluble fractions (WSF) of crude oil. WSF was more toxic to green than to purple sea urchins. Fertilization of green sea urchins was reduced by exposure to 3.2% WSF or greater. Weathered oil affected fertilization less. WSF to 32% was not toxic to purple sea urchin embryons. WSF greater than or equal to 56% was toxic to green sea urchin embryos. Results indicated possible effects of the *Exxon Valdez* oil spill on sea urchin populations, but hydrocarbon contents of sediment and WSF samples were not determined preventing conclusive impact assessment.

Key Words: *Exxon Valdez* oil spill, green sea urchin, hydrocarbon toxicity, purple sea urchin, *Strongylocentrus droebachiensis, Strongylocentrus purpuratus,* water soluble fraction.

Project Data: Archival location of data is unknown. All available information is included in this report.

Citation:

Donaldson, W., and S. Byersdorfer. 1998. Sea urchin injury: Assessment of impacts of oil on green sea urchins, *Strongylocentrotus* droebachiensis, in the Kodiak Island area, *Exxon Valdez* Oil Spill State/Federal Natural Resource Damage Assessment Annual Report (Fish/Shellfish Study Number 26), Alaska Department of Fish and Game, Division of Commercial Fisheries, Kodiak, Alaska.

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Executive Summary

A total of 4719 green sea urchins, Strongylocentrotus droebachiensis, were examined from 2 oiled and 4 non-oiled sites around Kodiak Island. Urchins ranged in size and estimated age from 8 mm (0.75 yrs) to 85 mm (6.0+ yrs.). Histological and hydrocarbon tissue samples were collected at 6 sites and await laboratory analysis. A collection of 20-40 urchins will be assembled in the spring of 1990 for larval bioassay analysis. Divers did not observe any subtital oil.

Objectives

1. To estimate the relative abundance of green sea urchins in oiled and non-oiled areas.

2. To estimate the gonad production of urchins in oiled and non-oiled areas such that differences of + or - 5% can be determined between the two impact levels 95% of the time.

3. To estimate the incidence of abnormalities in ovarian development in urchins in oiled and non-oiled areas such that differences of + or - 5% can be determined between the two impact levels 95% of the time.

4. To estimate recruitment of young urchins, as a percentage of all urchins in the sample area, in oiled and non-oiled areas.

5. To estimate the toxicity of crude oil to urchin larvae.

6. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS/DATA ANALYSIS

This project is being conducted in two phases, phase 1: field work and phase 2: laboratory work. Objectives 1 through 4; abundance estimates, roe production estimates, ovarian development abnormalities, and recruitment estimates will be conducted wholly or partially in the field while the larval bioassay work (objective 5) and a portion of the ovarian development work will be conducted in a laboratory. The larval bioassays and the ovarian development work will be conducted by an independent contractor.

STUDY SITES

Five oiled and four non-oiled areas of green sea urchin habitat were selected for study in the Chiniak and Uyak Bay areas, Figure 1. Specific site data recorded includes: site name, site orientation (N-NE etc.), latitude, longitude, dominate substrate composition in percent, surface and bottom water temperature and salinity, weather conditions, wave action and oil conditions.

SAMPLE DESIGN

Where conditions permitted, transect lines were established when sufficient numbers of urchins were found by scuba divers. The sites were sampled during (November and December, 1989). Transects were established perpendicular to the water's edge and parallel to each other along the bottom where urchins occurred. Distance between transects was determined on site by the distribution of urchins with a distance of 7-15 meters between transects. Scuba divers surveyed each transect within 1-3 meters on either side of the transect mid-line.

At sites where sufficient numbers of urchins were not available to conduct transect work or conditions limited dive time, adaptations to transect methodology were implemented to sample each site. These changes are described below in the site by site descriptions. Presence of oil, kelp and kelp condition (alive, dead,oiled, unoiled) were recorded. A minimum sample of 200 urchins were measured from each transect line and non-transect sites.

Along each transect and at non-transect sites, a random sample of ten urchins were collected, and the diameter, live weight, and roe weight of these animals were measured. A random sample of gonads from 10 urchins at each site was prepared for histological examination for abnormalities. The gonads were preserved in 10% buffered formalin (Dr. Ted Meyers, ADF&G Juneau, pers. comm.). Three randomly selected composite samples of three gonads each were selected from transects or sites in each area for hydrocarbon analysis.

During the spring of 1990, 20-40 live urchins will be shipped to a contractor for laboratory bioassay experiments on toxicity of oil to urchin larvae. The urchins will be transported in a dry condition in ice chests lined with kelp and maintained as close to normal ambient temperature as possible.

In order to assign an age structure to the populations studied, we made the following assumptions based on the limited data available for the Kodiak area: 1) Egg hatch occurs in April in all areas surveyed and 2) growth rates are similar between areas.

Based on unpublished modal size frequency data from observations of green sea urchin populations in the Chiniak Bay area of Kodiak Is. (collected by E. Munk, NMFS, Kodiak), we estimated the age composition of both Chiniak and Uyak Bay urchin samples.

Gonadal indexes were computed as: average gonad weight / average whole weight x 100. The indexes compare the size of gonads to the average size of whole animals at each site, and are a measure of reproductive activity over time.

DATA ANALYSIS

Survey areas were stratified into those impacted and not impacted by oil. Once the laboratory analyses are complete, statistics on analysis of variance will be computed to assess any differences in hydrocarbon content, incidence of gonad abnormalities, changes in relative abundance and any differences in parameters describing relationships between gonad weight and urchin diameter or gonad weight and total weight. Trend analysis of the relative abundance of young of the year urchins may be used to detect potential recruitment failures associated with oil impact.

STUDY RESULTS

A total of 24 sites within 9 areas of Chiniak and Uyak Bays were explored by scuba divers for the presence of green sea urchins. These sites are depicted on Figures 2-9. Urchins were found at 12 sites (50%). Six sites had sufficient numbers of urchins to sample. Two of these sites, numbers 21 and 23 were identified as light and very lightly oiled. The remaining 10 sites with urchins were identified as non-oiled, Table 1.

A total of 4719 urchins were sampled (1837 in Chiniak, 2882 in Uyak) ranging in size and estimated age from 8 mm (0.75 yr.) to 85 mm (6.0+ yr.). The average size and age at a site varied between 37.7 mm (2.75 yr.) at site 23 in Uyak Bay to 54.1 mm (3.75 yr.) at site 3 in Chiniak Bay. Samples of size distributions at the six sampling sites are shown in Figures 10-15.

Average gonad indices are presented in Table 2. Indices ranged from 16.9 at site 21 to 23.1 at site 3. This difference reflects that the average diameter of urchins at site 3 was 20 mm greater than at site 21. These indices will be more meaningful biologically when a temporal distribution of indices is made available with future studies.

Hydrocarbon and histological urchin tissue samples were collected at 6 sites (2 oiled, 4 unoiled), Table 1, and are being readied for analysis. No definitive data on injuries to urchins from the oil spill is available until the results from the tissue analysis is complete. Divers did not observe any signs of oiling on subtidal marine life or substrates.

Table 1. List of sites, urchins, oil observations and hydrocarbon and histological sampling.

Site number	Urchin observations	* Oil Observations	Samples Hydro.	Samples Histo.
1	urchins	no oil	no	no
2	urchins	no oil	yes	yes
3	urchins	no oil	yes	yes
4	no urchins	no oil	no	no
5	urchins	no oil	no	no
6	no urchins	no oil	no	no
7	urchins	no oil	no	no
8	no urchins	no oil	no	no
9	no urchins	no oil	no	no
10	few urchins	no oil	no	no
11	few urchins	no oil	no	no
12	no urchins	heavy oil	no	no
13	no urchins	heavy oil	no	no
14	no urchins	heavy oil	no	no
15	no urchins	heavy oil	no	no
16	no urchins	heavy oil	no	no
17	no urchins	very light oil	no	no
18	urchins	no oil	yes	yes
19	no urchins	no oil	no	no
20	urchins	no oil	yes	yes
21	urchins	light oil	yes	yes
22	no urchins	very light oil	no	no
23	urchins	very light oil	yes	yes
24	few urchins	no oil	no	no

*oiling data obtained from Kodiak Island Borough map July 21,1989

Table 2. Urchin measurements from collection sites

	Site #2	Site #3	Site #18	Site #20	Site #21	Site #23
Avg. Test Diameter	49mm	60.6mm	43.7mm	43.1mm	40.3mm	41.7mm
Avg. Live Weight	49.6g	81.7g	39.4g	30.7g	27.7g	29.3g
Avg. Roe Weight	10.3g	18.9g	8.1g	5.5g	4.7g	6.3g
Avg. Gonad Index	20.8	23.1	20.6	17.9	16.9	21.5

STATUS OF INJURY ASSESSMENT

The progress made to date in meeting each of the objectives has been variable. A significant factor affecting objective 1 was the absence of urchins at sites 12-16 adjacent to the heaviestly oiled beaches in the study area. Green sea urchins were documented at several locations along these beaches in July and August 1989 by Dames and Moore Biological Consultants (Mark Blakesly, pers. comm.). During our November and December dives we returned to the same sites and found no urchins. Another confounding factor is the presence of sea otters, Enhydra lutris, a predator of urchins, in the Uyak Bay area. We have no estimates of the rate of predation on urchins since the arrival of oil and therefore we cannot discriminate between oil and otter related mortalities at this time. Therefore we cannot discount the possibility that oil contamination caused a decline in abundance of sea urchins between July/August and November/December 1989. It is doubtful that a large increase in sea otter predation occurred over this time interval. While a few otters have been sighted in the Chiniak Bay area their numbers appear to be much lower.

Most of the commercial harvest from Kodiak waters occurs in the vicinity of sites 1-3 in the Chiniak Bay area. Very little commercial activity occurs in the Uyak area. Since commercial divers tend to remove the largest urchins any size comparisons and relative estimates of recruitment between areas becomes skewed. Data on removals are not precise enough to allow us to adjust our samples for the influence of commercial removals. These factors combined make it very difficult to assess oil's impact on the urchin populations through objectives 1,2 and 4.

The hydrocarbon and histological examination of tissues and the larval bioassay work scheduled for the spring of 1990, should provide the best information on oil-related impacts.

Following is a site by site description of the study area.

Site 1. Date: 11-16-89

Location: Kalsin Bay between Utesistoi and Svitlak Island
Latitude 57 37.7' Longitude 152 21.7'

Physical Characteristics: Bottom substrate was a mixture of sand, gravel, and rock. Larger rocks were covered in corraline algae. Sparse macroalgae observed.

Urchin population: Moderate urchin population seen. Urchins weren't collected due to commercial diver in the area.

Site 2. Date: 11-16-89

Location: Kalsin Bay along SW side of Isthmus Pt.
Latitude 57 37.6' Longitude 152 20.5'

Physical characteristics: (see Appendix 1)

Urchin Population: Moderate urchin population.

Collection methods: Four transects were laid down perpendicular to shore approximately 7 meters apart. A total of 200-300 urchins were collected from 1 meter on either side of the transect midline. The urchins were brought ashore to be measured, then a random sample of 10 urchins from each transect was chosen and live and roe weights taken. Random samples were saved for hydrocarbon and histology analysis.

Site 3. Date: 11-20-89

Location: Kalsin Bay along SW side of Isthmus Pt. (100m from site 2)
Latitude 57 37.5' Longitude 152 23.0'

Physical Characteristics: (see Appendix 2)

Urchin Populations: Moderate urchin population

Collection methods: Same as site 2

Site 4 Date: 11-20-89

Location: Middle Bay along NW side of Broad Pt.
Latitude 57 42.3' Longitude 152 24.4'

Physical Characteristics: Bottom substrate a mixture of pebbles, rocks and boulders with dense beds of macroalgae mainly Laminaria and Alaria. At a depth of 15-20 feet sandy areas were interspersed with above bottom type.

Urchin Populations: No urchins seen

Site 5 Date: 11-27-89

Location: Zaimka Island, East Side
Latitude 57 43.6' Longitude 152 27.9'

Physical Characteristics: Bottom substrate is a mixture of rock, pebble and sand. Scattered areas of dense macroalgae.

Urchin Population: Urchins were found in sparse concentrations under algal cover.

Site 6 Date: 11-27-89

Location: Cliff Point, NW side
Latitude 57 43.5' Longitude 152 28.0'

Physical Characteristics: Sand, silt, bottom.

Urchin Populations: No urchins seen.

Site 7 Date: 11-27-89

Location: Blodgett Island, N & E sides
Latitude 57 43.4' Longitude 152 29.3'

Physical Characteristics: Bottom substrate is pebble, rock, boulder with underlying sand. Large sand, silt areas interspersed occasionally. Moderate cover of macroalgae.

Urchin Population: Moderate urchin population scattered under cover of macroalgae and shell hash. No transects done due to ice cover.

Site 8 Date: 11-27-89

Location: Cliff Point across from Blodgett Island
Latitude 57 43.2' Longitude 152 29.3'

Physical Characteristics: Bottom substrate is rock, pebble, boulder with underlying sand. Occasional sand, silt areas. Moderate cover of macroalgae.

Urchin Population: No urchins seen.

Site 9 Date: 11-22-89

Location: West side of Woody Island
Latitude 58 47.4' Longitude 152 20.5'

Physical Characteristics: Visibility 0-2' not sure of bottom type.

Urchin Population: Unsure due to poor visibility.

Site 10 Date: 11-24-89

Location: Trident Basin between Near and Holiday Island
Latitude 58 47.1' Longitude 152 23.2'

Physical Characteristics: Bottom substrate a mixture of pebbles, rocks, and boulders. Large quantities of macroalge.

Urchin Populations: A few live urchins and some fresh cracked urchin tests observed. Sea otter seen in the area.

Site 11 Date: 11-24-89

Location: South Bay of Crooked Island
Latitude 58 46.4′ Longitude 152 24.0′

Physical Characteristics: Bottom type a rocky, shell hash.

Urchin Populations: Only a few urchins were observed. Two years previous this area held large quantities of urchins but was commercially harvested.

Site 12 Date: 11-13-89

Location: Spiridon Bay, Hook Point
Latitude 57 41.1′ Longitude 153 46.7′

Physical characteristics: On the east side of Hook Point the bottom sediment was sandy, rock rubble covered with a moderate amount of macroalgae. On the west side of Hook Point the bottom sediment varied from boulder reef along the intertidal to rocky, cobble, shell hash offshore. Heavy macroalgae covered the reef grading to moderate offshore, occasional eelgrass beds interspersed.

Urchin Population: Few urchin tests seen, but no live urchins.

Site 13 Date: 11-7-89

Location: Spiridon Bay, Egg Island, NE side
Latitude 57 41.9′ Longitude 153 51.4′

Physical Characteristics: Large boulder reefs along upper intertidal, sandy, shell hash offshore. Moderate macroalgae cover.

Urchin Population: Few urchin tests seen, but no live urchins.

Site 14 Date: 11-8-89

Location: Spiridon Bay, NW of Egg Island
Latitude 57 42.3′ Longitude 153 52.6′

Physical Characteristics: Bottom Substrate small pebbles with sandy interstices. Light macroalgae cover, mostly Agarum, with some Laminaria. Otter cracked bivalves seen.

Urchin Population: One pale urchin seen.

Site 15 Date: 12-3-89

Location: Spiridon Bay, Egg Island, SW side
Latitude 57 42.0′ Longitude 153 51.6′

Physical Characteristics: Bottom substrate is a pebble, rock, boulder reef, with interspersed shell hash. Heavy macroalgae cover of Agarum, Laminaria, Corralina, and Rhodomenia.

Urchin Population: No urchins seen.

Site 16 Date: 12-4-89

Location: Chief Cove and North of Chief Cove
From Latitude 57 43.9′ Longitude 153 56.2′
To Latitude 57 42.1′ Longitude 153 54.4′

Physical Characteristics: Most of the intertidal area around Prominance mound was solid rocky reef. Moderate Concentrations of macroalgae, mostly Agarum and Laminaria and misc. filamentous reds. Otter cracked shells seen. South towards Chief Point the boulder reef gave way to unconsolidated rock, pebble areas. Rocks were encrusted with corraline algae and clumps of brown and red algae but no large beds seen.

Urchin Population: Few old urchin tests seen, but no live urchins.

Site 17 Date: 12-5-89

Location: Coves across from Harvester Island
Latitude 57 38.3′ Longitude 154 0′

Physical Characteristics: Bottom sediment mostly rock, pebble interspersed with large sandy areas. Rocks were encrusted with corraline algae and sporadic clumps of Laminaria and filamentous red algae. Occasional eelgrass beds throughout sandy areas.

Urchin Population: Few urchin tests seen but no live urchins.

Site 18 Date: 11-4-89

Location: Larsen Bay Cannery
Latitude 57 32.0′ Longitude 153 59.0′

Physical Characteristics: (see Appendix 3)

Urchin Concentrations: Moderate to heavy concentrations of urchins seen.

Collection Methods: Four transects were laid down perpendicular to shore and approximately 7 meters apart. A total of 200-300 urchins were measured underwater in 1 meter band on either side of the transect mid-line. A random sample of 10 urchins was then collected from each transect for live and roe weights and for hydrocarbon and histology analysis.

Site 19 Date: 11-6-89

Location: Uyak Bay, East side of Amook Island
Latitude 57 28.9' Longitude 153 49.0'

Physical Characteristics: Bottom sediment mostly gravel, silt and shell hash. Sparse macroalgae, mainly Laminaria. Offshore starting at 20' was a rock ledge and boulder terraces to 30'. Dense macroalgae on rocks and boulders.

Urchin Populations: No urchins seen.

Site 20 Date: 11-9-89 & 12-6-89

Location: Amook Island
Latitude 57 31.0' Longitude 153 50.0'

Physical Characteristics: (see Appendix 4)

Urchin Population: Large urchin population

Collection Methods: Transects were done on 12-6-89. They were laid out perpendicular to shore, 15 m apart starting at the low tide mark. In a 4 m band along each tranect, 200 to 300 urchins were taken to shore to be measured. A random sample of 10 urchins from each transect was then chosen for live and roe weights. A random sample of urchin for hydrocarbon analysis was previously taken on 11-9-89.

Site 21 Date: 12-5-89

Location: Larsen Bay (head)

Physical Characterisstics: (see Appendix 5)

Urchin Population: Moderate urchin population in a narrow band parallel to shore.

Collection Methods: Two transects were laid down parallel to shore. One transect was along the 12 ft. depth contour and the second was along the 16 ft. depth contour. From a 4 m band along transect 1, 200 - 300 urchins were collected, and from a 2 m band along transect 2, 200 - 300 urchins were collected. All urchins were brought to shore to sample. A random sample was taken for live and roe weights and for hydrocarbon and histological analysis.

Site 22 Date: 12-6-89

Location: Zacher Bay, east of Carlsen Pt.
Latitude 57 34.0' Longitude 153 48.8'

Physical Characteristics: Bottom sediment a mixture of rock, pebbles and sandy silt. Scarce macroalgae was noted but an algal scum covered much of the area.

Urchin Population: A few urchin tests were found but no live urchins.

Site 23 Date: 12-9-89

Location: Cove SW of Alf Island
Latitude 57 23.6' Longitude 153 51.2'

Physical Characteristics: (see Appendix 6)

Urchin Population: A small narrow band of urchins in shallow water parallel to shore.

Collection Methods: All urchins in a 5 by 30 m band parallel to shore were collected and measured on shore. From these urchins, a random sample was taken for live and roe weights and hydrocarbon and histological analysis.

Site 24 Date: 11-6-89

Location: Alf Island, SE cove
Latitude 57 24.6' Longitude 153 49.8'

Physical characteristics: The bottom type was slate shingle with sparse algae composed mostly of Laminaria.

Urchin Population: Lightly, distributed urchins under shingles and amongst blue mussels.

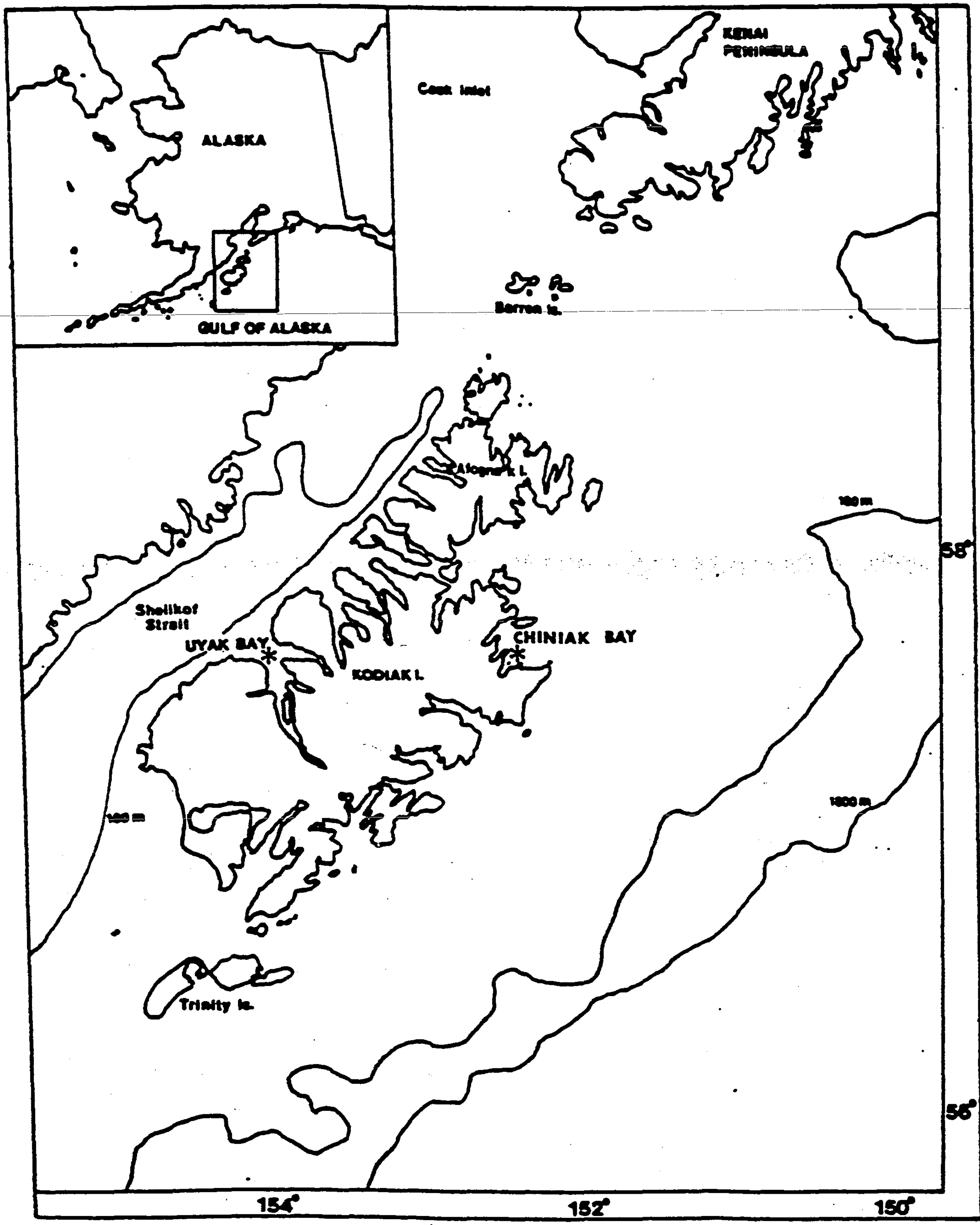

Figure 1. Kodiak Island, Alaska.

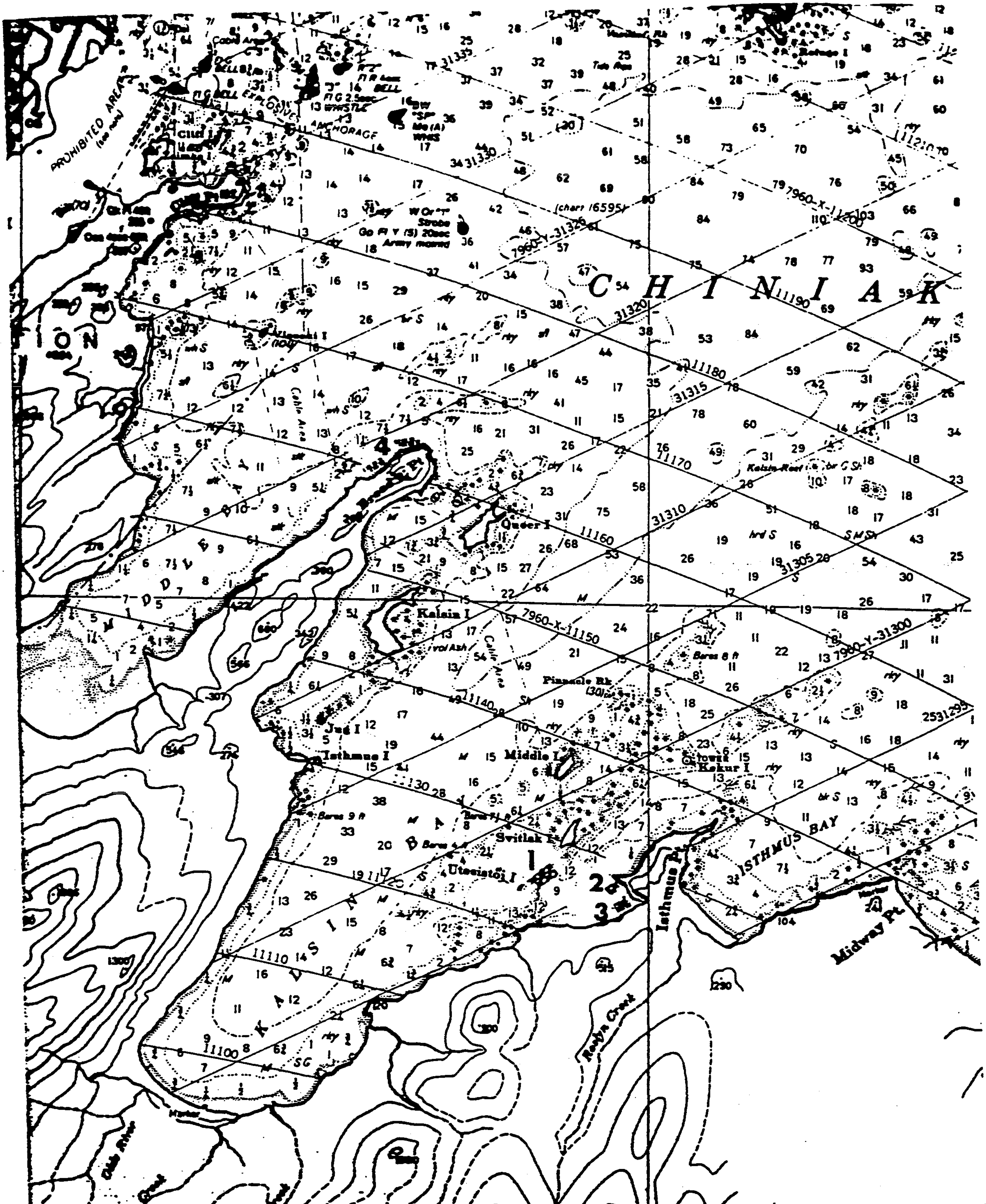

Figure 2. Sites 1-4 in Chiniak Bay Area.

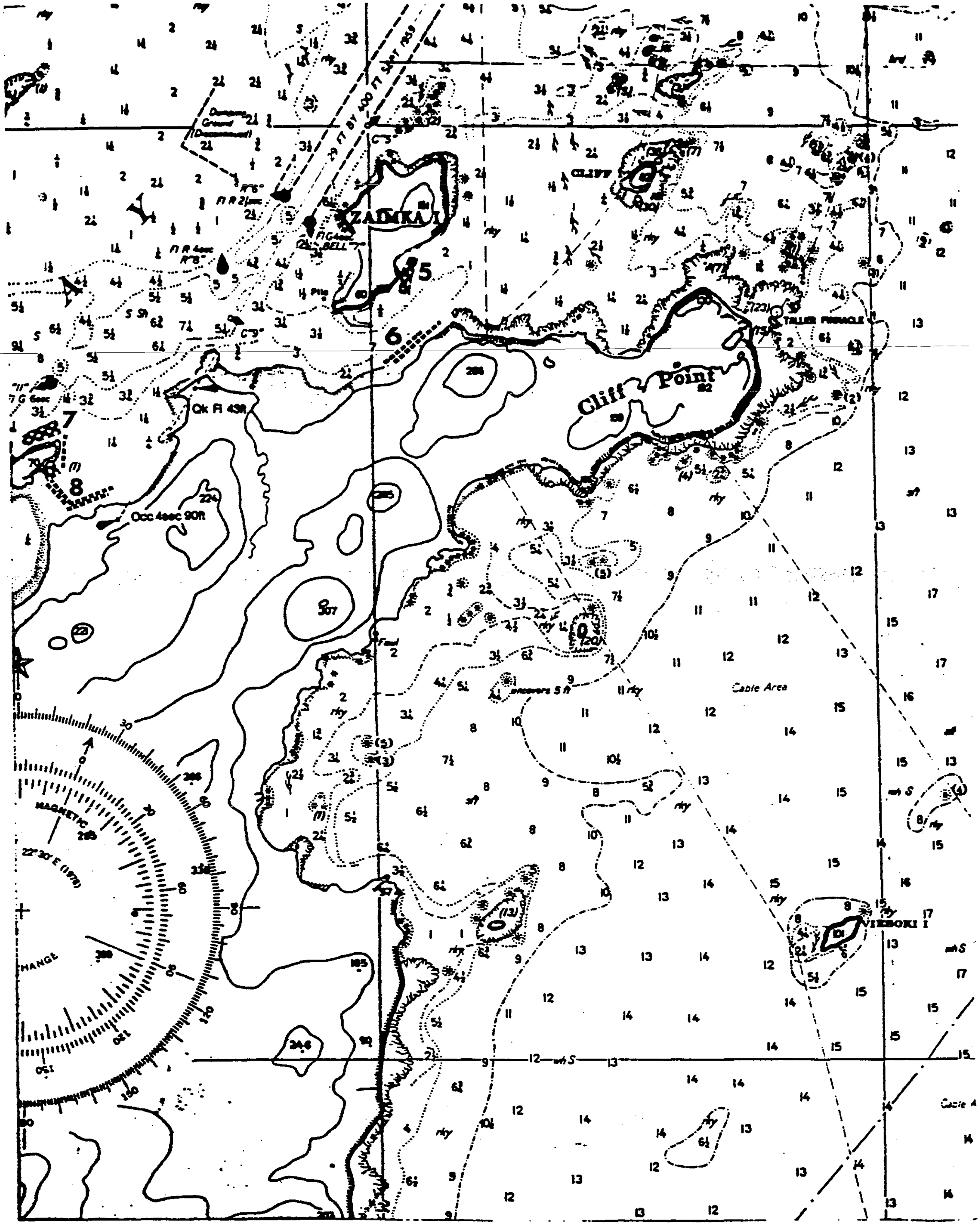

Figure 3. Sites 5-8 in Chiniak Bay Area.

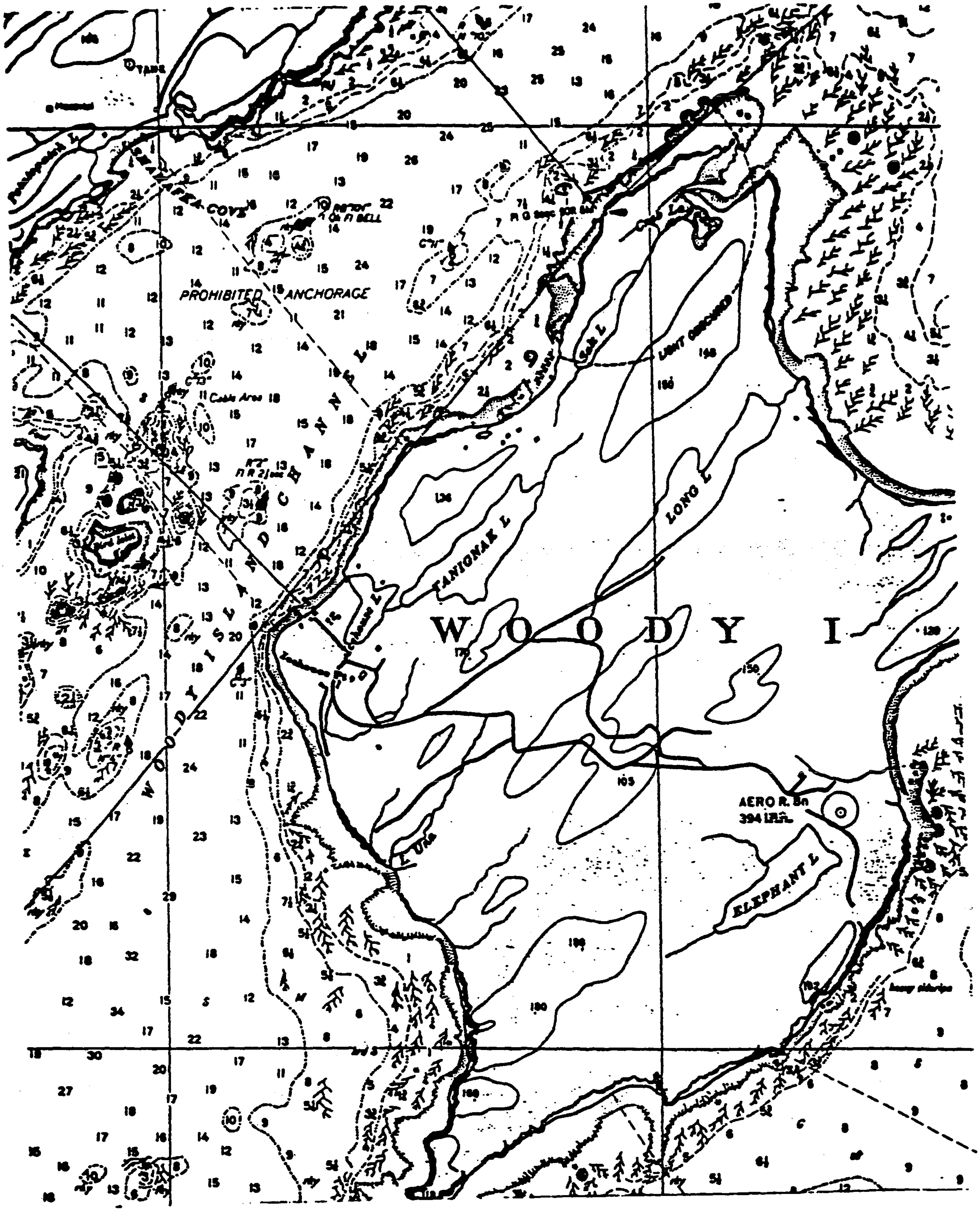

Figure 4. Site 9 in Chiniak Bay Area.

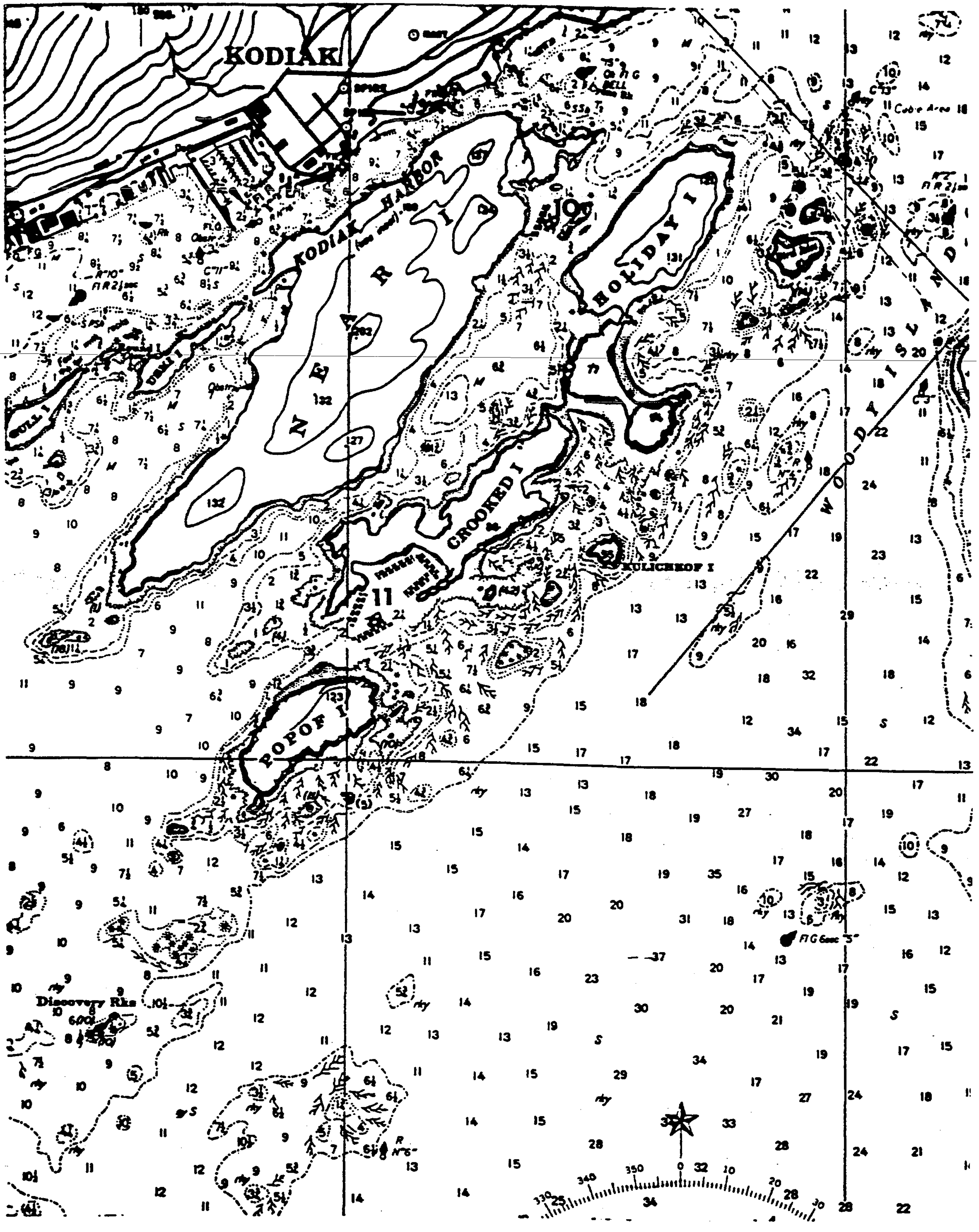

Figure 5. Sites 10-11 in Chiniak Bay Area.

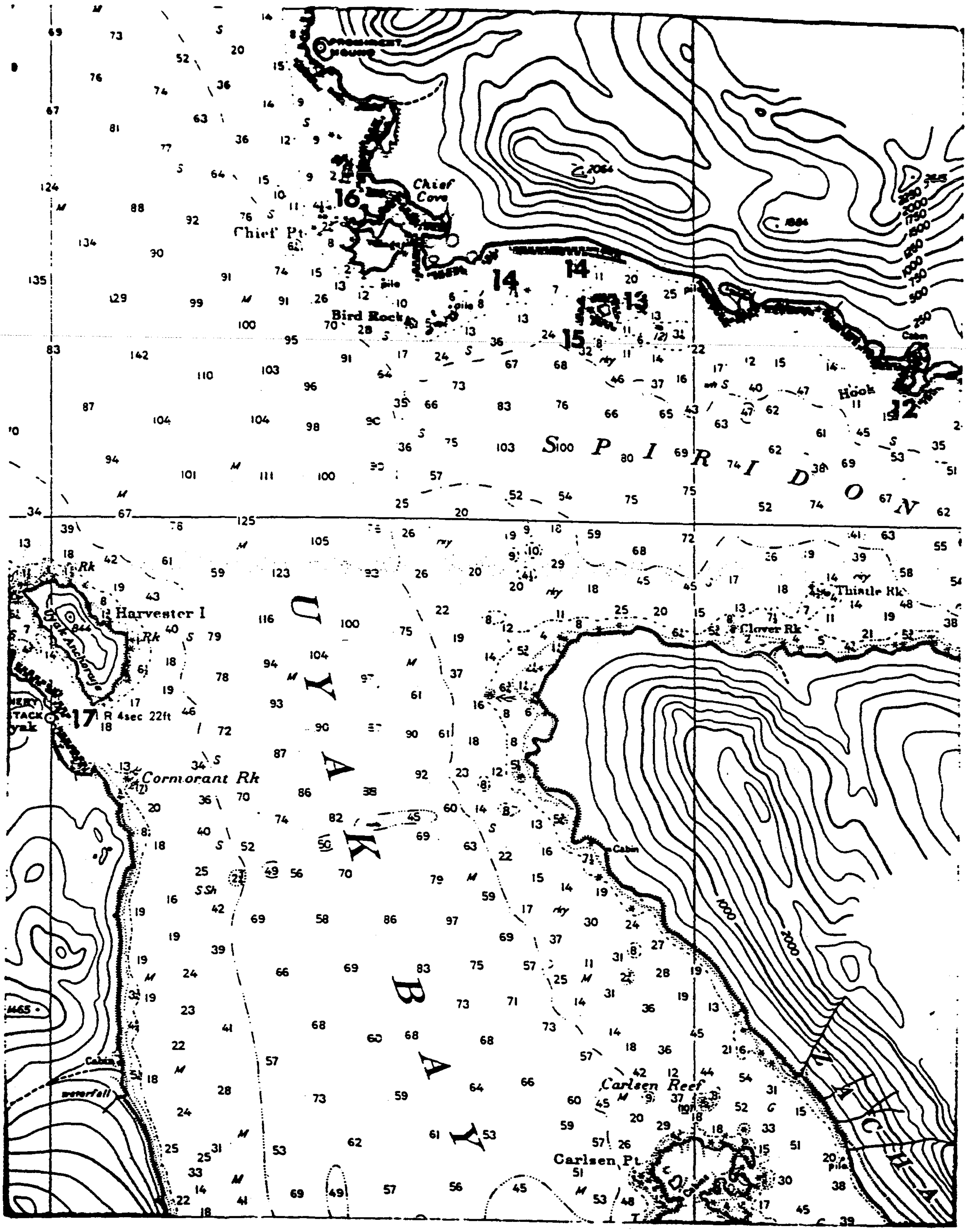

Figure 6. Sites 12-17 in Uyak Bay Area.

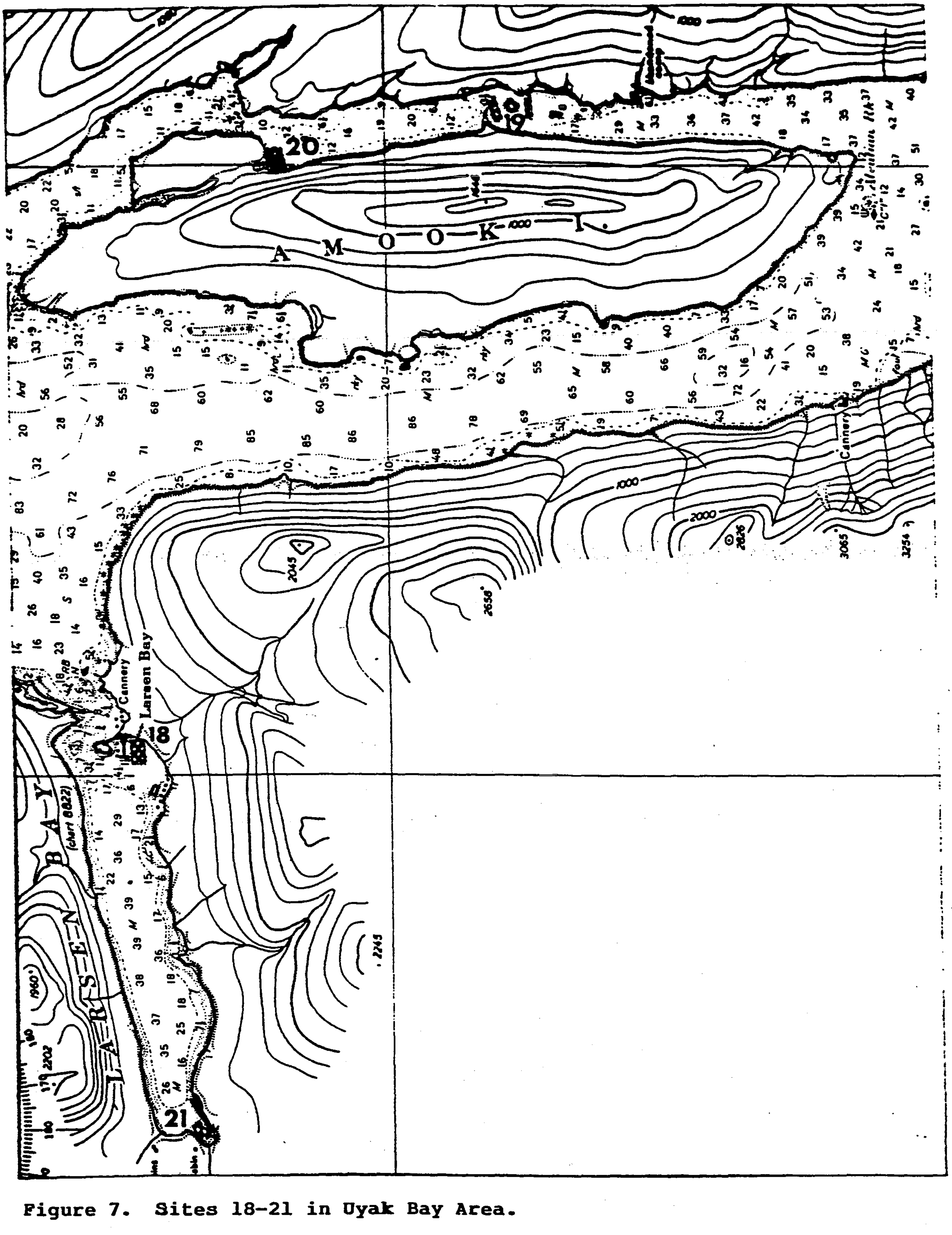

Figure 7. Sites 18-21 in Uyak Bay Area.

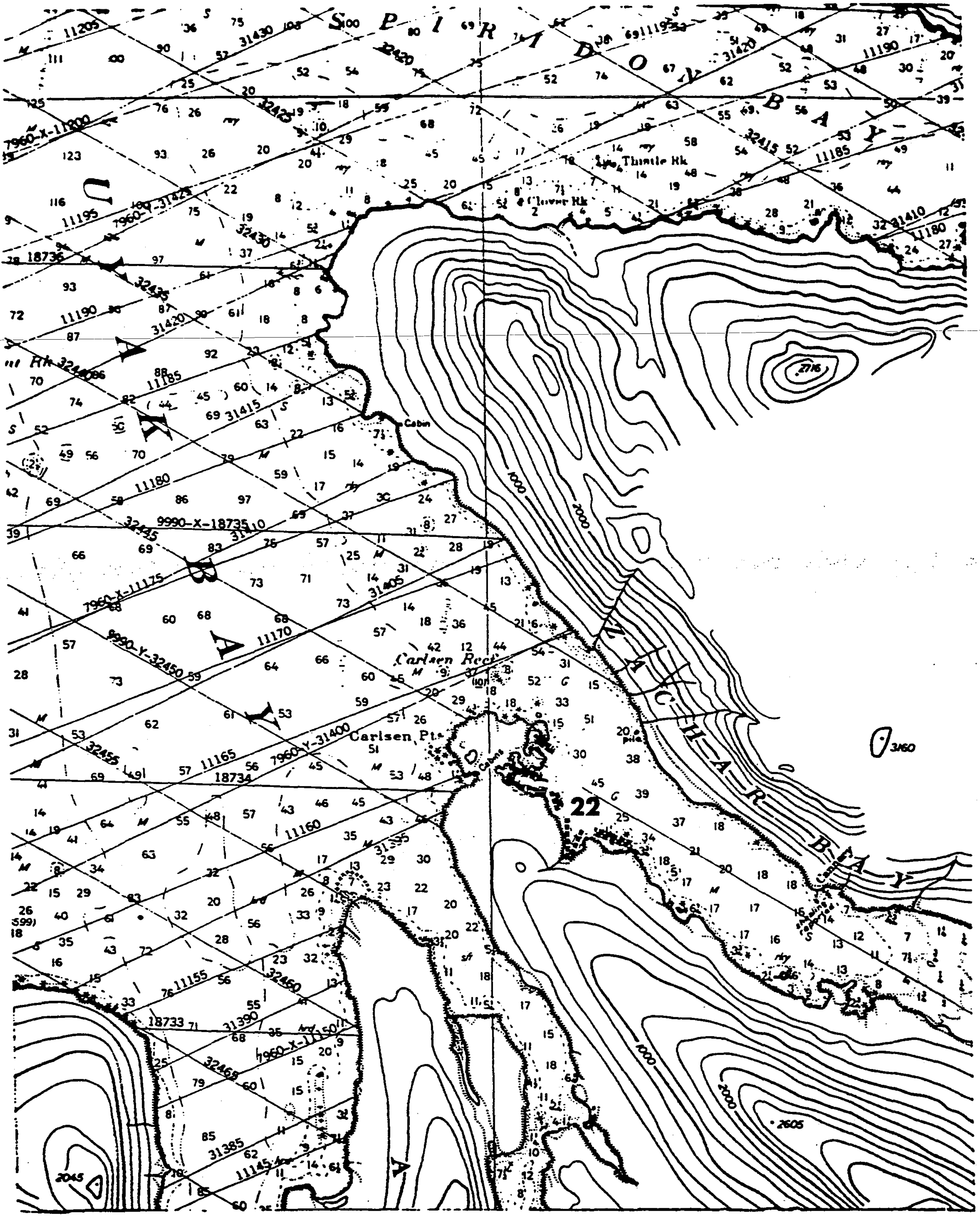

Figure 8. Site 22 in Uyak Bay Area.

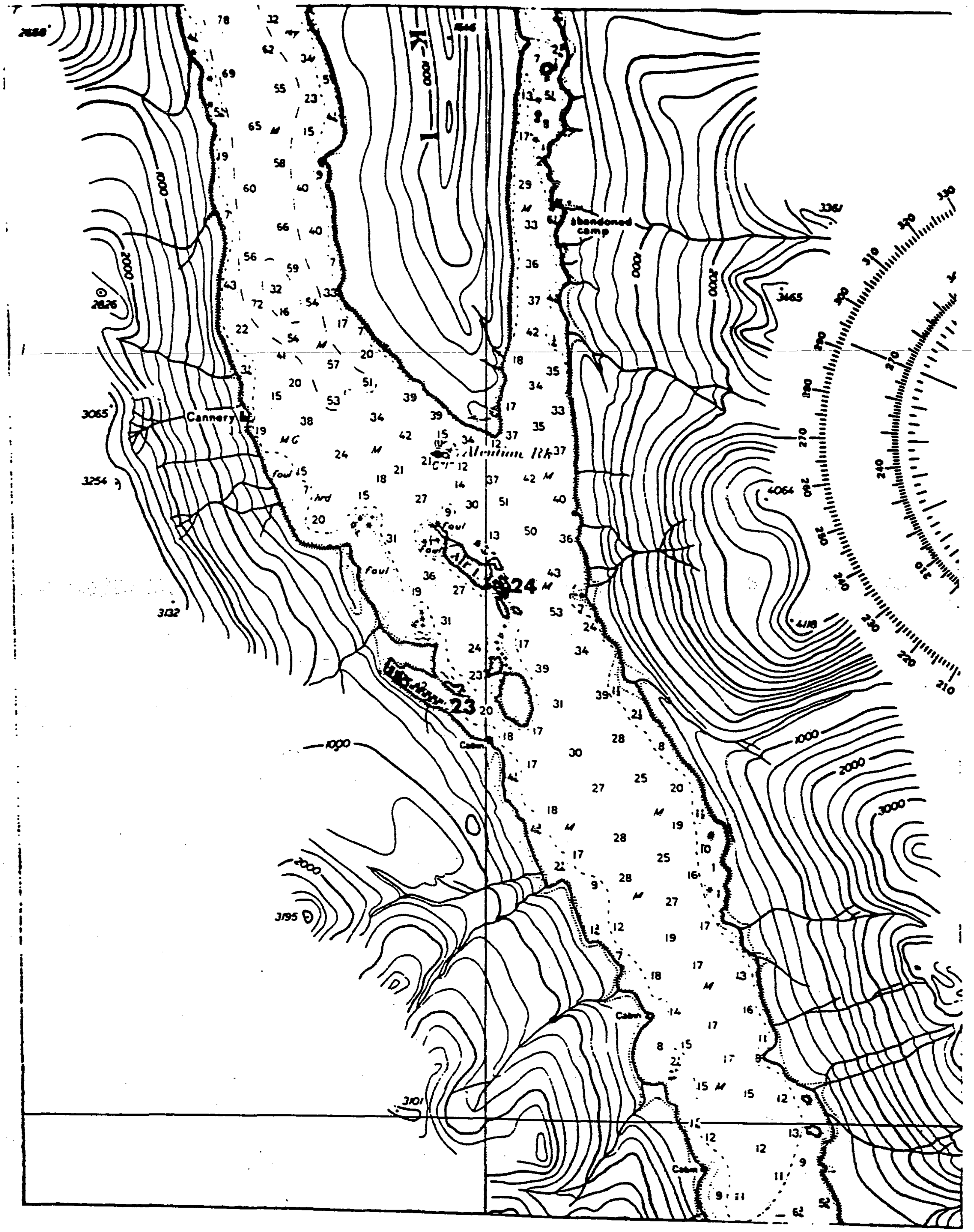

Figure 9. Sites 23-24 in Uyak Bay Area.

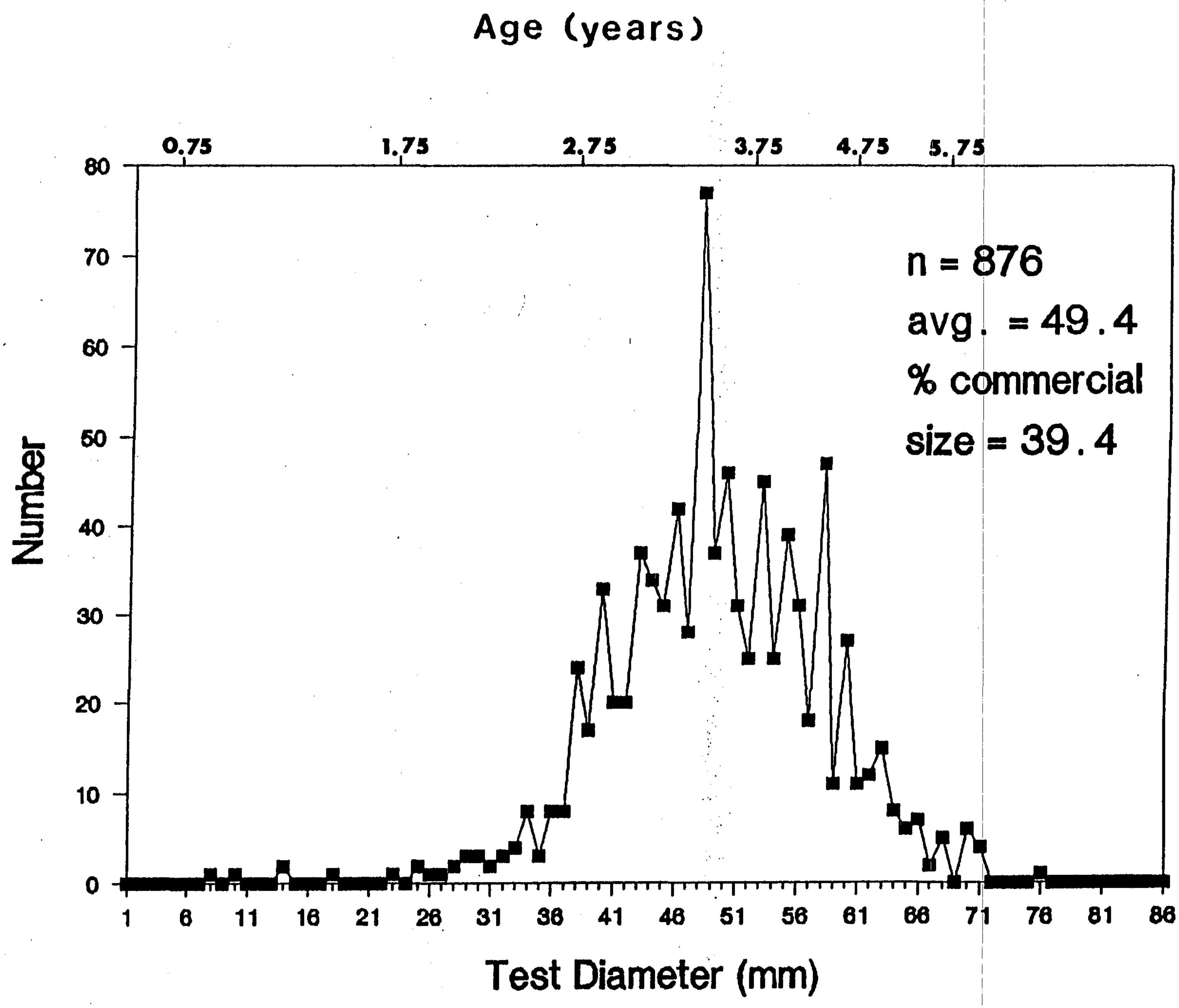

Figure 10. Site 2 green sea urchin test width frequency histogram.

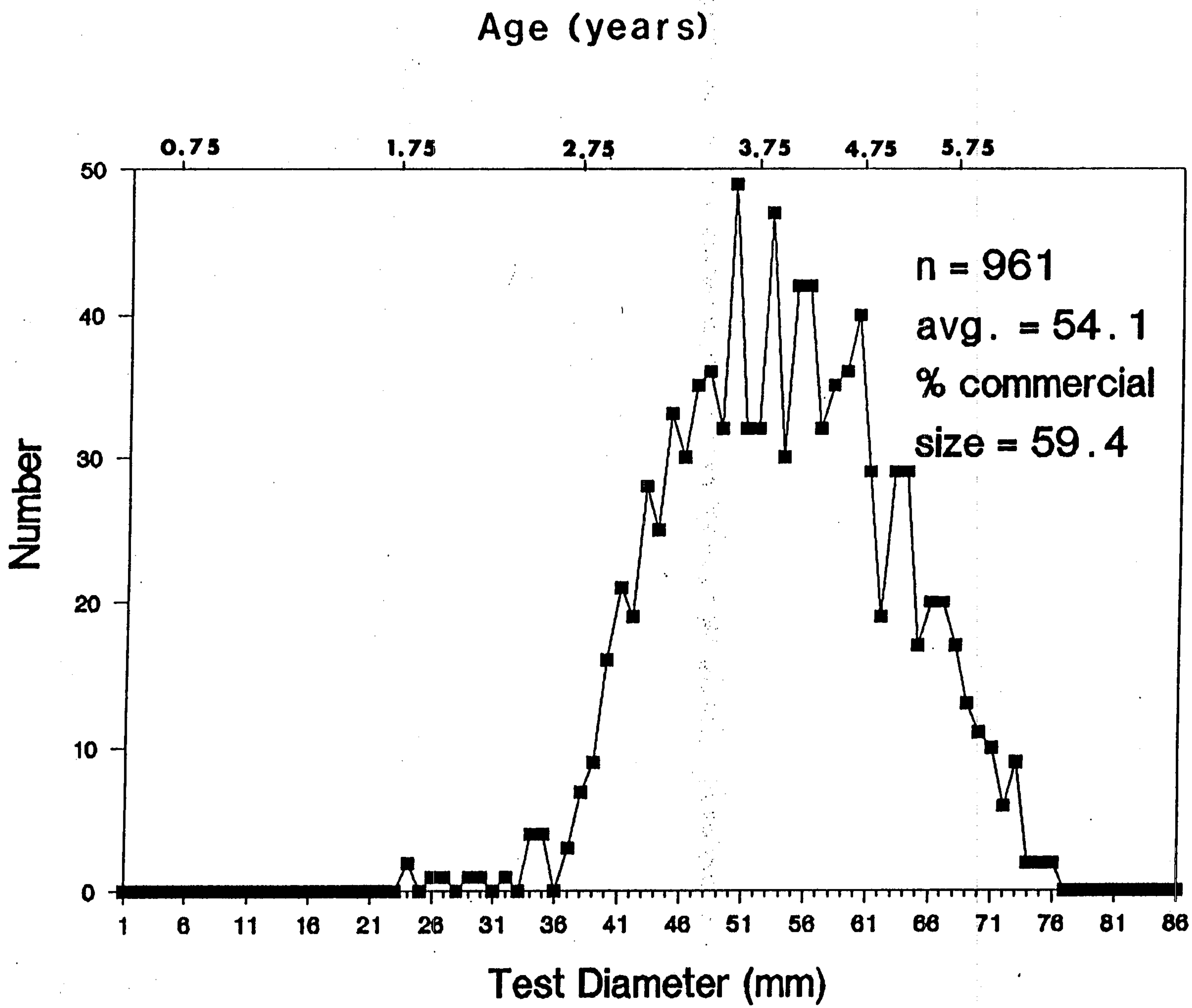

Figure 11. Site 3 green sea urchin test width frequency histogram.

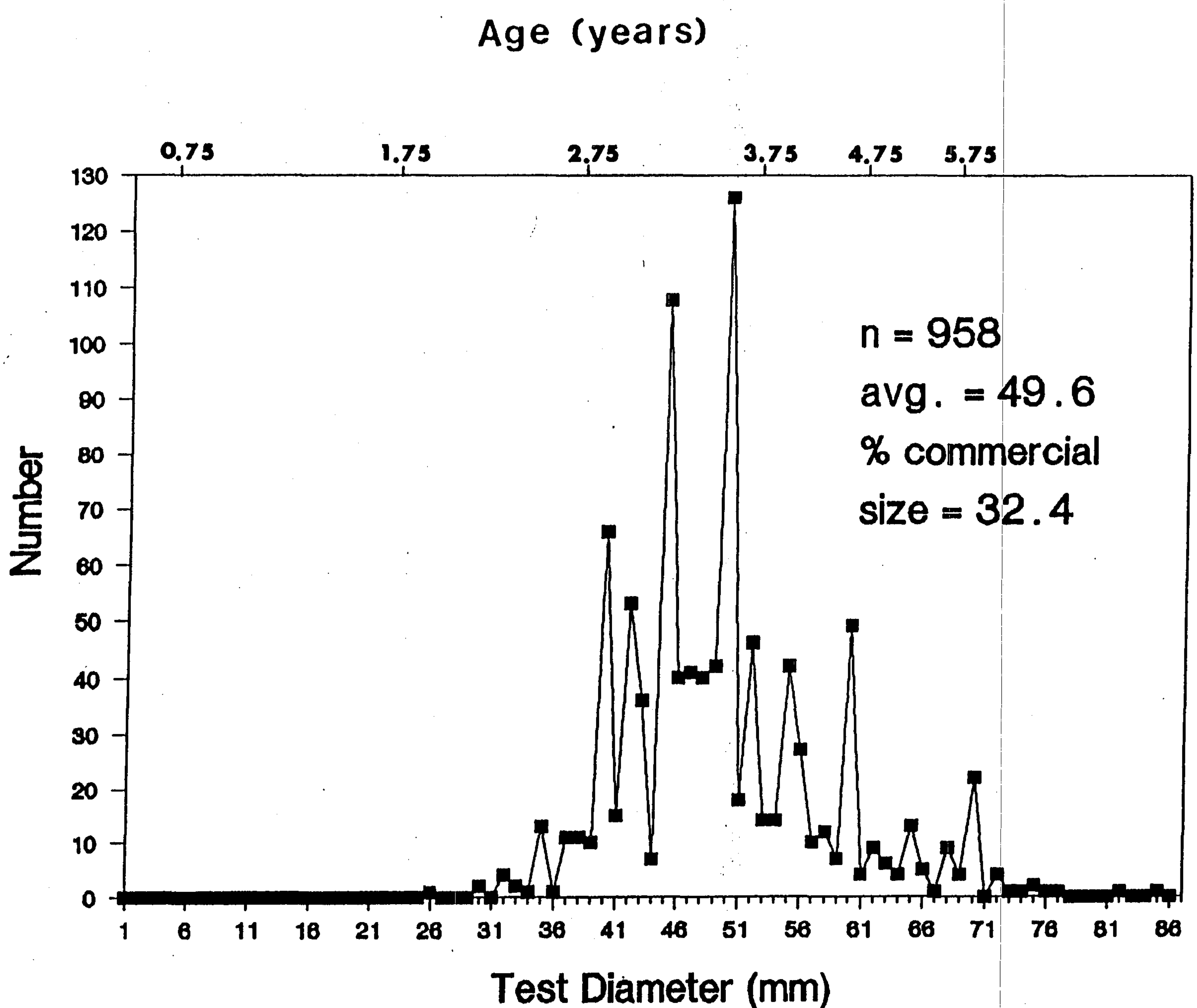

Figure 12. Site 18 green sea urchin test width frequency histogram.

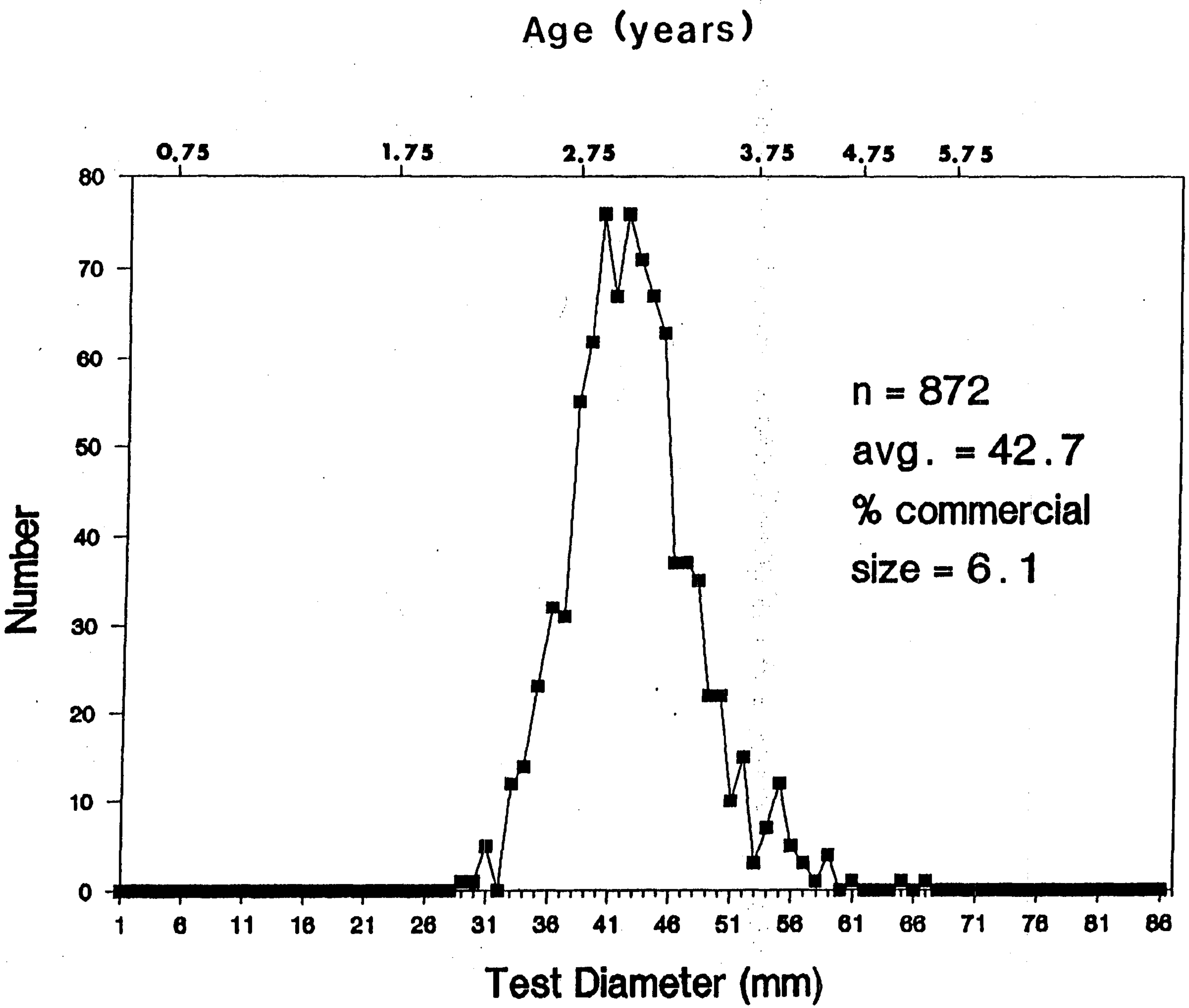

Figure 13. Site 20 green sea urchin test width frequency histogram.

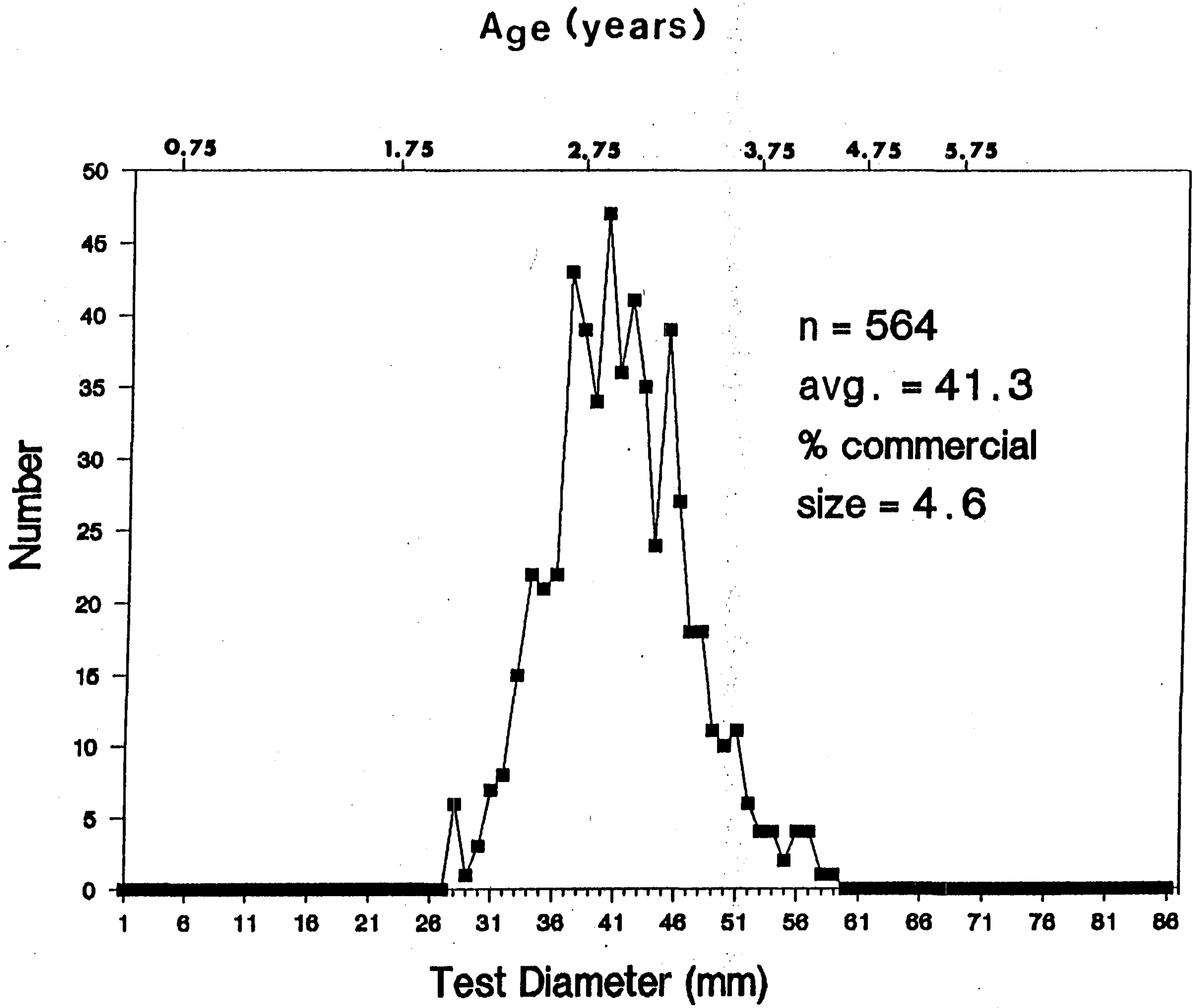

Figure 14. Site 21 green sea urchin test width frequency histogram.

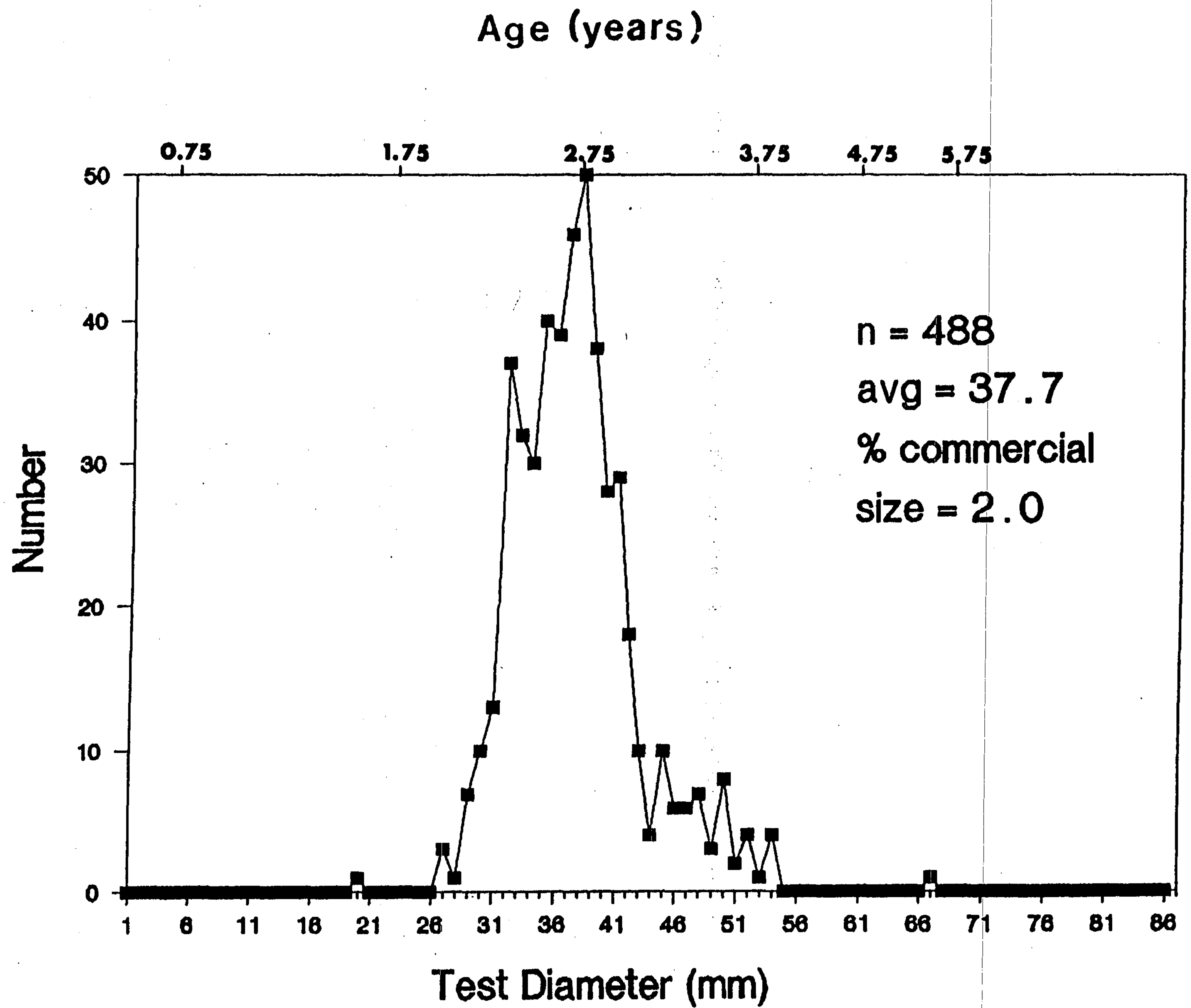

Figure 15. Site 23 green sea urchin test width frequency histogram.

Appendix 1.

ADF&G URCHIN OIL PROJECT SITE DESCRIPTION FIELD FORM

Site Name: Kalsin Bay - Site #2 **Date:** 11-16-89

Latitude: 57° 37.55' **Longitude:** 152° 20.5'

Recorder: Byersdorfer

	Code	Description
Waves	3	Wavelets
Weather	2	Partly cloudy

Air Temp 3.7°C **Surface Sea Temp** 3.0°C **Surface Salinity** 28.5°/oo

	%	Code	Description
Adjacent Beach Substrate	40	5,6	Pebble, rock fragments
	40	8	Rock
	20	4	Granule

Beach Slope 7% **Beach Orientation (e.g. N-NW)** NW-SE

1.	**S. Temp.**	2.8°C	____	____
2.	**B. Temp.**	1.7°C	____	____
3.	**Depth**	7'	____	____
4.	**S. Salinity**	18.5°/oo	____	____
5.	**B. Salinity**	29°/oo	____	____
6.	**Visibility**	7-10	____	____

Transect Length (meters): Transect #1,2,3 were 33.5m
#4 = 26.8m

Substrate (%):	Description:
50	Sand, granule
40	Pebbles
10	Rock fragments, rocks

Appendix 2.

ADF&G URCHIN OIL PROJECT SITE DESCRIPTION FIELD FORM

Site Name: Kalsin Bay - Site #3 **Date:** 11/20/89

Latitude: 57° 37.45 **Longitude:** 152° 23.0'

Recorder: Byersdorfer

	Code	Description
Waves	1	Glassy
Weather	1	Clear

Air Temp 0°C **Surface Sea Temp** 2.2°C **Surface Salinity** 28 °/oo

	%	Code	Description
Adjacent Beach Substrate	40	4,5	Granule, pebble
	60	6,8	Rock, rock fragments

Beach Slope 8° **Beach Orientation (e.g. N-NW)** NW-SE

1.	**S. Temp.**	2.2°C		
2.	**B. Temp.**	2.9°C		
3.	**Depth**	7-10'		
4.	**S. Salinity**	28 °/oo		
5.	**B. Salinity**	28.5 °/oo		
6.	**Visibility**	15-20		

Transect Length (meters): Transect #1 = 58m; Transect #2,3 = 33m; Transect #4 = 91m

Substrate (%):	**Description:**
10	Mud, silt
10	Sand
70	Rock fragments
10	

ADF&G URCHIN OIL PROJECT SITE DESCRIPTION FIELD FORM

Site Name: Larsen Bay Cannery-Site#18 **Date:** 11/05/89

Latitude: 57° 32.0' **Longitude:** 153° 59.0'

Recorder: Byersdorfer

	Code	Description
Waves	4	Slight 2-4'
Weather	2	Partly cloudy

Air Temp 4.72°C **Surface Sea Temp** 5.83°C **Surface Salinity** 29°/oo

	%	Code	Description
Adjacent Beach Substrate	10	4	Granule
	45	5	Pebble
	43	6	Rock fragments
	2	8	Rock

Beach Slope 7° **Beach Orientation (e.g. N-NW)** N-S

1.	S. Temp.	6°C	____	____
2.	B. Temp.	6°C	____	____
3.	Depth	12'	____	____
4.	S. Salinity	29°/oo	____	____
5.	B. Salinity	29.5°/oo	____	____
6.	Visibility	20'	____	____

Transect Length (meters): Transect #1 = 15m; Transect #2 = 6m; Transects 3,4 = 10m

Substrate (%):	Description:
1%	Sand
17 %	Granule
80%	Pebble
> 1 %	

Appendix 4.

ADF&G URCHIN OIL PROJECT SITE DESCRIPTION FIELD FORM

Site Name: Amook Island - Site #20 **Date:** 11/9/89 & 12/06/89

Latitude: 57° 31.0' **Longitude:** 153° 50.0'

Recorder: Byersdorfer

	Code	Description
Waves	1	Glassy
Weather	2	Partly cloudy

Air Temp 6°C **Surface Sea Temp** 5°C **Surface Salinity** 29 °/oo

	%	Code	Description
Adjacent Beach Substrate	20	6	Rock fragments
	50	7	Cobble
	26	8	Rock
	4	9	Boulder

Beach Slope 10-12° **Beach Orientation (e.g. N-NW)** W-E

1.	**S. Temp.**	5°C		
2.	**B. Temp.**	5°C		
3.	**Depth**	8'		
4.	**S. Salinity**	29°/oo		
5.	**B. Salinity**	30°/oo		
6.	**Visibility**	10-20'		

Transect Length (meters): Transect #1=16m; Transect #2=8m; Transect #3=17m; Transect #4=10m

Substrate (%):	Description:
45	Rock
45	Cobble shingle
10	Pebble

Appendix 5.

ADF&G URCHIN OIL PROJECT SITE DESCRIPTION FIELD FORM

Site Name: Larsen Bay-Head - Site #21 **Date:** 12/05/89
Latitude: 57° 31.7' **Longitude:** 154° 6'
Recorder: Byersdorfer

	Code	Description
Waves	4	Slight
Weather	5-6	Showers - squalls

Air Temp 7.2°C **Surface Sea Temp** 4.5°C **Surface Salinity** 28°/oo

	%	Code	Description
Adjacent Beach Substrate	5	5	Pebble
	23	6	Rock fragments
	30	7	Cobble
	40	8	Rookle
	2	9	Boulder

Beach Slope 10° **Beach Orientation (e.g. N-NW)** W-E

1.	**S. Temp.**	4.5°C	___	___
2.	**B. Temp.**	4°C	___	___
3.	**Depth**	12-16'	___	___
4.	**S. Salinity**	28°/oo	___	___
5.	**B. Salinity**	29.5°/oo	___	___
6.	**Visibility**	5'	___	___

Transect Length (meters): Transect #1=18m; Transect #2=56m

Substrate (%):	**Description:**
90	Mud
10	Pebble, rock

ADF&G URCHIN OIL PROJECT SITE
DESCRIPTION FIELD FORM

Site Name: Cove SW of Alf Is.-Site #23 **Date:** 12/09/89

Latitude: 57° 23.65' **Longitude:** 153° 51.2'

Recorder: Byersdorfer

	Code	Description
Waves	3	Wavelets
Weather	8	Rain

Air Temp 6.1°C **Surface Sea Temp** 4°C **Surface Salinity** 28°/oo

	%	Code	Description
Adjacent Beach Substrate	15	6	Rock fragments
	25	7	Cobble
	35	8	Rock
	25	9	Boulder

Beach Slope 35° **Beach Orientation (e.g. N-NW)** NW-SE

1.	**S. Temp.**	4°	____	____
2.	**B. Temp.**	5°	____	____
3.	**Depth**	5-17'	____	____
4.	**S. Salinity**	28°/oo	____	____
5.	**B. Salinity**	29.5°/oo	____	____
6.	**Visibility**	15'	____	____

Transect Length (meters): All urchins in the small 5x30m band parallel to shore were collected

Substrate (%):	Description:
5	Rock
40	Cobble shingle
40	Rock fragments
15	Pebble

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Appendix I:

Sea Urchin Fertilization and Larval Development Study

Supplement to:
Fish/Shellfish Study Number 26
Annual Report

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EXECUTIVE SUMMARY

These studies were conducted to determine the effects of the *Exxon Valdez* oil spill on fertilization and embryonic development of sea urchins. We examined the potential toxicity of elutriates from sediments collected from Prince William Sound, and the toxicity of water soluble fractions of Prudhoe Bay crude oil. The Fall 1990 experiments examined toxicity to purple sea urchins. Experiments with green sea urchins were performed in Spring 1991, the peak spawning period for this species. Both fertility and larval development assays were performed.

Sediment toxicity tests were performed using samples collected from 3 oiled (Northwest Bay, Herring Bay, and Bay of Isles) and 3 control sites (Cabin Bay, Lower Herring Bay, and Drier Bay). In studies conducted in Fall 1990, elutriates from sediments collected from Bay of Isles proved toxic to purple urchins, with respect to both fertilization and embryo development. None of the other sediments collected in Fall 1990 were toxic.

Sediment tests conducted in Spring 1991 revealed relatively little toxicity to sea urchin gametes or embryos. Some suggestion of toxicity was noted at Bay of Isles. The proportion of embryos developing normally was lower in sediment elutriates from Bay of Isles than in a filtered seawater control, but development did not differ significantly in elutriates from Bay of Isles vs elutriates from unoiled control sites. Some toxicity was also observed at Cabin Bay, an unoiled control.

Water soluble fractions (WSF) of crude oil, made in a 9:1 dilution of filtered sea water to oil, proved toxic to purple sea urchin sperm at concentrations equal to or greater than 18%. Water soluble fractions of oil were more toxic to green sea urchins than to purple urchins. Fertilization of green urchins was reduced significantly at concentrations greater than or equal to 3.2% WSF. Weathering of oil for 2 weeks (the approximate transit time of oil from the spill site to Kodiak Island) reduced toxicity of the oil by approximately 6 times. Water soluble fractions of weathered oil inhibited fertilization of green urchins at concentrations greater than or equal to 18%.

Embryonic development proved less sensitive than fertilization to water soluble extracts. In tests with purple urchins, no toxicity was observed with respect to embryonic development at concentrations up to 32% WSF, the highest concentration tested. Water soluble fractions of unweathered oil were toxic to green urchin embryo development at concentrations greater than or equal to 56%. No effect of weathered oil was observed at concentrations up to 56%.

Sediment samples and water soluble fraction samples were collected for hydrocarbon analysis and shipped to the Auke Bay Laboratory for analysis. The data from the hydrocarbon analyses are not yet available.

The results of this study suggest that there were possible effects of oil on sea urchin populations, especially in the days immediately after the spill. However, without data on the hydrocarbon content for water soluble fractions examined in this study, it is difficult to determine if concentrations of hydrocarbons in the water were sufficient to cause damage.

Draft Final Report - Sea Urchin Fertilization and Larval Development Study

1.0 Introduction

This study examines the possible effects of the *Exxon Valdez* oil spill on sea urchins. We examined effects of elutriates made from sediments collected from Prince William Sound, and of water soluble fractions of Prudhoe Bay crude oil on sperm, eggs, and larvae of both green urchins (*Strongylocentrotus droebachiensis*) and purple urchins (*Strongylocentrotus purpuratus*).

Previous studies with *S. droebachiensis* and other sea urchins indicate that the behavior and reproductive success of adult urchins are affected by oil. In simulated oil spill studies, adult *S. droebachiensis* avoided dispersed oil, and may have avoided untreated and dispersed oil in sediments up to 2 years after oiling (Cross et al, 1987). After the *Tampico Maru* spill of mineral oil, North et al (1964) noted high mortality among sea urchins and a reduction in sea urchin density was observed up to 2 years later. Adults of *S. nudus* that were exposed to hydrocarbons from diesel fuel had impaired reproductive success (Vashchenko, 1980; Vashchenko and Naidenko, 1989).

More direct effects of crude oil and other petroleum products on gametic stages and embryos have been reported for sea urchin species including *S. droebachiensis* (Kobayashi, 1981), *S. purpuratus* (Allen, 1971), and others (Kobayahsi, 1981; Greenwood, 1983). However, it is impossible to relate the results of these studies to the effects of the current spill in any quantitative sense. Previous studies have not measured the hydrocarbon content of the water soluble fractions tested.

2.0 Methods

2.1 Experiments with Purple Urchins

2.1.1. Design and Urchin Collection

Tests were performed to examine the toxicity of elutriates made from sediments collected from oiled and control sites in Prince William Sound, and to examine the toxicity of water soluble fractions of Prudhoe Bay crude oil on purple urchins.

Approximately forty purple urchins were collected from the Mission Bay Jetty in San Diego in October 1990. The urchins were maintained in our laboratory cold room (15°C) and fed kelp *ad libitum* until the time of the tests.

2.12 Elutriate Test Procedures

Toxicity tests were performed on elutriates made from the sediment samples and control water (filtered sea water from La Jolla, California). Both sperm cell (fertilization) and embryo development tests were performed. All tests were performed at 15°C.

Sediment samples were collected from 3 oiled and 3 control sites in Prince William Sound on October 4 and 5, 1990 (Figures 1 and 2). Oiled and control sites used were paired with regard to slope, sediment type, and general exposure. The oiled sites sampled were Herring Bay, Bay of Isles, and Northwest Bay. The control sites were in Lower Herring Bay, Drier Bay, and Cabin Bay. Samples were collected from heavily oiled areas at approximately the +1 m tide level. Six replicate sediment samples were collected from each site by scraping off the top 10 cm of sediment and then collecting 2 cm of sediments into a 500 ml pre-cleaned I-CHEM jar. Sampling stations at each site were separated by approximately 2 m. Two samples per site were frozen for later hydrocarbon analysis and one sample per site was frozen and delivered to our laboratory in Carlsbad, CA. for use in purple urchin bioassays. (Three samples per site were used in aborted experiments with green urchins in October 1990. See Section 2.2.2).

Three replicate elutriate samples were made from each of the 6 sediment samples. Elutriates were prepared by taking a 140 ml aliquot of sediment and adding this to 560 ml of filtered seawater to achieve a 1:4 ratio (v:v). The sediment/water mixture was placed in a 1 l Ehrlenmeyer flask and shaken for 2 minutes at 15°C. Each sample was allowed to settle for 60 min after shaking. The water used in the tests was then siphoned from below the surface and from above the sediments. A 10 ml aliquot was removed and placed into a test tube for sperm cell toxicity testing. A 100 ml aliquot was placed into a tissue culture flask for tests of embryo development.

The sperm cell (fertilization) test followed the procedures of Dinnel et al (1987). Release of the eggs and sperm from gravid sea urchins was induced by injection of 0.5 ml of 0.5 M KCl into the coelom of each individual sea urchin. Eggs were shed directly into beakers of chilled seawater. Sperm was collected with a minimum of seawater (dry condition) and refrigerated until used.

The test was initiated by adding 0.1 ml of sperm stock solution to each of 5 borosilicate glass test tubes per treatment, each containing 10 ml of elutriate sample. The sperm solution was adjusted to a density of 1×10^6 sperm per ml. Sperm density was determined by placing a sample of the sperm on a hemacytometer and examining it at 400x using a compound microscope. After 60 min exposure, 2,000 eggs were added to each sample. This provided a sperm to egg ratio of the recommended 200:1. Egg density was determined by counting replicate 0.1 ml samples of eggs and adjusting the volume of the egg suspension to achieve a density of 2000/ml.

Each sample was allowed 20 min for fertilization to occur and then preserved with 1 ml of formalin for later examination. The preserved eggs were examined using a light microscope (100 x). The proportion of eggs fertilized was determined for the first 100 eggs observed in an aliquot of each sperm exposure sample. Fertilization was indicated by the presence of a well-defined fertilization membrane around the egg.

The embryo toxicity test methods were modified from the procedures of Oshida et al (1981). Exposure of the embryos to the samples was initiated by inoculating each of five, 100 ml samples per treatment with 3,000 fertilized eggs. The contents of the beakers were held for 48 hours at 15°C.

The contents of the beakers were preserved for later analysis of percentage normal development. One hundred eggs were examined per sample. The numbers of embryos in the gastrula or blastula stage and without malformations were considered normal. Embryos exhibiting an unusual pattern of development, such as exogastrulation, blastula filled with cells, or unhatched embryos with abnormal cleavage were classified as abnormal.

2.1.3. Water Soluble Fraction Test Procedures

University of Alaska personnel delivered Prudhoe Bay crude oil to the laboratory in Seward for use in toxicity tests. A subsample of the oil was shipped to Carlsbad for purple urchin tests. Water soluble fractions (WSF) of oil were prepared using the methods of Anderson et al (1974). A solution was made by adding 300 ml of oil into a glass container and adding filtered seawater to achieve a final volume of 3 l. The mixture was stirred with a magnetic stirrer for 20 hours at 20°C and then was allowed to settle for 2 hours. The water soluble fraction was then siphoned from below the oil's surface.

Both fertilization and embryo development tests were performed. A dilution series was prepared ranging from 1.8% to 100% for the fertilization test and from 5.6% to 32% in the embryo tests. In addition, a reference test using copper was performed in conjunction with the fertilization test. Copper standards were made from a stock solution prepared by dissolving analytical grade copper chloride in filtered sea water. From this stock, solutions with copper concentrations of 1.25, 2.5, 5.0, 10, and 20 µg/l were made. There were five replicate samples made for each dilution and a control in both fertilization and embryo development tests.

2.1.4. Hydrocarbon Analyses

Twenty-two samples were shipped to the Auke Bay Laboratory for hydrocarbon analyses. (A list of samples shipped to Auke Bay and their present status is given in Appendix A). These included 12 frozen sediment samples and duplicate samples of each of 5 concentrations of methylene chloride extract of water soluble fractions: Control (filtered sea water), 1.8%, 5.6%, 18%, and 56% WSF.

All of the WSF samples were extracted with methylene chloride immediately following the tests. Nine hundred ml of the water soluble fraction were added to a separatory funnel that was washed and rinsed with methylene chloride. Fifty ml of methylene chloride were added to the funnel and the contents shaken for 2 minutes. The funnel was let stand for 5 minutes and the denser bottom layer of methylene chloride was decanted into a pre-cleaned, 150 ml I-CHEM jar. An additional 25 ml of methylene chloride were added and the sample was shaken, settled, and decanted as above. The procedure was repeated a third time with an additional 25 ml of methylene chloride, producing a final volume of 100 ml of extract.

2.2. Experiments with Green Urchins

2.2.1 Design and Sea Urchin Collection

Three tests were performed with green sea urchins. One examined the toxicity of elutriates made from sediments collected from oiled and control sites in Prince William Sound. The others examined the toxicity of water soluble fractions of both fresh and weathered Prudhoe Bay Crude oil.

Approximately 75 green sea urchins were collected from a lightly oiled location in Kelson Bay, on Kodiak Island by Mr. Bill Donaldson of Alaska Department of Fish and Game on July 12, 1990. The urchins were packed in seaweed and shipped to the Seward Marine Laboratory where they were unpacked and placed in a flowing seawater tank. All but 2 of the urchins arrived alive and apparently in good health. These urchins were fed fresh kelp *ad libitum* and kept in a seawater tank at ambient sea water temperature.

We attempted to spawn the urchins in October 1990. A total of 34 males and 0 females spawned. As a result, experiments using green urchins were postponed until April 1991, during the peak of the sea urchin spawning season.

2.2.2. Elutriate Test Procedures

Toxicity tests were performed on elutriates made from the 18 sediment samples and control water (filtered sea water from the Seward Marine Laboratory). Both sperm cell (fertilization) and embryo development tests were performed.

Sediment samples were collected from 3 oiled and 3 control sites in Prince William Sound in April 1991. Oiled and control sites used were the same as sampled in Fall 1990. Samples were collected from Bay of Isles, Drier Bay, Lower Herring Bay and Herring Bay on April 27 and from Cabin and Northwest Bays on April 30. The hiatus in sampling was caused by poor weather in the Sound. Samples were collected from heavily oiled areas at approximately the +1 m tide level. Six replicate sediment samples were collected from each site by scraping off the top 10 cm of sediment and then collecting 2 cm of sediment into a 500 ml pre-cleaned I-CHEM jar. Two samples each were collected from 3 holes dug on the beach. The holes were approximately 2 m apart. Oil sheens were observed in holes dug at all oiled sites.

After sampling, the samples were kept in a cooler with blue ice and delivered to the Seward Marine Laboratory on April 30. Three samples per site (one per sampling hole) were frozen for later hydrocarbon analysis. The three other samples per site were kept in a refrigerator and used in elutriate tests the following day.

One elutriate sample was made from each of 3 sediment samples per site. Elutriates were prepared by taking a 140 ml aliquot of sediment and adding this to 560 ml of filtered seawater to achieve a 1:4 ratio (v:v). The sediment/water mixture was placed in 1 l Ehrlenmeyer flask and shaken. After one hour, the shaker table failed to operate, and samples were removed and shaken by hand for one minute at 5 and 10 hours after mixing. All elutriates were kept in a coldroom maintained at 10°C. Each sample was allowed to settle for 10 hours after shaking.

The methods used to make elutriates for experiments with green urchins differed from those used with purple urchins for several reasons. First, we had hoped to employ a mechanical shaker available at the Seward Marine Laboratory and provide a longer shaking time (20 hours) in order to insure that any substance in the sediments could be mixed in the elutriate water. Upon failure of the shaker table, we were forced to improvise somewhat, and resorted to occasional manual shaking. The longer settling period was necessary because the mechanical shaker put fine sediments into solution that required several hours to settle out.

The water used in the tests was then siphoned from the below the surface and from above the sediments. A 10 ml aliquot was removed from each flask and placed into a test tube for sperm cell toxicity testing. A 50 ml aliquot was placed into a tissue culture flask for tests of embryo development. A 250 ml aliquot was extracted for later hydrocarbon analyses.

The sperm cell (fertilization) test followed the procedures of Dinnel et al (1987) as described above for purple sea urchins. The only modifications to the procedure were that the sperm solution was adjusted to a density of 1×10^7 sperm per ml in order to obtain a sperm to egg ratio of 2000 to 1 as recommended for green sea urchins, the tests were performed at 10°C, and 3 replicate tubes were used for each elutriate sample.

The embryo toxicity test methods were modified from the procedures of Oshida et al (1981). Test procedures were as described for purple sea urchins, except that a 50 ml sample volume was used and the tests were performed at 10°C.

2.2.3. Water Soluble Fraction Test Procedures

Water soluble fractions (WSF) of Prudhoe Bay crude oil were prepared using the methods of Anderson et al (1974) as described above for purple sea urchins. Separate water soluble fraction tests were conducted using both fresh and weathered oil. The weathered oil was mixed in a 9:1 ratio with filtered seawater on April 10, 1991 and placed in a wide mouth 1 gal glass jar. The jar was left uncapped and was placed out of doors (but under a roof). The container was capped with a Teflon lined lid and shaken for 1 minute twice daily until 1 day prior to the preparation of water soluble fractions.

Both fertilization and embryo development tests were performed for both new and weathered oil. A dilution series was prepared ranging from 1.0% to 56% for the fertilization test and from 5.6% to 56% in the embryo tests. (A higher concentration (56%) was used for embryo tests with green urchins than with purple because results of purple urchin tests failed to indicate a response at 32%). In addition, a reference test using copper was performed in conjunction with the fertilization test. Copper standards were made from a stock solution prepared by dissolving analytical grade copper chloride in filtered sea water. From this stock, solutions with copper concentrations of 1.25, 2.5, 5.0, 10, and 20 µg/l were made. There were five replicate samples made for each dilution and a control in both fertilization and embryo development tests.

2.2.4. Hydrocarbon Analyses

A total of 55 samples were shipped to the Auke Bay Laboratory for hydrocarbon analyses. These included 12 frozen sediment samples, duplicate methylene chloride extracts of each of 5 concentrations (5.6%, 10%, 18%, 32%, and 56%) of water soluble fractions of new and weathered oil, duplicate samples of the control (extracts of filtered sea water), and 1 methylene chloride extract for each elutriate sample made from Prince William Sound sediments. The extracts of elutriates were damaged in shipment and will not be analyzed.

All of the WSF samples were extracted with methylene chloride immediately following the tests using the procedures outlined in section 2.1.4 above, except that only 700 ml of WSF was used. Elutriate sample extractions were made by adding 25 ml of methylene chloride to 250 ml of sample, shaking for 2 minutes, settling for 5 minutes, and decanting the methylene chloride portion into a pre-cleaned I-CHEM jar. An additional 25 ml of methylene chloride were added and the above procedure repeated, producing 50 ml of final extract.

2.3 Statistical Analyses

For the elutriate assays, we tested the null hypotheses that there was no difference between an oiled site and its paired control site with respect to toxicity of elutriates. T-tests were performed to test for differences in mean fertilization rates and mean proportion of embryos developing normally at oiled and control site pairs. In addition, we compared mean percent fertilization and percent normal development of each elutriate sample with a filtered sea water control using Bonferroni's multiple comparison test (Milliken and Johnson, 1984). For analyses of fertilization rate in Spring 1991 in which we replicated the tests for each elutriate sample, mean values for each sediment elutriate were used in the tests.

The results of the water soluble fraction tests were analyzed to determine No Observed Effect Concentrations (NOEC = the highest concentration that did not differ significantly from the control) and the EC_{50} (the concentration at which 50% of the experimental population was affected). NOEC values were determined for percent fertilization and percent normal development using multiple comparison tests. In cases where there were equal numbers of replicates among the treatments, we tested for differences of each elutriate vs the control using Dunnett's test (Zar, 1974). In cases where there was unequal replication, we used Bonferroni's test. EC_{50} values were determined using probit analysis (Finney, 1971). Percent fertilization and normal development values were arcsin transformed prior to all analyses.

Nominal concentrations of oil (expressed as % dilution of the WSF) and copper (expressed as µg/l Cu) were used in the analysis. No data on total hydrocarbon levels or analytically determined copper levels were available.

3.0 Results.

3.1 Elutriate Tests with Purple Urchins

All elutriates made from sediments collected from Prince William Sound in Fall 1990 developed oil sheens indicating the obvious presence of oil. Toxicity was observed in only those elutriates made from sediments collected from Bay of Isles. Fertility in the Bay of Isles sample was only 63% compared to 85% in the control samples from Drier Bay (Table 1). These values differed significantly at $P<0.10$ (Table 1). Fertilization in the Bay of Isles elutriates was also significantly lower than the filtered sea water control ($P\leq0.05$, t-test). The proportion of embryos developing normally was also significantly lower in the Bay of Isles sample than in the control site sample ($P<0.01$, Table 2) and in filtered sea water ($P<0.05$, t-test). All elutriate samples had test temperatures that were similar for all treatments (Table 3). The salinity and pH from the Bay of Isles elutriates were slightly lower than at the other sites, but all were well within the range for normal fertilization and development.

3.2 Elutriate Tests with Green Urchins

Oil sheens were also observed at all oiled sites where sediments were collected in late April 1991. However, there was little toxicity of oil indicated in any of the elutriate tests conducted with green urchins. No toxicity was indicated in sediments collected at any of the oiled sites relative to their paired unoiled control sites, with respect to either fertilization (Table 4) or embryonic development (Table 5). Only the elutriate sample from Bay of Isles had significantly lower rates of normal development vs the filtered sea water control in the embryo tests ($P<0.05$, t-test), and none of the elutriates from oiled sites differed from the sea water control in fertilization tests ($P>0.05$, t-tests).

There were indications of toxicity at the presumably unoiled Cabin Bay site (Tables 4 and 5). Rates of normal development were significantly lower at Cabin Bay than its paired oiled site (Northwest Bay). The mean fertilization rate was also substantially lower at Cabin Bay than at Northwest Bay (41% vs 82%), but this difference was not statistically significant.

All samples had pH, salinity, and temperature values that were within a normal range for fertilization and development (Table 6).

3.3 Water Soluble Fraction Tests with Purple Urchins

Fertility tests indicated that concentrations of the water soluble fraction of 18% or greater were toxic to the sperm of purple sea urchins (Table 7 and Figure 3). The NOEC for this test was 10% and the EC_{50} was 52.5%. No toxicity was observed in embryo development tests, even at the highest concentration used (32%) (Table 7 and Figure 9).

3.4 Reference Tests with Purple Urchins

The copper reference assays showed slightly less sensitivity than is the norm for purple sea urchin fertilization tests conducted in our laboratory, but well within the normal range of values previously observed. The NOEC level for copper (nominal concentration) was 10 µg/l (Table 8), slightly higher than the median of 5 µg/l for prior sea urchin sperm cell tests conducted in our laboratory. However, the estimated EC_{50} was 23 µg/l, compared with an average of 24 µg/l reported by Nacci and Jackim (1985) for other laboratories.

3.5 Physical/Chemical Parameters for Water Soluble Fractions - Purple Urchins

All physical/chemical factors measured were within the range for normal fertilization and development (Table 9).

3.6 Water Soluble Fraction Tests with Green Urchins

Fertility tests indicated that concentrations of the water soluble fractions of unweathered oil of 3.2% or greater were toxic to the sperm of green sea urchins (Table 10 and Figure 3). The NOEC level for new (unweathered) oil was 1.8% and the EC_{50} was 21.8 %.

The weathering of oil significantly reduced toxicity of the oil. Concentrations of the water soluble fraction of weathered oil of 18% or greater were toxic to the sperm of green sea urchins (Table 11 and Figure 3) and the NOEC level for weathered oil was 10%. While there was a relatively large difference between new and weathered oil with respect to NOEC levels, the EC_{50} values differed only slightly, 21.8 % for new oil and 28.6% for weathered oil.

As observed for purple urchins, embryo development was less sensitive to oil than fertilization. The NOEC values for normal embryo development in new and weathered oil were 32% and >56% respectively (Table 10 and 11, and Figure 4). For new oil, the NOEC value for embryo development was an order of magnitude greater than for fertilization.

3.7 Reference Tests with Green Urchins

The copper reference fertilization assay resulted in an EC_{50} value of 147.2 µg/l (Table 12 and Figure 3). This is somewhat higher than the average value of 58 µg/l reported in the literature (Nacci and Jackim, 1985). Reference results for the embryo tests indicated substantially lower sensitivity than has been previously observed for green urchins. Nacci and Jackin (1985) report an EC_{50} of 21 µg/l copper. We observed no effect at concentrations up to 40 µg/l (Table 12 and Figure 4). The reasons for the discrepancies in our tests and previously reported tests may be the result of different sources of dilution water. Bioavailability of divalent metals such as copper can change appreciably in different sea water sources.

3.8 Physical/Chemical Parameters for Reference Tests

All physical/chemical factors measured were within the range for normal fertilization and development (Table 9).

4.0 Discussion

The studies conducted here suggest that the oil spilled by the *Exxon Valdez* may have had a direct impact on sea urchin populations by inhibiting fertilization or development. The spill occurred at a time of year when spawning takes place, and it seems likely that gametes were being released by green urchins in Prince William Sound and off Kodiak Island during or shortly after the spill. However, without data on the hydrocarbon content of test solutions used in our tests, it is difficult to determine if concentrations of hydrocarbons in the water were sufficient to cause damage. Possible effects of oil were also suggested by the toxicity of sediments collected from an oiled area in Bay of Isles in Fall 1990.

The toxic effects of oil appear to ameliorate with weathering. Oil weathered for two weeks had reduced toxicity relative to fresh oil, as evidenced in the comparison of dose responses for the 2 oil types in Figure 3. Furthermore, there was relatively little evidence of toxicity in oiled sediments collected 1.5 to 2 years after the spill in spite of the still obvious presence of oil sheens in the sediments. The reduction of toxicity with weathering is probably the result of volatilization of lighter fractions of oil that tend to be more toxic (Davis et al, 1985).

The results reported here indicate that fertilization is more sensitive to oil than embryonic development. This is in contrast to previous studies with both purple urchins (Allen, 1971) and green urchins (Kobayashi, 1981) that suggest that oil was more toxic to embryo development than to fertilization. These differences likely resulted from the different methods used. Both Allen (1971) and Kobayashi (1981) used extremely short exposure times (3 to 5 minutes) for estimation of effects on fertilization, compared with the 1 hour exposure used here. Also, both previous studies made no attempt to regulate sperm to egg ratio, and any spermicidal effects may have been obscured by an excess of sperm.

Our results also indicated that green sea urchins were more sensitive to oil than purple urchins. This is somewhat surprising since this and previous studies with both purple and green sea urchins (reviewed in Nacci and Jackim, 1985 and Nacci et al, 1986) suggest that green urchins are less sensitive than purple urchins to most heavy metals. However, sensitivity that is both species and toxicant specific has been widely observed.

It is impossible to compare, in any quantitative way, the results obtained here with previous studies of the toxicity of oil to sea urchins. Methods for the preparation of water soluble fractions varied considerably among studies, and perhaps more importantly, there have been no previous attempts to chemically verify concentrations of hydrocarbons in the water soluble fractions. Previous studies indicate toxicity of water soluble fractions of crude oil in concentrations ranging from 0.02% (Kobayashi, 1981) to <6.25% (Allen, 1981). How these numbers translate to levels of hydrocarbon, and how this compares to our effect levels (that are on the order of 1 to 10% WSF) is unknown.

4.0 References

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Table 1. Results of t-tests of differences in fertilization rates of purple sea urchins in elutriates made from sediments collected from oiled vs control sites in Prince William Sound. T-tests were performed on data that were transformed (arcsin square root). Untransformed means and standard errors are tabulated. Probabilities with an asterisk were corrected for unequal variances among treatments. Variances were equal for all other comparisons.

Site Name	Oil/Control	% Fertilization. Mean	SE	t	df	P
Control Water		98.7	0.67			
Herring Bay	O	91.0	8.00			
				0.99	2.1	0.42*
Lower Herring Bay	C	86.0	1.53			
Bay of Isles	O	62.7	7.17			
				2.32	4.0	0.08
Drier Bay	C	85.0	6.56			
Northwest Bay	O	96.7	1.20			
				1.78	4.0	0.15
Cabin Bay	C	90.0	4.04			

Table 2. Results of t-tests of differences in the proportion of purple sea urchin embryos that developed normally in elutriates made from sediments collected from oiled vs control sites in Prince William Sound. T-tests were performed on data that were transformed (arcsin square root). Untransformed means and standard errors are tabulated.

Site Name	Oil/Control	% Normal Mean	SE	t	df	P
Control Water		80.0	4.00			
Herring Bay	O	85.7	4.84	0.08	4.0	0.94
Lower Herring Bay	C	85.3	4.06			
Bay of Isles	O	42.0	8.71	5.41	4.0	<0.01
Drier Bay	C	88.7	2.40			
Northwest Bay	O	83.0	5.00	0.52	4.0	0.63
Cabin Bay	C	86.3	5.24			

Table 3. Physical chemical parameters for elutriates made with sediments from Prince William Sound used in toxicity tests with purple sea urchins.

Site Name	Oil/Control	pH	Temp (°C)	Salinity (ppt)
Control		8.1	13.5	33
Herring Bay	Oil	7.5	13.3	33
Lower Herring bay	Control	7.5	13.3	33
Bay of Isles	Oil	7.3	13.5	31
Drier Bay	Control	7.5	13.4	32
Northwest Bay	Oil	7.6	13.3	32
Cabin Bay	Control	7.5	13.3	32

Table 4. Results of t-tests of differences in fertilization rates of green sea urchins in elutriates made from sediments collected from oiled vs control sites in Prince William Sound. T-tests were performed on data that were transformed (arcsin square root). Untransformed means and standard errors are tabulated.

Site Name	Oil/Control	% Fertilization Mean	SE	t	df	P
Control Water		94.2	0.40			
Herring Bay	O	91.0	1.64	1.84	4.0	0.14
Lower Herring Bay	C	91.9	0.89			
Bay of Isles	O	91.1	0.59	0.48	4.0	0.66
Drier Bay	C	93.9	1.24			
Northwest Bay	O	82.0	10.2	1.54	4.0	0.20
Cabin Bay	C	41.3	23.3			

Table 5. Results of t-tests of differences in the proportion of green sea urchin embryos that developed normally in elutriates made from sediments collected from oiled vs control sites in Prince William Sound. T-tests were performed on data that were transformed (arcsin square root). Untransformed means and standard errors are tabulated.

Site Name	Oil/Control	% Normal		t	df	P
		Mean	SE			
Control Water		50.0	3.21			
Herring Bay	O	44.3	0.88	0.89	4.0	0.43
Lower Herring Bay	C	39.7	5.24			
Bay of Isles	O	19.7	7.84	2.03	4.0	0.11
Drier Bay	C	41.0	4.93			
Northwest Bay	O	50.7	3.53	3.07	4.0	0.04
Cabin Bay	C	36.0	3.21			

Table 6. Physical chemical parameters for elutriates made with sediments from Prince William Sound used in toxicity tests with green sea urchins. Values are given for each of the three replicate samples taken per site.

Site Name	Oil/Control	pH	Temp (°C)	Salinity (ppt)
Control		7.5	10.0	31
Control		7.6	10.0	31
Control		7.5	10.0	31
Herring Bay	Oil	7.3	10.0	29
Herring Bay	Oil	7.4	10.0	29
Herring Bay	Oil	7.4	10.0	29
Lower Herring Bay	Control	7.3	10.0	28
Lower Herring Bay	Control	7.3	10.0	28
Lower Herring Bay	Control	7.4	10.0	29
Bay of Isles	Oil	7.2	10.0	30
Bay of Isles	Oil	7.7	10.0	32
Bay of Isles	Oil	7.1	10.0	28
Drier Bay	Control	7.5	10.0	30
Drier Bay	Control	7.5	10.0	30
Drier Bay	Control	7.4	10.0	30
Northwest Bay	Oil	7.3	10.0	30
Northwest Bay	Oil	7.5	10.0	29
Northwest Bay	Oil	7.4	10.0	30
Cabin Bay	Control	7.4	10.0	29
Cabin Bay	Control	7.5	10.0	30
Cabin Bay	Control	7.2	10.0	31

Table 7. Mean % fertilization and % of normal embryos of purple sea urchins developing normally in different concentrations of water soluble fractions made from Prudhoe Bay Crude Oil. Underlined values differed significantly from the control at $P<0.05$. NOEC and EC_{50} values are also given.

WSF Conc.	% Fertilization Mean	% Fertilization SE	% Normal Mean	% Normal SE
Control	82.0	3.53	87.6	0.88
1.8%	80.6	3.70	-	-
3.2%	77.4	1.81	86.0	2.65
5.6%	75.2	4.34	87.3	2.43
10%	71.6	3.43	89.6	0.51
18%	63.6	3.43	94.0	2.06
32%	66.2	5.97	86.0	0.41
56%	45.6	7.06	-	-
100%	1.60	0.40	-	-
	NOEC = 10%		NOEC = 32%	
	EC_{50}= 52.5		EC_{50}= -	

Table 8. Mean % fertilization in different concentrations of copper. Underlined values differed significantly from the control at $P<0.05$. NOEC and EC_{50} values are also given.

Cu Conc.	% Fertilization	
	Mean	SE
0	79.0	3.70
1.25	91.0	2.77
2.50	85.8	3.96
5.00	87.0	3.45
10.0	91.2	2.56
20.0	72.8	3.53

NOEC = 10 µg/l Cu

EC_{50}= -

Table 9. Physical chemical parameters for purple sea urchin fertilization tests with water soluble fractions of Prudhoe Bay crude oil and copper reference solutions.

Water Soluble Fraction Test

Conc. %	pH	Temperature (°C)	Salinity (ppt)
Control	8.0	14.6	32
1.8%	8.0	14.4	32
3.2%	8.0	14.1	32
5.6%	8.0	14.0	32
10%	8.0	14.2	32
18%	8.1	14.3	32
32%	8.0	14.5	32
56%	8.1	15.0	32
100%	8.0	15.2	32

Copper Reference Tests

Conc. (µg/l)	pH	Temperature (°C)	Salinity (ppt)
0.00	8.0	13.9	32
1.25	8.0	14.1	33
2.50	8.0	14.1	32
5.00	8.0	14.2	33
10.0	8.0	14.2	33
20.0	8.0	14.3	33

Table 10. Mean % fertilization and % of normal embryos of green sea urchins developing normally in different concentrations of water soluble fractions made from fresh Prudhoe Bay Crude Oil. Underlined values differed significantly from the control at $P < 0.05$. NOEC and EC_{50} values are also given.

WSF Conc.	% Fertilization		% Normal	
	Mean	SE	Mean	SE
Control	90.4	1.08	75.8	2.52
1.0%	83.2	3.25	-	-
1.8	82.4	3.94	-	-
3.2%	<u>65.6</u>	<u>1.69</u>	-	-
5.6%	<u>80.4</u>	<u>3.85</u>	65.0	3.24
10%	<u>59.8</u>	<u>3.64</u>	69.2	3.44
18%	<u>42.2</u>	<u>6.65</u>	73.5	0.87
32%	<u>19.6</u>	<u>2.66</u>	61.4	5.37
56%	<u>8.2</u>	<u>1.36</u>	<u>9.6</u>	<u>2.20</u>
	NOEC = 1.8%		NOEC = 32%	
	EC_{50}= 21.8		EC_{50}= 40.6	

Table 11. Mean % fertilization and % of normal embryos of green sea urchins developing normally in different concentrations of water soluble fractions made from weathered Prudhoe Bay Crude Oil. Underlined values differed significantly from the control at $P < 0.05$. NOEC and EC_{50} values are also given.

WSF Conc.	% Fertilization Mean	% Fertilization SE	% Normal Mean	% Normal SE
Control	90.4	1.08	75.8	2.52
1.0%	91.4	3.37	-	-
1.8%	87.0	2.39	-	-
3.2%	90.0	1.14	-	-
5.6%	91.6	1.44	74.6	1.75
10%	89.3	2.87	75.5	2.36
18%	<u>56.4</u>	<u>3.85</u>	76.6	1.86
32%	<u>34.8</u>	<u>4.83</u>	74.8	2.63
56%	<u>3.80</u>	<u>0.86</u>	69.8	4.08
	NOEC = 10.0		NOEC = 56	
	EC_{50}= 28.6		EC_{50}= -	

Table 12. Mean % fertilization in different concentrations of copper. Underlined values differed significantly from the control at $P<0.05$. NOEC and EC_{50} values are also given.

Cu Conc.	% Fertilization Mean	% Fertilization SE	% Normal Mean	% Normal SE
0	90.4	1.08	75.8	2.52
2.50	89.2	1.85	67.4	2.34
5.00	91.8	2.33	69.2	1.91
10.0	91.4	2.34	69.5	2.97
20.0	88.8	1.39	68.6	3.08
40.0	<u>79.0</u>	<u>4.74</u>	66.4	2.01
	NOEC = 20		NOEC = 40	
	EC_{50}= 22.6		EC_{50}= -	

Table 13. Physical chemical parameters for green sea urchin fertilization tests with water soluble fractions of Prudhoe Bay crude oil and copper reference solutions.

Water Soluble Fraction Test - Unweathered Oil

Conc. %	pH	Temperature (°C)	Salinity (ppt)
Control	7.0	10.0	31
1.8%	-	10.0	-
3.2%	-	10.0	-
5.6%	7.1	10.0	31
10%	7.0	10.0	31
18%	7.1	10.0	31
32%	6.9	10.0	31
56%	6.9	10.0	30

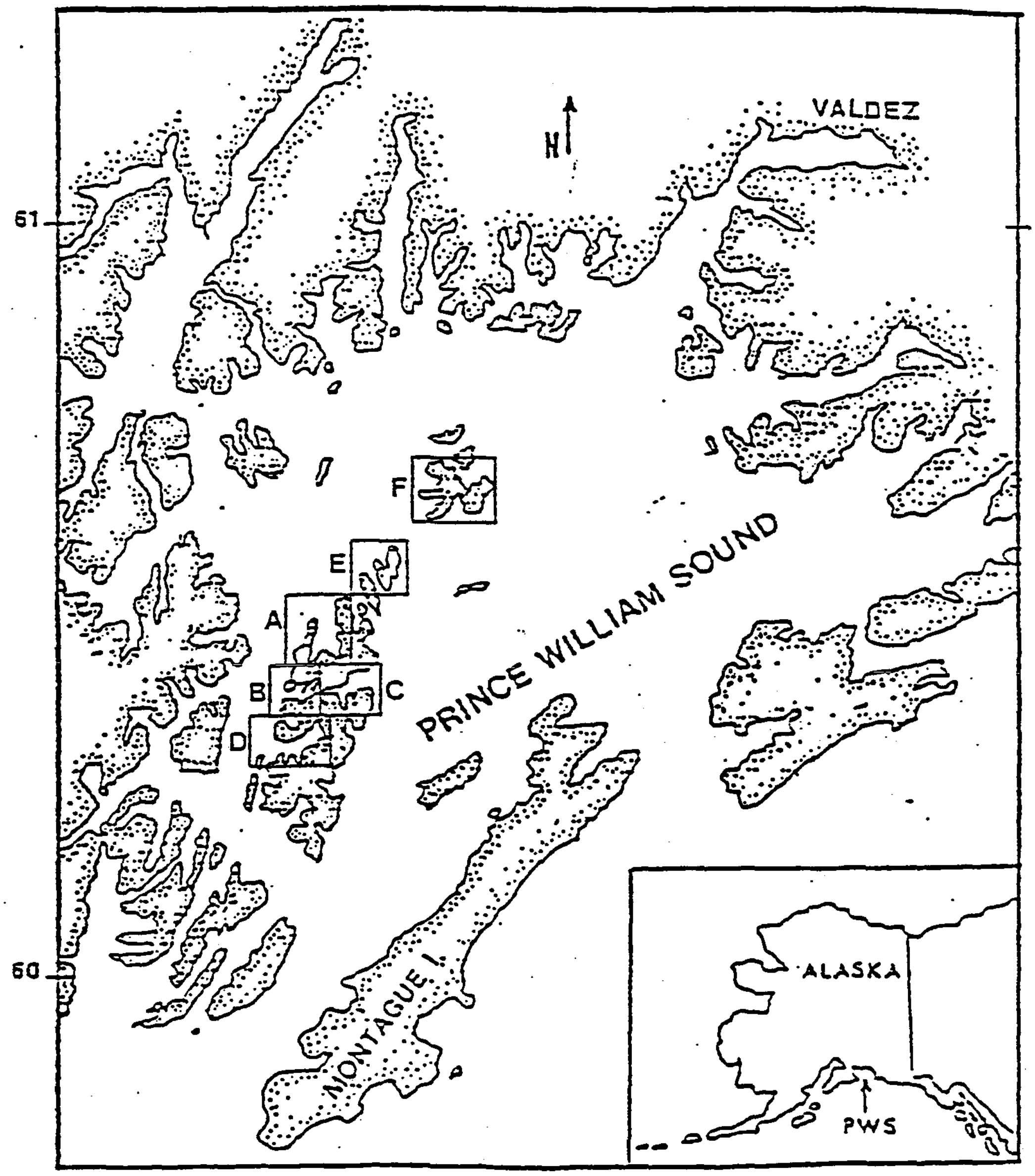
Water Soluble Fraction Test - Weathered Oil

Conc. %	pH	Temperature (°C)	Salinity (ppt)
Control	7.0	10.0	31
1.8%	-	10.0	-
3.2%	-	10.0	-
5.6%	7.0	10.0	31
10%	7.1	10.0	31
18%	7.1	10.0	31
32%	6.8	10.0	31
56%	7.2	10.0	31

Copper Reference Tests

Conc. (μg/l)	pH	Temperature (°C)	Salinity (ppt)
0.00	7.0	10.0	31
1.25	-	10.0	-
2.50	-	10.0	-
5.00	-	10.0	-
10.0	-	10.0	-
20.0	-	10.0	-

Figure 1. Map of Prince William Sound showing the general location of sampling sites of sediments used in elutriate tests. More detailed maps and sampling locations are given in Figure 2.



The letters on each map correspond to general locations shown in Figure 1.

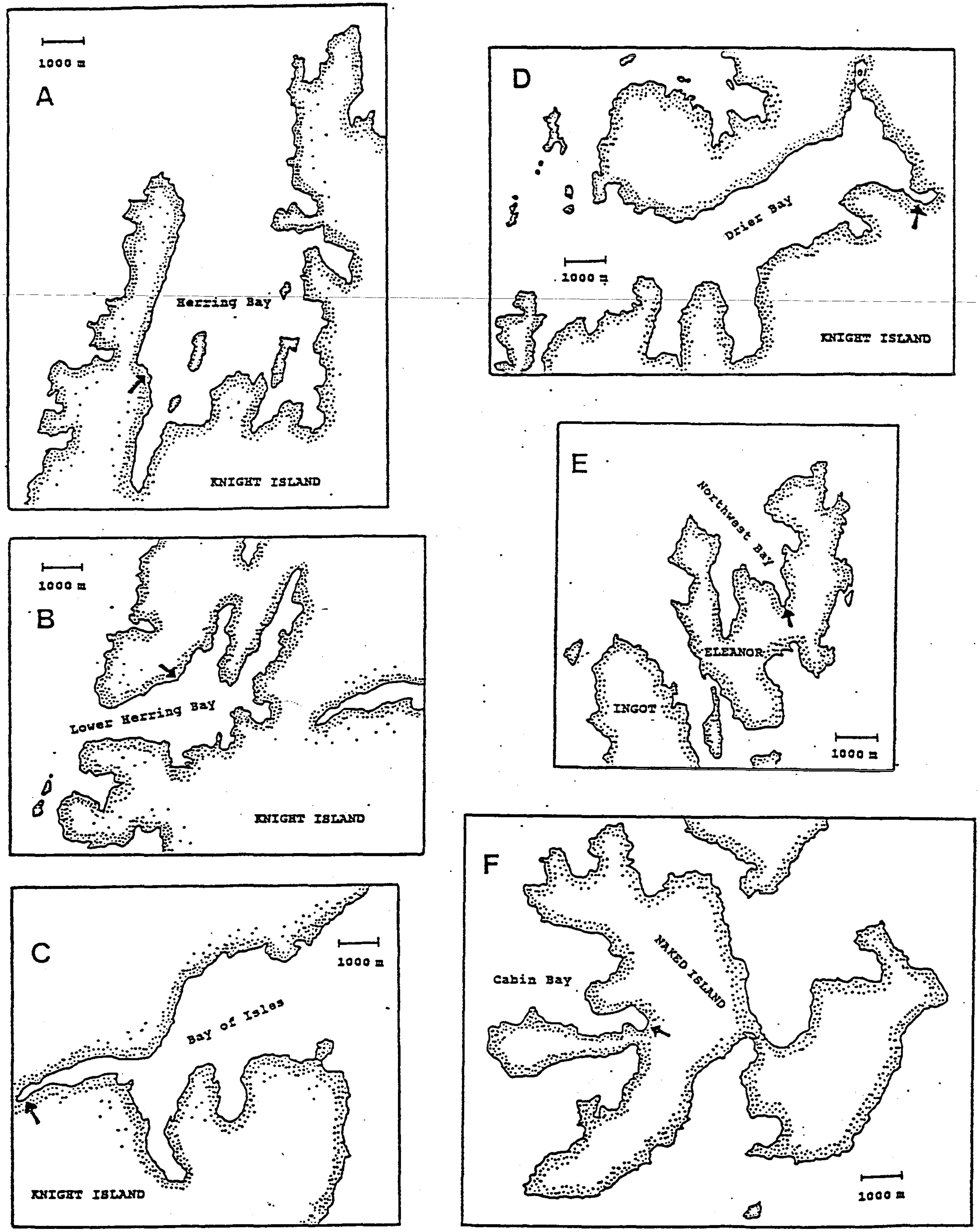
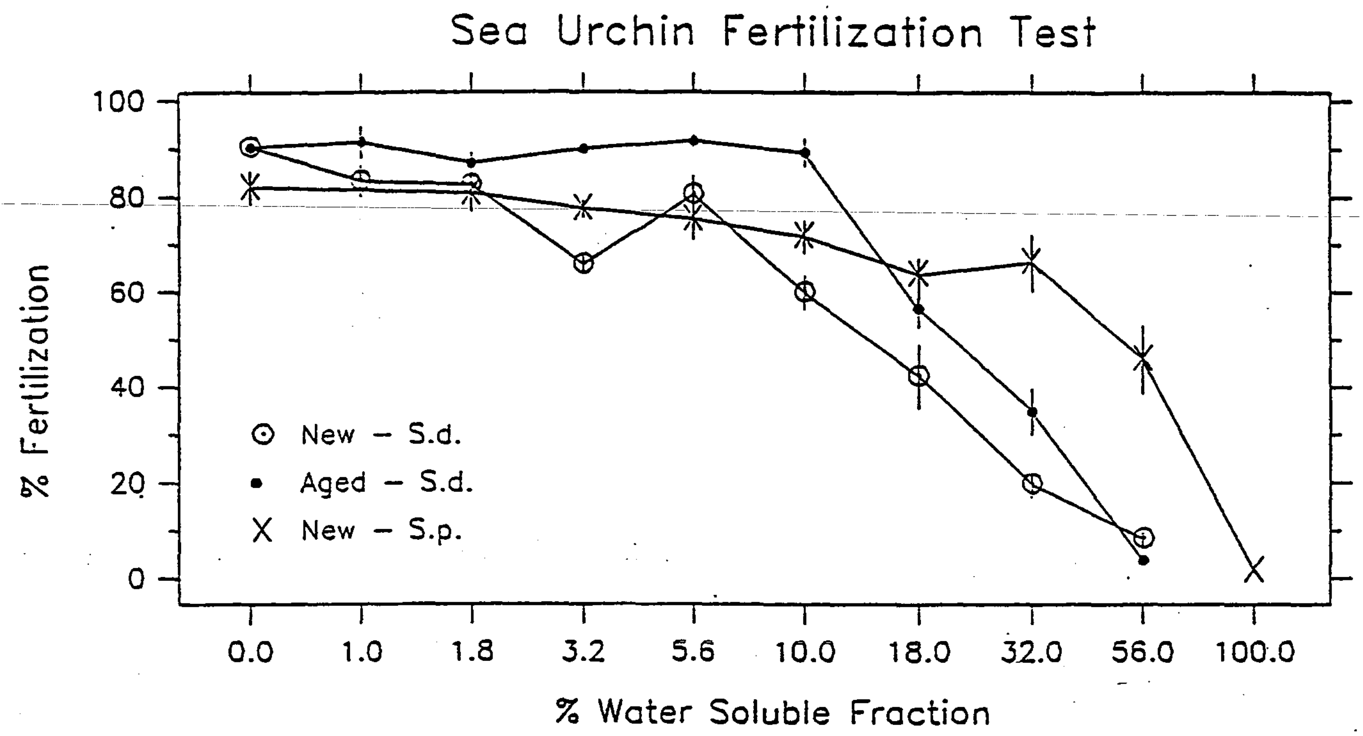


Figure 3. Dose response curves for sea urchin fertilization tests of: A. Water soluble fractions of new and weathered Prudhoe Bay crude oil, and B. Copper reference standards. Results are given for purple sea urchins (S.p.) and green sea urchins (S.d.). Means plus or minus 1 standard error are plotted.



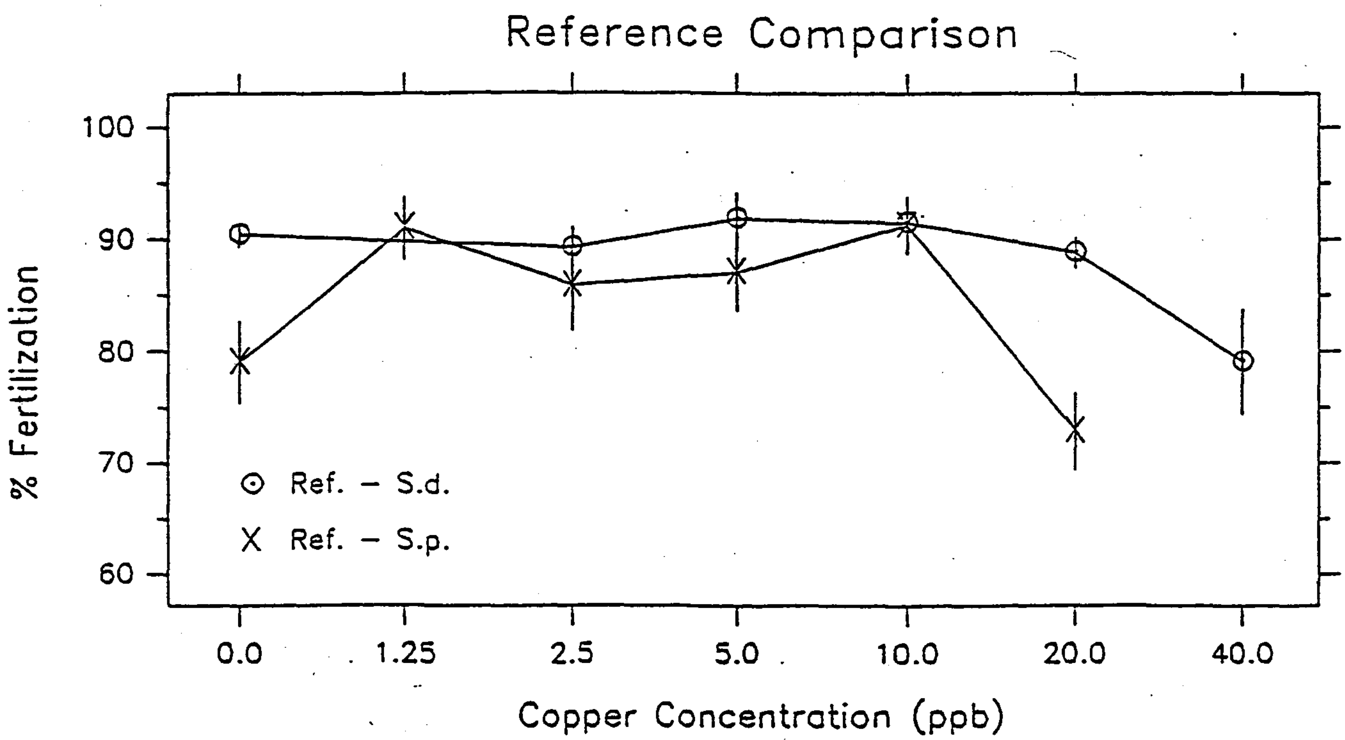
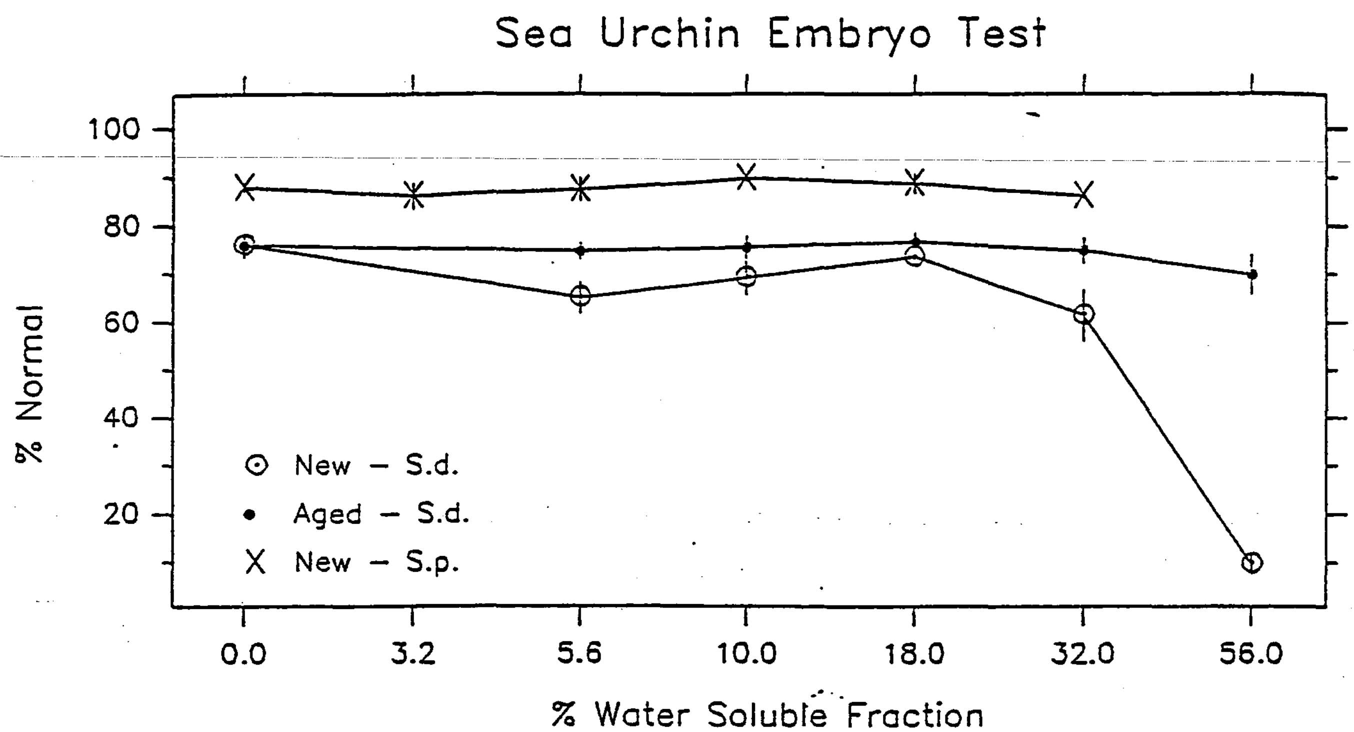
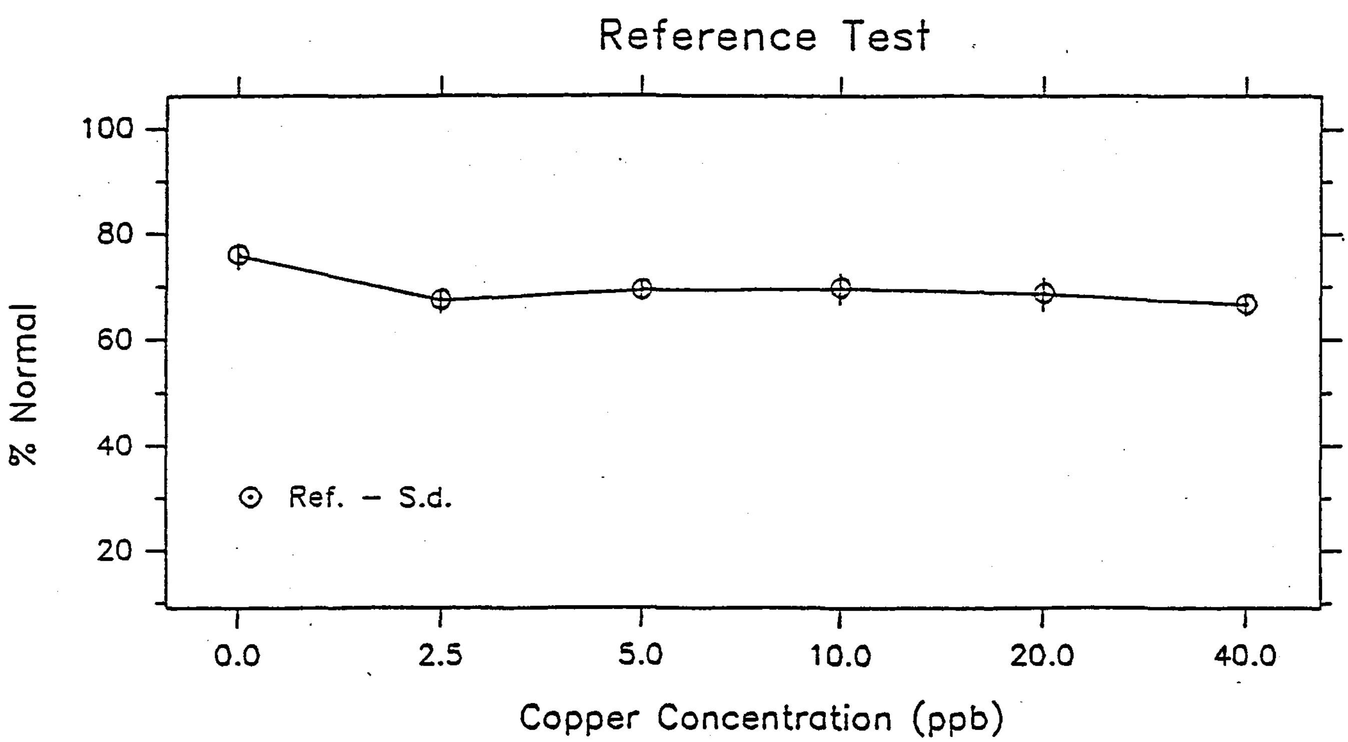


Figure 4. Dose response curves for sea urchin development tests of: A. Water soluble fractions of new and weathered Prudhoe Bay crude oil, and B. Copper reference standards. Results are given for purple sea urchins (S.p.) and green sea urchins (S.d.). Means plus or minus 1 standard error are plotted.





Appendix A. List of samples shipped to the Auke Bay Laboratory for hydrocarbon analysis and their status upon arrival.

Sediments Collected October 1991

Auke Bay#	CRA#	Site	Sample Hole	Status
2112-19	3	Bay of Isles	6	Fine
2112-20	2	Bay of Isles	3	cracked*
2112-21	9	Drier Bay	5	Fine
2112-22	10	Drier Bay	2	cracked*
2112-23	15	Lower Herring	5	Fine
2112-24	17	Lower Herring	4	Fine
2112-25	21	Herring Bay	5	Fine
2112-26	19	Herring Bay	1	cracked*
2112-27	28	Northwest Bay	2	Fine
2112-28	26	Northwest Bay	3	Fine
2112-29	31	Cabin Bay	1	Fine
2112-30	33	Cabin Bay	3	Fine

Sediments Collected October 1991

Auke Bay#	CRA#	Site	Sample Hole	Status
2112-01	2	Bay of Isles	1	Fine
2112-02	4	Bay of Isles	2	Fine
2112-03	6	Bay of Isles	3	Fine
2112-04	8	Drier Bay	1	Fine
2112-05	10	Drier Bay	2	cracked*
2112-06	12	Drier Bay	3	Fine
2112-07	14	Lower Herring	1	Fine
2112-08	16	Lower Herring	2	Fine
2112-09	18	Lower Herring	3	Fine
2112-10	20	Herring Bay	1	Fine
2112-11	22	Herring Bay	2	Fine
2112-12	24	Herring Bay	3	Fine
2112-13	26	Northwest Bay	1	Fine
2112-14	28	Northwest Bay	2	Fine
2112-15	30	Northwest Bay	3	cracked*
2112-16	32	Cabin Bay	1	Fine
2112-17	34	Cabin Bay	2	Fine
2112-18	36	Cabin Bay	3	Fine

*Bottles were cracked upon reciept. Contents were placed in a ziploc bag & refrozen. It is possible that this may effect hydrocarbon content. Results from these hydrocarbon tests should be flagged in all future analyses or discussions.

Appendix A, cont.

1990 Extracts of Water Soluble Fractions
For Purple Urchins

Auke Bay#	CRA#	Vol. Extracted(mls)	Status
1101-16	WSF 56%	900	Fine
1101-17	WSF 56%	900	Fine
1101-18	WSF 18%	900	Fine
1101-19	WSF 18%	900	Fine
1101-20	WSF 5.6%	900	Fine
1101-21	WSF 5.6%	900	Fine
1101-22	WSF 1.8%	900	Fine
1101-23	WSF 1.8%	900	Fine
1101-24	Seawater Blank	900	Fine
1101-25	Seawater Blank	900	Fine

1991 Extracts of Water Soluble Fractions
For Green Urchins

Auke Bay#	CRA#	Vol. Extracted (mls)	Status
2113-03	S1	250	Fine
2113-04	S2	250	Fine
2113-05	S3	250	Fine
2113-06	S4	250	Fine
2113-07	S5	250	Fine
2113-08	S6	250	Fine
2113-09	S7	250	Fine
2113-10	S8	250	Fine
2113-11	S9	250	Fine
2113-12	S10	250	Fine
2113-13	S11	250	Fine
2113-14	S12	250	Fine
2113-15	S13	250	Fine
2113-16	S14	250	Fine
2113-17	S15	250	Fine
2113-18	S16	250	Fine
2113-19	S17	250	Fine
2113-20	S18	250	Fine
2113-21	S19	250	Fine
2113-22	S20	250	Fine
2113-23	S21	250	Fine

Appendix A, cont.

1991 Extracts of Elutriates
For Green Urchins

Auke Bay#	CRA#	Volume Extracted (mls)	Status
2112-31	3	700	Damaged*
2112-32	18	700	Damaged*
2112-33	22	700	Damaged*
2112-34	2	700	Damaged*
2112-35	8	700	Damaged*
2112-36	20	700	Damaged*
2112-37	11	700	Damaged*
2112-38	5	700	Damaged*
2112-39	14	700	Damaged*
2112-40	13	700	Damaged*
2112-41	17	700	Damaged*
2112-42	7	700	Damaged*
2112-43	10	700	Damaged*
2112-44	1	700	Damaged*
2112-45	6	700	Damaged*
2112-46	9	700	Damaged*
2112-47	4	700	Damaged*
2112-48	15	700	Damaged*
2112-49	16	700	Damaged*
2112-50	21	700	Damaged*
2113-01	12	700	Damaged*
2113-02	19	700	Damaged*

*One of the sample bottles leaked in transit. Methylene chloride from this dissolved the plastic lids of the other bottles, thus contaminating them. Unfortunately all these samples were unusable.

Appendix B. Observations of the number of fertilized and unfertilized ova in sea urchin fertilization tests with elutriates of sediments from Prince William Sound. Concentrations are % for WSF tests and ppb Copper for reference tests.

Test Species	Site	Sample Number	Rep. Number	Number Fertile	Number Non-fertile
S. purpuratus	Control	1	1	100	0
S. purpuratus	Control	1	2	98	2
S. purpuratus	Control	1	3	98	2
S. purpuratus	Bay of Is.	1	1	56	44
S. purpuratus	Bay of Is.	1	2	77	33
S. purpuratus	Bay of Is.	1	3	55	45
S. purpuratus	Drier Bay	1	1	93	7
S. purpuratus	Drier Bay	1	2	90	10
S. purpuratus	Drier Bay	1	3	72	28
S. purpuratus	Herring B.	1	1	75	25
S. purpuratus	Herring B.	1	2	99	1
S. purpuratus	Herring B.	1	3	99	1
S. purpuratus	L.Herring	1	1	87	13
S. purpuratus	L.Herring	1	2	88	12
S. purpuratus	L.Herring	1	3	83	17
S. purpuratus	N.W. Bay	1	1	95	5
S. purpuratus	N.W. Bay	1	2	99	1
S. purpuratus	N.W. Bay	1	1	96	4
S. purpuratus	Cabin Bay	1	1	82	18
S. purpuratus	Cabin Bay	1	2	95	5
S. purpuratus	Cabin Bay	1	3	93	7
S. droebachiensis	Control	1	1	93	7
S. droebachiensis	Control	1	2	97	3
S. droebachiensis	Control	1	1	95	5
S. droebachiensis	Control	2	1	95	5
S. droebachiensis	Control	2	2	96	4
S. droebachiensis	Control	2	3	90	10
S. droebachiensis	Control	3	1	96	4
S. droebachiensis	Control	3	2	94	6
S. droebachiensis	Control	3	3	92	8
S. droebachiensis	Bay of Is.	1	1	90	10
S. droebachiensis	Bay of Is.	1	2	91	9
S. droebachiensis	Bay of Is.	1	3	95	5
S. droebachiensis	Bay of Is.	2	1	82	18
S. droebachiensis	Bay of Is.	2	2	91	9
S. droebachiensis	Bay of Is.	2	3	97	3
S. droebachiensis	Bay of Is.	3	1	92	8
S. droebachiensis	Bay of Is.	3	2	89	11
S. droebachiensis	Bay of Is.	3	3	93	7
S. droebachiensis	Drier Bay	1	1	96	4
S. droebachiensis	Drier Bay	1	2	94	6
S. droebachiensis	Drier Bay	1	3	99	1

Appendix B. (Continued)

S. droebachiensis	Drier Bay	2	1	91	9
S. droebachiensis	Drier Bay	2	2	95	5
S. droebachiensis	Drier Bay	2	3	91	9
S. droebachiensis	Drier Bay	3	1	88	12
S. droebachiensis	Drier Bay	3	2	95	5
S. droebachiensis	Drier Bay	3	3	96	4
S. droebachiensis	Herring B.	1	1	93	7
S. droebachiensis	Herring B.	1	2	94	6
S. droebachiensis	Herring B.	1	3	94	6
S. droebachiensis	Herring B.	2	1	91	9
S. droebachiensis	Herring B.	2	2	86	4
S. droebachiensis	Herring B.	2	3	87	3
S. droebachiensis	Herring B.	3	1	92	8
S. droebachiensis	Herring B.	3	2	93	7
S. droebachiensis	Herring B.	3	1	89	11
S. droebachiensis	L.Herring B.	1	1	90	10
S. droebachiensis	L.Herring B.	1	2	95	5
S. droebachiensis	L.Herring B.	1	3	96	4
S. droebachiensis	L.Herring B.	2	1	92	8
S. droebachiensis	L.Herring B.	2	2	91	9
S. droebachiensis	L.Herring B.	2	3	90	10
S. droebachiensis	L.Herring B.	3	1	91	9
S. droebachiensis	L.Herring B.	3	2	94	6
S. droebachiensis	L.Herring B.	3	3	88	12
S. droebachiensis	N.W. Bay	1	1	91	9
S. droebachiensis	N.W. Bay	1	2	96	4
S. droebachiensis	N.W. Bay	1	3	89	11
S. droebachiensis	N.W. Bay	2	1	93	7
S. droebachiensis	N.W. Bay	2	2	92	8
S. droebachiensis	N.W. Bay	2	3	100	0
S. droebachiensis	N.W. Bay	3	1	94	6
S. droebachiensis	N.W. Bay	3	2	93	7
S. droebachiensis	N.W. Bay	3	3	9	1
S. droebachiensis	Cabin Bay	1	1	23	77
S. droebachiensis	Cabin Bay	1	2	16	84
S. droebachiensis	Cabin Bay	1	3	12	88
S. droebachiensis	Cabin Bay	2	1	21	79
S. droebachiensis	Cabin Bay	2	2	19	81
S. droebachiensis	Cabin Bay	2	3	17	83
S. droebachiensis	Cabin Bay	3	1	90	10
S. droebachiensis	Cabin Bay	3	2	89	11
S. droebachiensis	Cabin Bay	3	3	85	15

Appendix C. Observations of the number of normal and abnormal embryos in sea urchin fertilization tests with elutriates of sediments from Prince William Sound. Concentrations are % for WSF tests and ppb Copper for reference tests.

Test Species	Site	Sample Number	Rep Number	Number Normal	Number Abnormal
S. purpuratus	Control	1	1	84	16
S. purpuratus	Control	1	2	.	.
S. purpuratus	Control	1	3	76	24
S. purpuratus	Bay of Is.	1	1	44	56
S. purpuratus	Bay of Is.	1	2	26	74
S. purpuratus	Bay of Is.	1	3	56	44
S. purpuratus	Drier Bay	1	1	92	8
S. purpuratus	Drier Bay	1	2	84	16
S. purpuratus	Drier Bay	1	3	90	10
S. purpuratus	Herring B.	1	1	91	9
S. purpuratus	Herring B.	1	2	90	10
S. purpuratus	Herring B.	1	3	76	24
S. purpuratus	L.Herring B.	1	1	78	22
S. purpuratus	L.Herring B.	1	2	86	14
S. purpuratus	L.Herring B.	1	3	92	8
S. purpuratus	N.W. Bay	1	1	73	27
S. purpuratus	N.W. Bay	1	2	88	12
S. purpuratus	N.W. Bay	1	1	88	12
S. purpuratus	Cabin Bay	1	1	85	15
S. purpuratus	Cabin Bay	1	2	96	4
S. purpuratus	Cabin Bay	1	3	78	22
S. droebachiensis	Control	1	1	27	73
S. droebachiensis	Control	2	1	28	72
S. droebachiensis	Control	3	1	4	96
S. droebachiensis	Bay of Is.	1	1	42	58
S. droebachiensis	Bay of Is.	2	1	35	65
S. droebachiensis	Bay of Is.	3	1	31	69
S. droebachiensis	Drier Bay	1	1	56	44
S. droebachiensis	Drier Bay	2	1	49	51
S. droebachiensis	Drier Bay	3	1	45	55
S. droebachiensis	Herring B.	1	1	49	51
S. droebachiensis	Herring B.	2	1	32	68
S. droebachiensis	Herring B.	3	1	42	58
S. droebachiensis	L.Herring B.	1	1	46	54
S. droebachiensis	L.Herring B.	2	1	43	57
S. droebachiensis	L.Herring B.	3	1	44	56
S. droebachiensis	N.W. Bay	1	1	48	52
S. droebachiensis	N.W. Bay	2	1	30	70
S. droebachiensis	N.W. Bay	3	1	41	59
S. droebachiensis	Cabin Bay	1	1	44	56
S. droebachiensis	Cabin Bay	2	1	52	48
S. droebachiensis	Cabin Bay	3	1	56	44

Appendix D. Observations of the number of fertilized and unfertilized ova in sea urchin fertilization tests with water soluble fractions of Prudhoe Bay crude oil and a copper reference toxicant. Concentrations are % for WSF test and ppb Copper for reference tests.

Test Type	Species	Concentration	Number Fertile	Number Non-fertile
New Oil	S. droebachiensis	0.00	89	11
New Oil	S. droebachiensis	0.00	91	9
New Oil	S. droebachiensis	0.00	92	8
New Oil	S. droebachiensis	0.00	87	13
New Oil	S. droebachiensis	0.00	93	7
New Oil	S. droebachiensis	1.00	86	14
New Oil	S. droebachiensis	1.00	75	25
New Oil	S. droebachiensis	1.00	76	24
New Oil	S. droebachiensis	1.00	91	9
New Oil	S. droebachiensis	1.00	88	12
New Oil	S. droebachiensis	1.80	68	32
New Oil	S. droebachiensis	1.80	87	13
New Oil	S. droebachiensis	1.80	91	9
New Oil	S. droebachiensis	1.80	85	15
New Oil	S. droebachiensis	1.80	81	19
New Oil	S. droebachiensis	3.20	64	36
New Oil	S. droebachiensis	3.20	70	30
New Oil	S. droebachiensis	3.20	64	36
New Oil	S. droebachiensis	3.20	61	39
New Oil	S. droebachiensis	3.20	69	31
New Oil	S. droebachiensis	5.60	77	23
New Oil	S. droebachiensis	5.60	69	31
New Oil	S. droebachiensis	5.60	90	10
New Oil	S. droebachiensis	5.60	88	12
New Oil	S. droebachiensis	5.60	78	22
New Oil	S. droebachiensis	10.00	62	38
New Oil	S. droebachiensis	10.00	52	48
New Oil	S. droebachiensis	10.00	70	30
New Oil	S. droebachiensis	10.00	51	49
New Oil	S. droebachiensis	10.00	64	36
New Oil	S. droebachiensis	18.00	31	69
New Oil	S. droebachiensis	18.00	45	55
New Oil	S. droebachiensis	18.00	43	57
New Oil	S. droebachiensis	18.00	65	35
New Oil	S. droebachiensis	18.00	27	73
New Oil	S. droebachiensis	32.00	17	83
New Oil	S. droebachiensis	32.00	19	81
New Oil	S. droebachiensis	32.00	12	88
New Oil	S. droebachiensis	32.00	22	78
New Oil	S. droebachiensis	32.00	28	72
New Oil	S. droebachiensis	56.00	12	88
New Oil	S. droebachiensis	56.00	4	96
New Oil	S. droebachiensis	56.00	8	92
New Oil	S. droebachiensis	56.00	10	90

Appendix D. (continued)

Test Type	Species	Concentration	Number Fertile	Number Non-fertile
New Oil	S. droebachiensis	56.00	7	93
New Oil	S. purpuratus	0.00	86	14
New Oil	S. purpuratus	0.00	77	23
New Oil	S. purpuratus	0.00	94	6
New Oil	S. purpuratus	0.00	75	25
New Oil	S. purpuratus	0.00	78	22
New Oil	S. purpuratus	1.80	77	23
New Oil	S. purpuratus	1.80	68	32
New Oil	S. purpuratus	1.80	83	17
New Oil	S. purpuratus	1.80	87	13
New Oil	S. purpuratus	1.80	88	12
New Oil	S. purpuratus	3.20	73	27
New Oil	S. purpuratus	3.20	75	25
New Oil	S. purpuratus	3.20	80	20
New Oil	S. purpuratus	3.20	76	24
New Oil	S. purpuratus	3.20	83	17
New Oil	S. purpuratus	5.60	81	19
New Oil	S. purpuratus	5.60	69	31
New Oil	S. purpuratus	5.60	72	28
New Oil	S. purpuratus	5.60	89	11
New Oil	S. purpuratus	5.60	65	35
New Oil	S. purpuratus	10.00	73	27
New Oil	S. purpuratus	10.00	76	24
New Oil	S. purpuratus	10.00	71	29
New Oil	S. purpuratus	10.00	79	21
New Oil	S. purpuratus	10.00	59	41
New Oil	S. purpuratus	18.00	68	32
New Oil	S. purpuratus	18.00	59	41
New Oil	S. purpuratus	18.00	59	41
New Oil	S. purpuratus	18.00	75	25
New Oil	S. purpuratus	18.00	57	43
New Oil	S. purpuratus	32.00	72	28
New Oil	S. purpuratus	32.00	71	29
New Oil	S. purpuratus	32.00	83	17
New Oil	S. purpuratus	32.00	53	47
New Oil	S. purpuratus	32.00	52	48
New Oil	S. purpuratus	56.00	25	75
New Oil	S. purpuratus	56.00	45	55
New Oil	S. purpuratus	56.00	68	32
New Oil	S. purpuratus	56.00	51	48
New Oil	S. purpuratus	56.00	39	61
New Oil	S. purpuratus	100.00	2	98
New Oil	S. purpuratus	100.00	2	98
New Oil	S. purpuratus	100.00	2	98
New Oil	S. purpuratus	100.00	0	100
New Oil	S. purpuratus	100.00	2	98
Aged Oil	S. droebachiensis	0.00	89	11
Aged Oil	S. droebachiensis	0.00	91	9
Aged Oil	S. droebachiensis	0.00	92	8
Aged Oil	S. droebachiensis	0.00	87	13
Aged Oil	S. droebachiensis	0.00	93	7

Appendix D. (continued)

Test Type	Species	Concentration	Number Fertile	Number Non-fertile
Aged Oil	S. droebachiensis	1.00	99	1
Aged Oil	S. droebachiensis	1.00	93	7
Aged Oil	S. droebachiensis	1.00	91	9
Aged Oil	S. droebachiensis	1.00	95	5
Aged Oil	S. droebachiensis	1.00	79	21
Aged Oil	S. droebachiensis	1.80	86	14
Aged Oil	S. droebachiensis	1.80	83	17
Aged Oil	S. droebachiensis	1.80	93	7
Aged Oil	S. droebachiensis	1.80	92	8
Aged Oil	S. droebachiensis	1.80	81	19
Aged Oil	S. droebachiensis	3.20	87	13
Aged Oil	S. droebachiensis	3.20	93	7
Aged Oil	S. droebachiensis	3.20	90	10
Aged Oil	S. droebachiensis	3.20	88	12
Aged Oil	S. droebachiensis	3.20	92	8
Aged Oil	S. droebachiensis	5.60	95	5
Aged Oil	S. droebachiensis	5.60	88	12
Aged Oil	S. droebachiensis	5.60	90	10
Aged Oil	S. droebachiensis	5.60	95	5
Aged Oil	S. droebachiensis	5.60	90	10
Aged Oil	S. droebachiensis	10.00	97	3
Aged Oil	S. droebachiensis	10.00	86	14
Aged Oil	S. droebachiensis	10.00	90	10
Aged Oil	S. droebachiensis	10.00	84	16
Aged Oil	S. droebachiensis	18.00	48	52
Aged Oil	S. droebachiensis	18.00	63	37
Aged Oil	S. droebachiensis	18.00	68	32
Aged Oil	S. droebachiensis	18.00	51	49
Aged Oil	S. droebachiensis	18.00	52	48
Aged Oil	S. droebachiensis	32.00	47	53
Aged Oil	S. droebachiensis	32.00	23	77
Aged Oil	S. droebachiensis	32.00	38	62
Aged Oil	S. droebachiensis	32.00	42	58
Aged Oil	S. droebachiensis	32.00	24	76
Aged Oil	S. droebachiensis	56.00	3	97
Aged Oil	S. droebachiensis	56.00	3	97
Aged Oil	S. droebachiensis	56.00	4	96
Aged Oil	S. droebachiensis	56.00	2	98
Aged Oil	S. droebachiensis	56.00	7	93
Reference	S. droebachiensis	0.00	89	11
Reference	S. droebachiensis	0.00	91	9
Reference	S. droebachiensis	0.00	92	8
Reference	S. droebachiensis	0.00	87	13
Reference	S. droebachiensis	0.00	93	7
Reference	S. droebachiensis	2.50	84	16
Reference	S. droebachiensis	2.50	86	14
Reference	S. droebachiensis	2.50	94	6
Reference	S. droebachiensis	2.50	90	10
Reference	S. droebachiensis	2.50	92	8
Reference	S. droebachiensis	5.00	88	12

Appendix D. (continued)

Test Type	Species	Concentration	Number Fertile	Number Non-fertile
Reference	S. droebachiensis	5.00	94	6
Reference	S. droebachiensis	5.00	94	6
Reference	S. droebachiensis	5.00	98	2
Reference	S. droebachiensis	5.00	85	15
Reference	S. droebachiensis	10.00	88	12
Reference	S. droebachiensis	10.00	89	11
Reference	S. droebachiensis	10.00	97	3
Reference	S. droebachiensis	10.00	86	14
Reference	S. droebachiensis	10.00	97	3
Reference	S. droebachiensis	20.00	84	16
Reference	S. droebachiensis	20.00	89	11
Reference	S. droebachiensis	20.00	88	12
Reference	S. droebachiensis	20.00	92	8
Reference	S. droebachiensis	20.00	91	9
Reference	S. droebachiensis	40.00	81	19
Reference	S. droebachiensis	40.00	64	36
Reference	S. droebachiensis	40.00	73	27
Reference	S. droebachiensis	40.00	87	13
Reference	S. droebachiensis	40.00	90	10
Reference	S. purpuratus	0.00	86	14
Reference	S. purpuratus	0.00	81	19
Reference	S. purpuratus	0.00	68	32
Reference	S. purpuratus	0.00	73	27
Reference	S. purpuratus	0.00	87	13
Reference	S. purpuratus	1.25	86	14
Reference	S. purpuratus	1.25	91	9
Reference	S. purpuratus	1.25	95	5
Reference	S. purpuratus	1.25	84	16
Reference	S. purpuratus	1.25	99	1
Reference	S. purpuratus	2.50	75	25
Reference	S. purpuratus	2.50	95	5
Reference	S. purpuratus	2.50	86	14
Reference	S. purpuratus	2.50	94	6
Reference	S. purpuratus	2.50	79	21
Reference	S. purpuratus	5.00	80	20
Reference	S. purpuratus	5.00	85	15
Reference	S. purpuratus	5.00	97	3
Reference	S. purpuratus	5.00	93	7
Reference	S. purpuratus	5.00	80	20
Reference	S. purpuratus	10.00	90	10
Reference	S. purpuratus	10.00	95	5
Reference	S. purpuratus	10.00	86	14
Reference	S. purpuratus	10.00	99	1
Reference	S. purpuratus	10.00	86	14
Reference	S. purpuratus	20.00	85	15
Reference	S. purpuratus	20.00	67	33
Reference	S. purpuratus	20.00	75	25
Reference	S. purpuratus	20.00	72	28
Reference	S. purpuratus	20.00	65	35

Appendix E. Observations of the number of normal and abnormal embryos in sea urchin embryo tests with water soluble fractions of Prudhoe Bay crude oil and a copper reference toxicant. Concentrations are % for WSF test and ppb Copper for reference tests.

Test Type	Species	Concentration	Number Normal	Number Abnormal
New Oil	S. purpuratus	0.00	86	14
New Oil	S. purpuratus	0.00	89	11
New Oil	S. purpuratus	0.00	88	12
New Oil	S. purpuratus	32.00	87	13
New Oil	S. purpuratus	32.00	86	14
New Oil	S. purpuratus	32.00	85	15
New Oil	S. purpuratus	32.00	86	14
New Oil	S. purpuratus	18.00	83	17
New Oil	S. purpuratus	18.00	92	8
New Oil	S. purpuratus	18.00	94	6
New Oil	S. purpuratus	18.00	85	15
New Oil	S. purpuratus	18.00	89	11
New Oil	S. purpuratus	10.00	90	10
New Oil	S. purpuratus	10.00	91	9
New Oil	S. purpuratus	10.00	89	11
New Oil	S. purpuratus	10.00	90	10
New Oil	S. purpuratus	10.00	88	12
New Oil	S. purpuratus	5.60	89	11
New Oil	S. purpuratus	5.60	90	10
New Oil	S. purpuratus	5.60	80	20
New Oil	S. purpuratus	5.60	90	10
New Oil	S. purpuratus	3.20	85	15
New Oil	S. purpuratus	3.20	91	9
New Oil	S. purpuratus	3.20	82	18
New Oil	S. droebachiensis	0.00	81	19
New Oil	S. droebachiensis	0.00	75	25
New Oil	S. droebachiensis	0.00	82	18
New Oil	S. droebachiensis	0.00	72	28
New Oil	S. droebachiensis	0.00	69	31
New Oil	S. droebachiensis	5.60	70	30
New Oil	S. droebachiensis	5.60	58	42
New Oil	S. droebachiensis	5.60	71	29
New Oil	S. droebachiensis	5.60	61	39
New Oil	S. droebachiensis	10.00	70	30
New Oil	S. droebachiensis	10.00	64	36
New Oil	S. droebachiensis	10.00	72	28
New Oil	S. droebachiensis	10.00	80	20
New Oil	S. droebachiensis	10.00	60	40
New Oil	S. droebachiensis	18.00	74	26
New Oil	S. droebachiensis	18.00	74	26
New Oil	S. droebachiensis	18.00	75	25
New Oil	S. droebachiensis	18.00	71	29

Appendix E. (continued)

Test Type	Species	Concentration	Number Normal	Number Abnormal
New Oil	S. droebachiensis	32.00	59	41
New Oil	S. droebachiensis	32.00	74	26
New Oil	S. droebachiensis	32.00	55	45
New Oil	S. droebachiensis	32.00	46	54
New Oil	S. droebachiensis	32.00	73	27
New Oil	S. droebachiensis	56.00	17	83
New Oil	S. droebachiensis	56.00	12	88
New Oil	S. droebachiensis	56.00	8	92
New Oil	S. droebachiensis	56.00	6	94
New Oil	S. droebachiensis	56.00	5	95
Aged Oil	S. droebachiensis	0.00	81	19
Aged Oil	S. droebachiensis	0.00	75	25
Aged Oil	S. droebachiensis	0.00	82	18
Aged Oil	S. droebachiensis	0.00	72	28
Aged Oil	S. droebachiensis	0.00	69	31
Aged Oil	S. droebachiensis	5.60	74	26
Aged Oil	S. droebachiensis	5.60	72	28
Aged Oil	S. droebachiensis	5.60	71	29
Aged Oil	S. droebachiensis	5.60	75	25
Aged Oil	S. droebachiensis	5.60	81	19
Aged Oil	S. droebachiensis	10.00	75	25
Aged Oil	S. droebachiensis	10.00	69	31
Aged Oil	S. droebachiensis	10.00	79	21
Aged Oil	S. droebachiensis	10.00	79	21
Aged Oil	S. droebachiensis	18.00	80	20
Aged Oil	S. droebachiensis	18.00	79	21
Aged Oil	S. droebachiensis	18.00	79	21
Aged Oil	S. droebachiensis	18.00	70	30
Aged Oil	S. droebachiensis	18.00	75	25
Aged Oil	S. droebachiensis	32.00	84	16
Aged Oil	S. droebachiensis	32.00	72	28
Aged Oil	S. droebachiensis	32.00	75	25
Aged Oil	S. droebachiensis	32.00	68	32
Aged Oil	S. droebachiensis	32.00	75	25
Aged Oil	S. droebachiensis	56.00	74	26
Aged Oil	S. droebachiensis	56.00	60	40
Aged Oil	S. droebachiensis	56.00	60	40
Aged Oil	S. droebachiensis	56.00	76	24
Aged Oil	S. droebachiensis	56.00	79	21
Reference	S. droebachiensis	0.00	81	19
Reference	S. droebachiensis	0.00	75	25
Reference	S. droebachiensis	0.00	82	18
Reference	S. droebachiensis	0.00	72	28
Reference	S. droebachiensis	0.00	69	31
Reference	S. droebachiensis	2.50	79	31
Reference	S. droebachiensis	2.50	74	26
Reference	S. droebachiensis	2.50	65	35
Reference	S. droebachiensis	2.50	64	36
Reference	S. droebachiensis	2.50	62	38
Reference	S. droebachiensis	5.00	65	35

Appendix E. (continued)

Test Type	Species	Concentration	Number Normal	Number Abnormal
Reference	S. droebachiensis	5.00	71	29
Reference	S. droebachiensis	5.00	65	35
Reference	S. droebachiensis	5.00	75	25
Reference	S. droebachiensis	5.00	70	30
Reference	S. droebachiensis	10.00	74	26
Reference	S. droebachiensis	10.00	65	35
Reference	S. droebachiensis	10.00	79	21
Reference	S. droebachiensis	10.00	67	37
Reference	S. droebachiensis	10.00	65	35
Reference	S. droebachiensis	20.00	69	31
Reference	S. droebachiensis	20.00	57	43
Reference	S. droebachiensis	20.00	75	25
Reference	S. droebachiensis	20.00	72	28
Reference	S. droebachiensis	20.00	70	30
Reference	S. droebachiensis	40.00	66	34
Reference	S. droebachiensis	40.00	66	34
Reference	S. droebachiensis	40.00	63	37
Reference	S. droebachiensis	40.00	63	37
Reference	S. droebachiensis	40.00	74	26

Appendix F. Photographs of sampling sites for sediments used in Elutriate test.

Appendix F
Photographs of Sampling Sites for Sediments Used in Elutriate Test

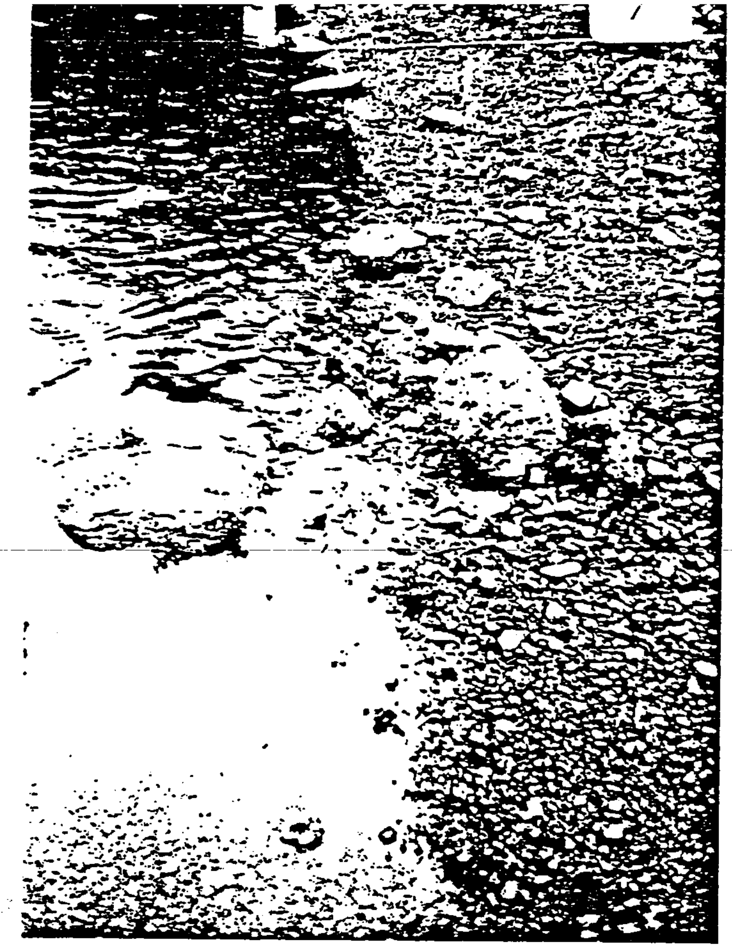
Submitted to: The Alaska Department of Fish and Game

By: Coastal Resources Associates

May 30, 1991

1990 ADF&G URCHIN/SEDIMENT COLLECTION PHOTOS

PHOTO #
1 Site #3 Bay of Isles
2 Site #3 Bay of Isles
3 Site #3 Bay of Isles. Closeup of site
4 Site #3 Bay of Isles. Distant view
5 Site #4 Drier Bay. Site closeup
6 Site #4 Drier Bay. Sample hole
7 Site #4 Drier Bay. Sample sediment in jar
8 Site #4 Drier Bay. Distant view
9 Site #2 Lower Herring. Site closeup
10 Site #2 Lower Herring. Sample hole
11 Site #2 Lower Herring. Sediment - coarse gravel
12 Site #2 Lower Herring. Site long shot
13 Site #1 Herring Bay. Sediment collection
14 Site #1 Herring Bay. Sediment collection
15 Site #1 Herring Bay. Fine texture sediment
16 Site #1 Herring Bay. Sampling after dark
17 Site #5 Northwest Bay. Distant view
18 Site #5 Northwest Bay. Oil sheen floating up when sampling holes were dug
19 Site #5 Northwest Bay. Oil sheen floating up when sampling holes were dug
20 Site #5 Northwest Bay. Oil sheen floating up when sampling holes were dug
21 Site #5 Northwest Bay. Sampling holes in a line
22 Site #6 Cabin Bay. Site from distance
23 Site #6 Cabin Bay. Note fine texture of sediment
24 Site #6 Cabin Bay. Sediment & hole
25 Site #6 Cabin Bay. Line of sampling holes
26 Site #6 Cabin Bay. Site closeup



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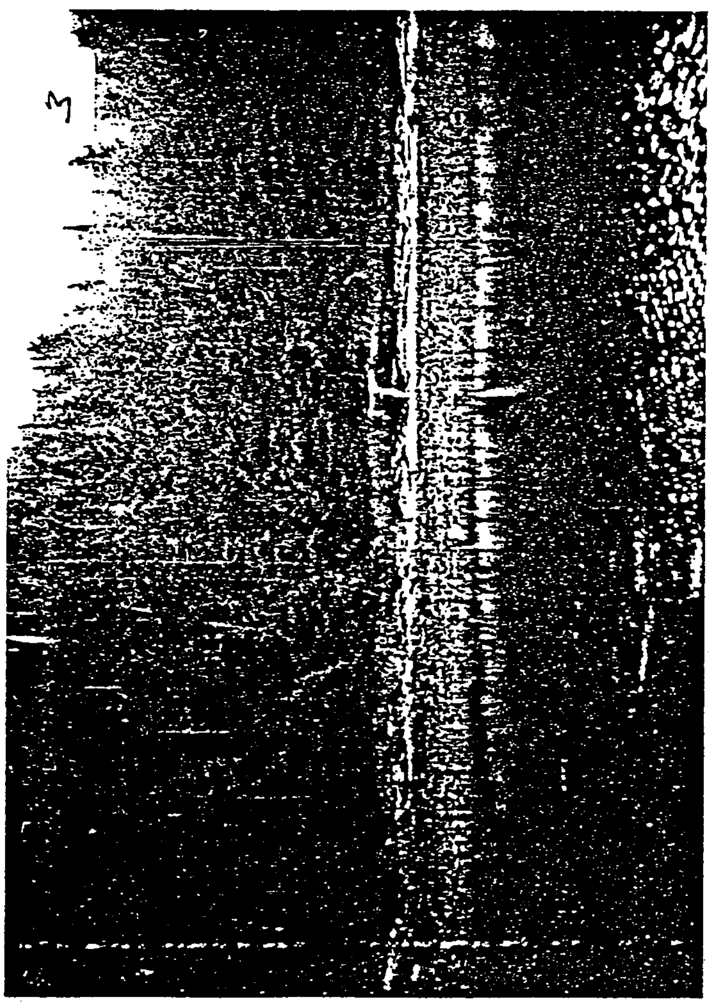

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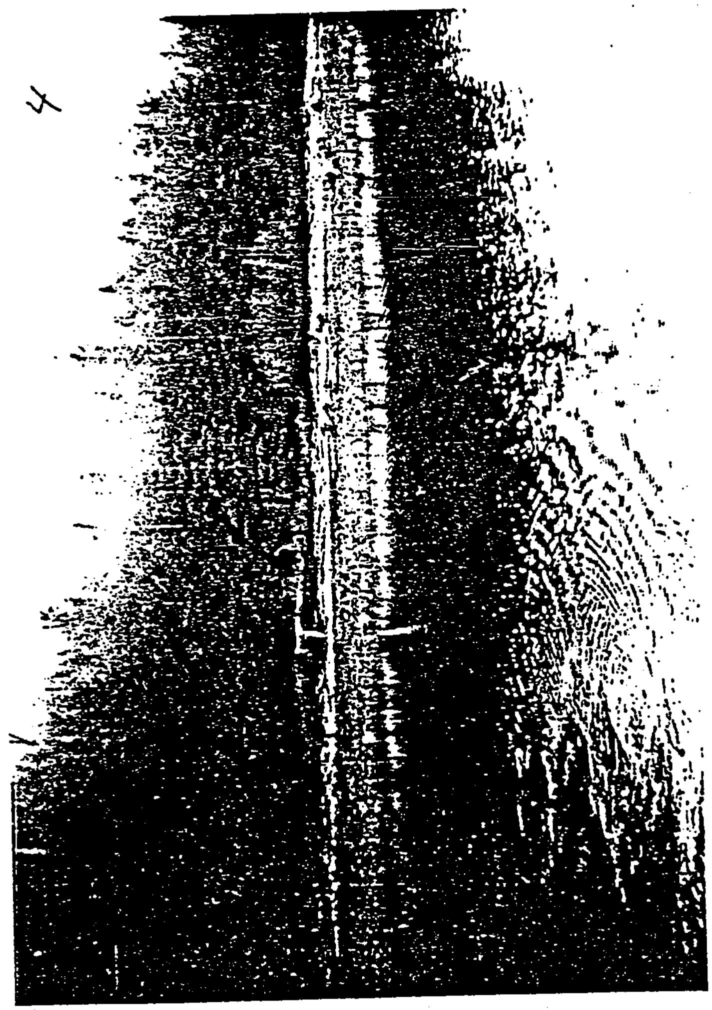

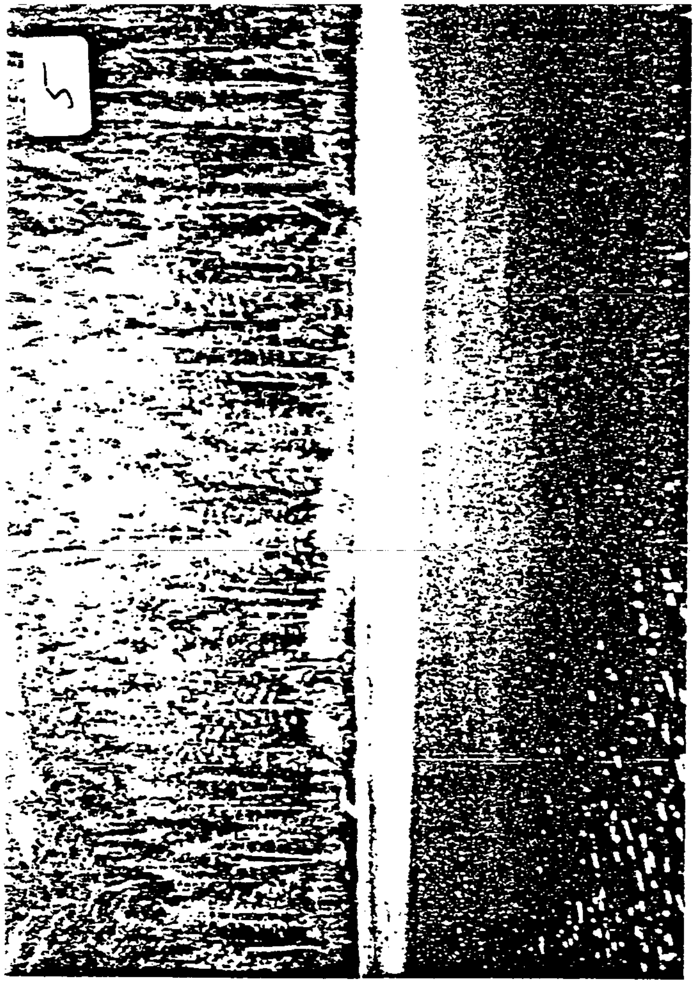

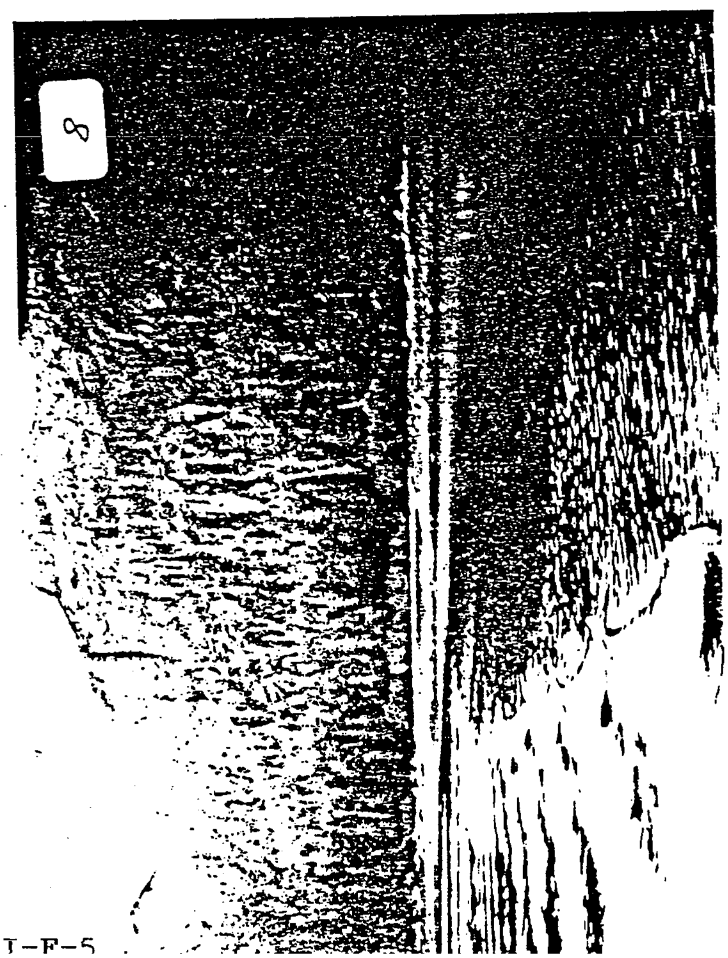


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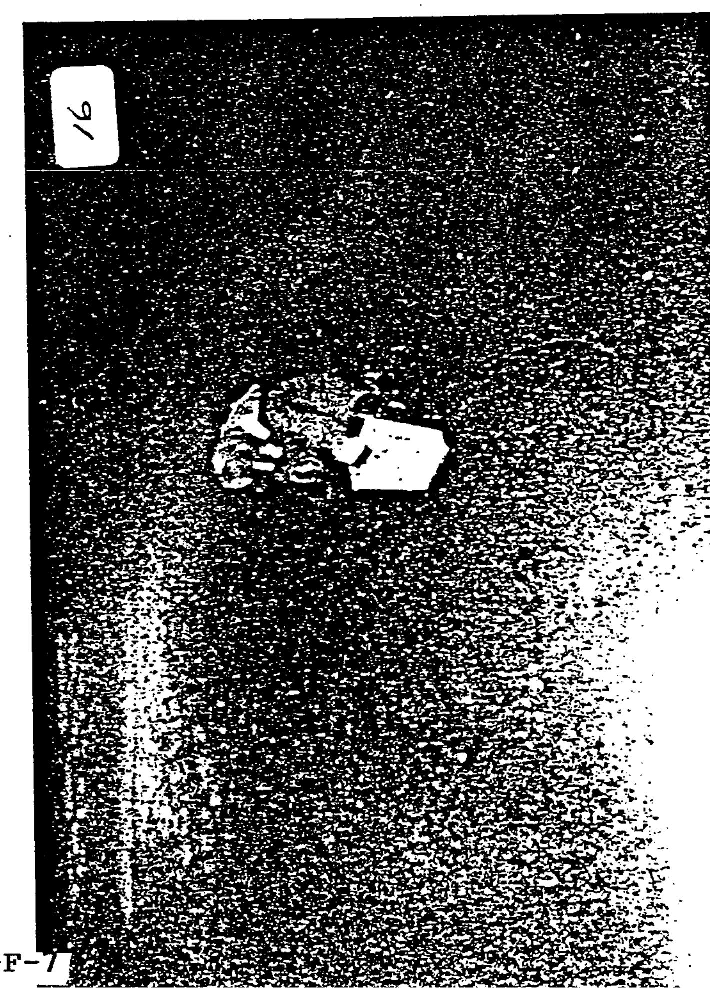
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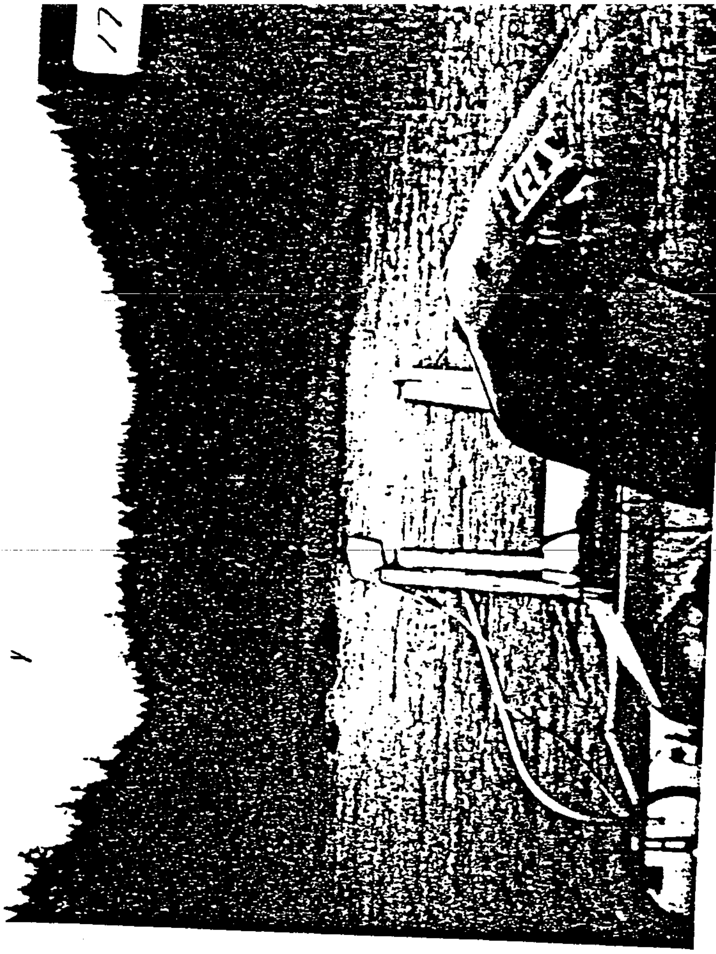
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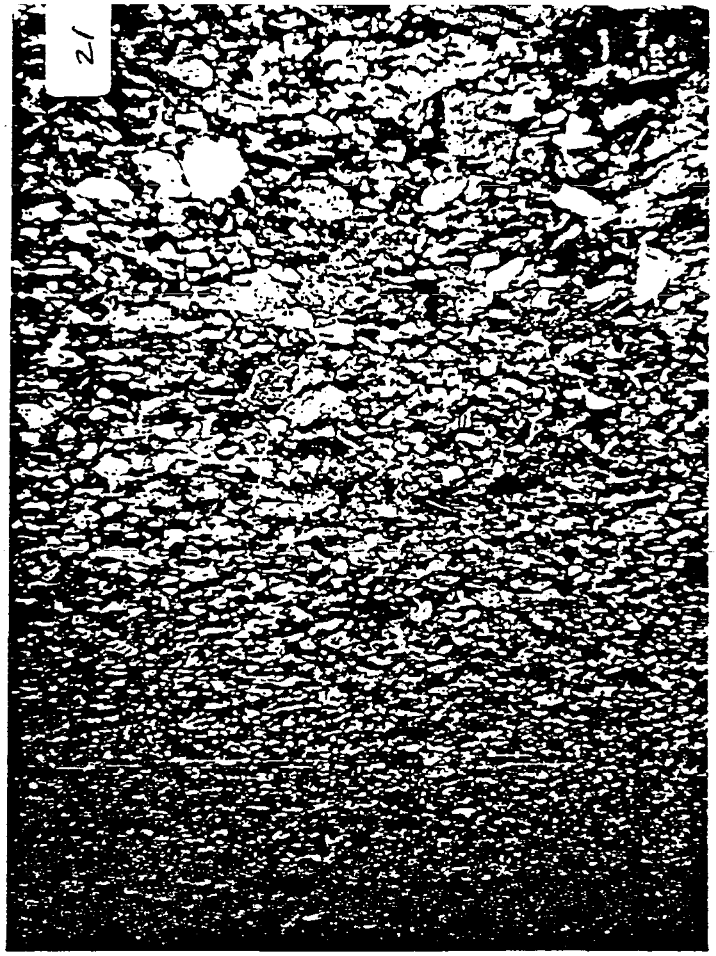

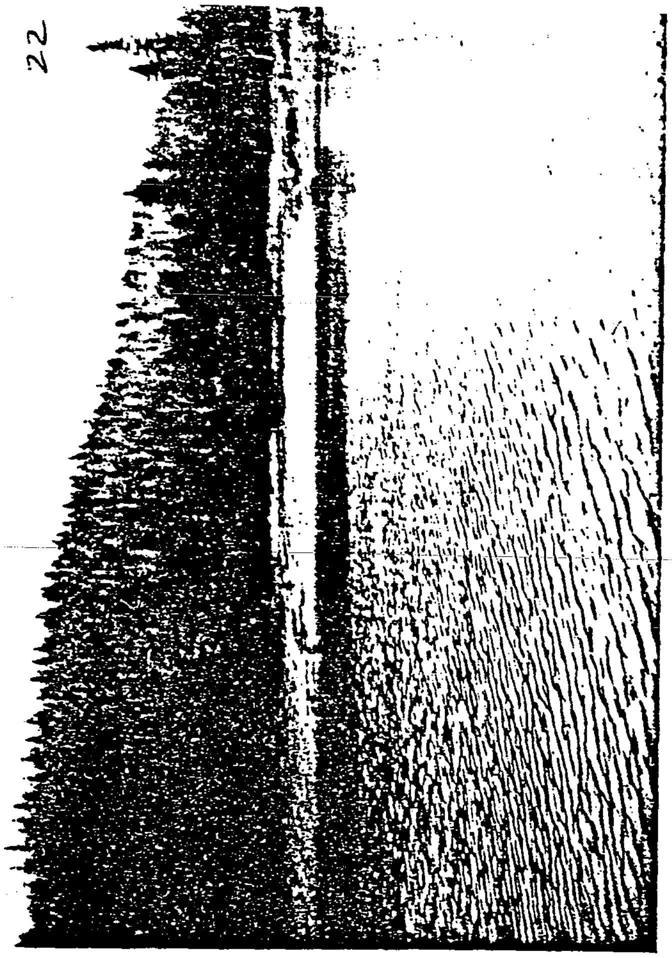
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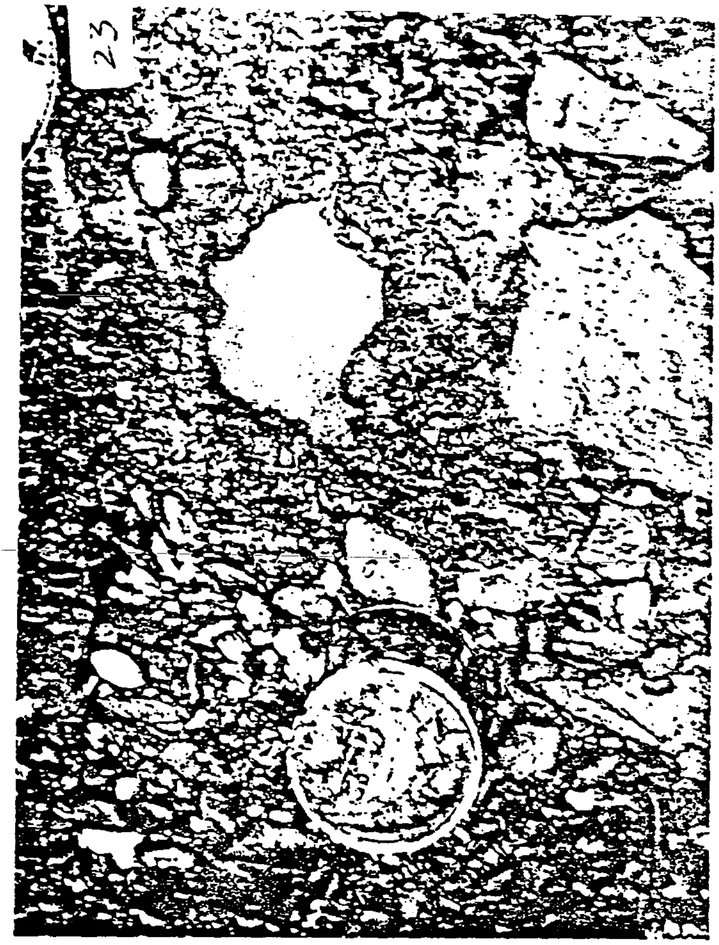
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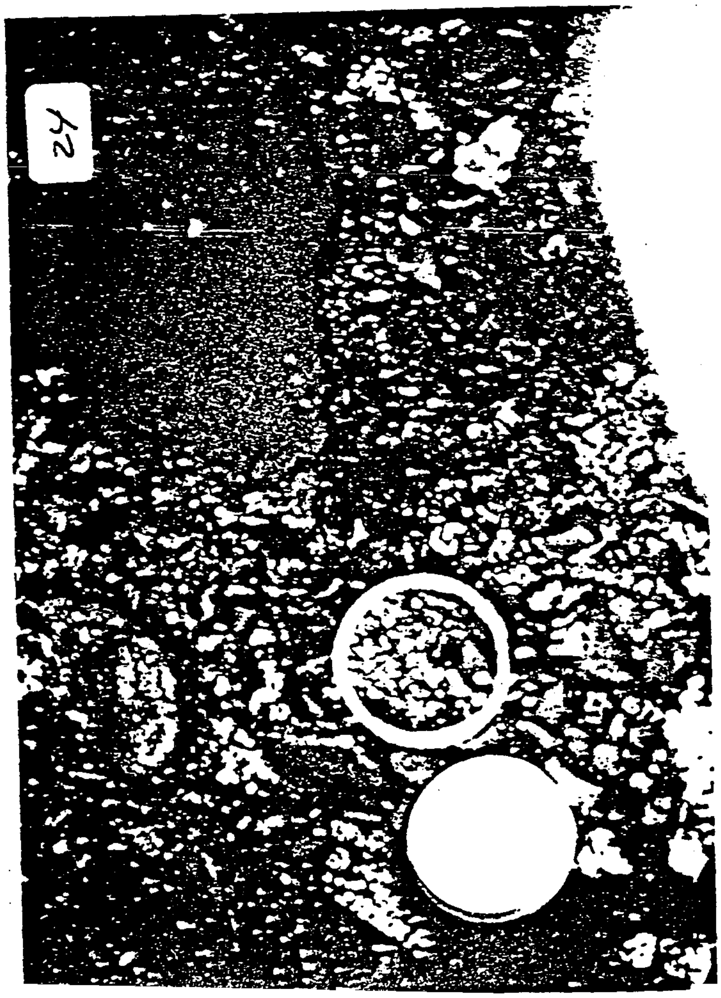
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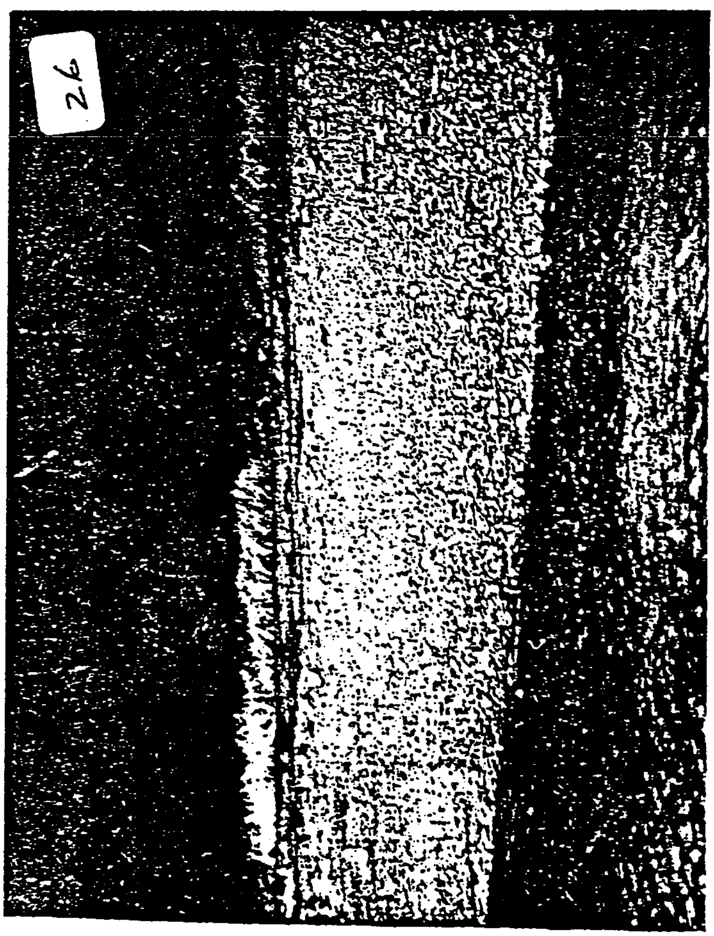
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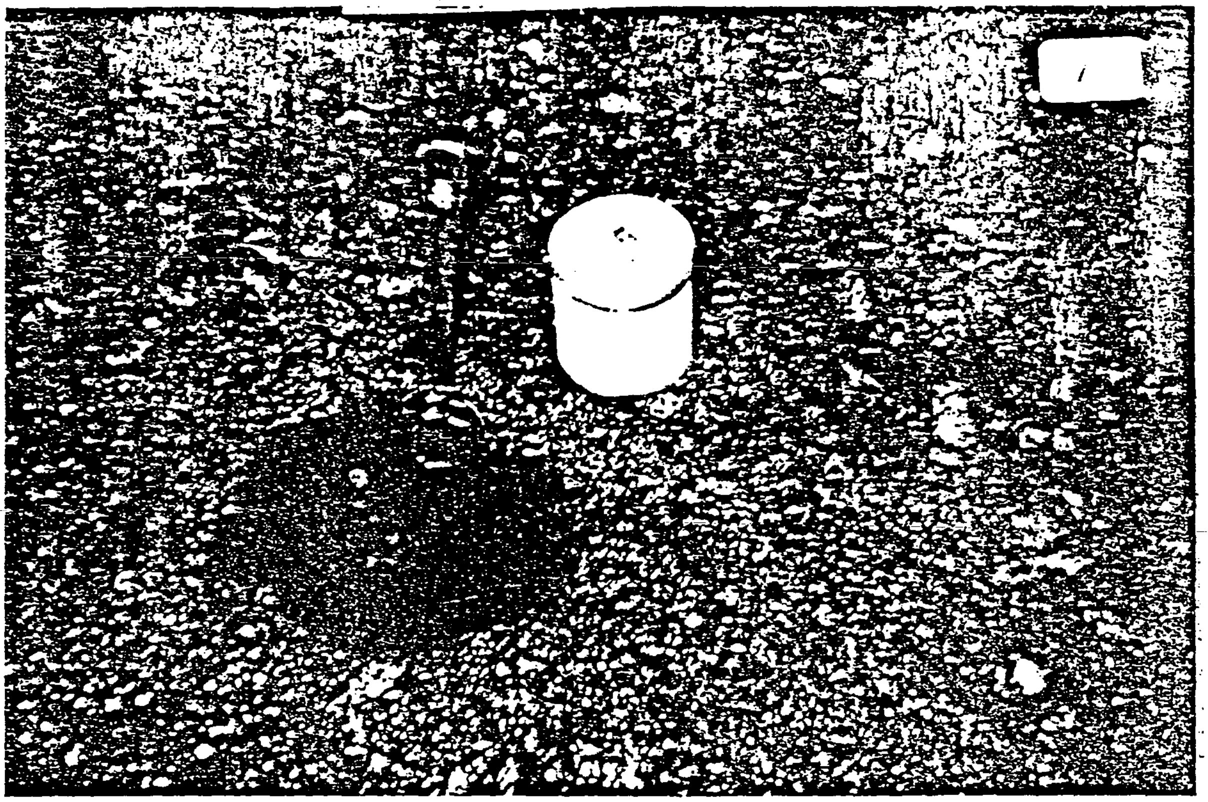
1991 ADF&G URCHIN/SEDIMENT COLLECTION PHOTOS

PHOTO #
1 Site #3 Bay of Isles. Sample hole
3 Site #3 Bay of Isles. Oil sheen adjacent to sampling hole
6 Site #3 Bay of Isles. Distant view of site
7 Site #4 Drier Bay. Sample hole
8 Site #4 Drier Bay. Spacing of sampling holes.
9 Site #4 Drier Bay. Sample collection
10 Site #4 Drier Bay. Site closeup
11 Site #4 Drier Bay. Distant view
12 Site #2 Lower Herring Bay. Sample hole
13 Site #2 Lower Herring Bay. Sediment sample & hole
14 Site #2 Lower Herring Bay. Site closeup
15 Site #2 Lower Herring Bay. Distant view
16 Site #1 Herring Bay. Oil sheen coming up from sampling hole
17 Site #1 Herring Bay. Two sampling holes
18 Site #1 Herring Bay. Oil sheen & sampling hole
19 Site #1 Herring Bay. Closeup of site
20 Site #1 Herring Bay. Distant view of site
23 Site #5 Northwest Bay. Sample hole
24 Site #5 Northwest Bay. Sample hole
25 Site #5 Northwest Bay. Oil sheen at water's edge
26 Site #5 Northwest Bay. Oil sheen at water's edge
27 Site #5 Northwest Bay. Site closeup
28 Site #5 Northwest Bay. Distant view
29 Site #6 Cabin Bay. Sediment texture & hole
30 Site #6 Cabin Bay. Sediment texture & hole
31 Site #6 Cabin Bay. Sediment texture & hole
32 Site #6 Cabin Bay. Sample hole
33 Site #6 Cabin Bay. 2 sample holes & freshwater flushing from stream
34 Site #6 Cabin Bay. Closeup of site
35 Site #6 Cabin Bay. Distant view of site



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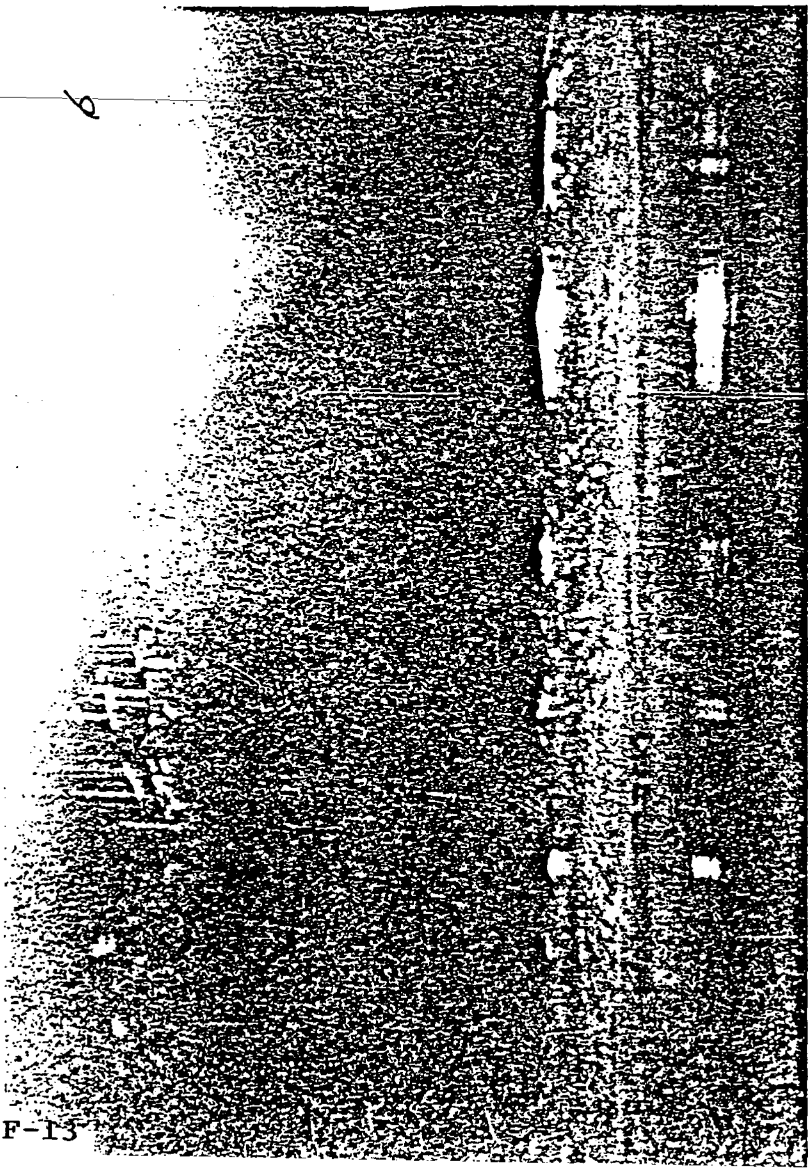


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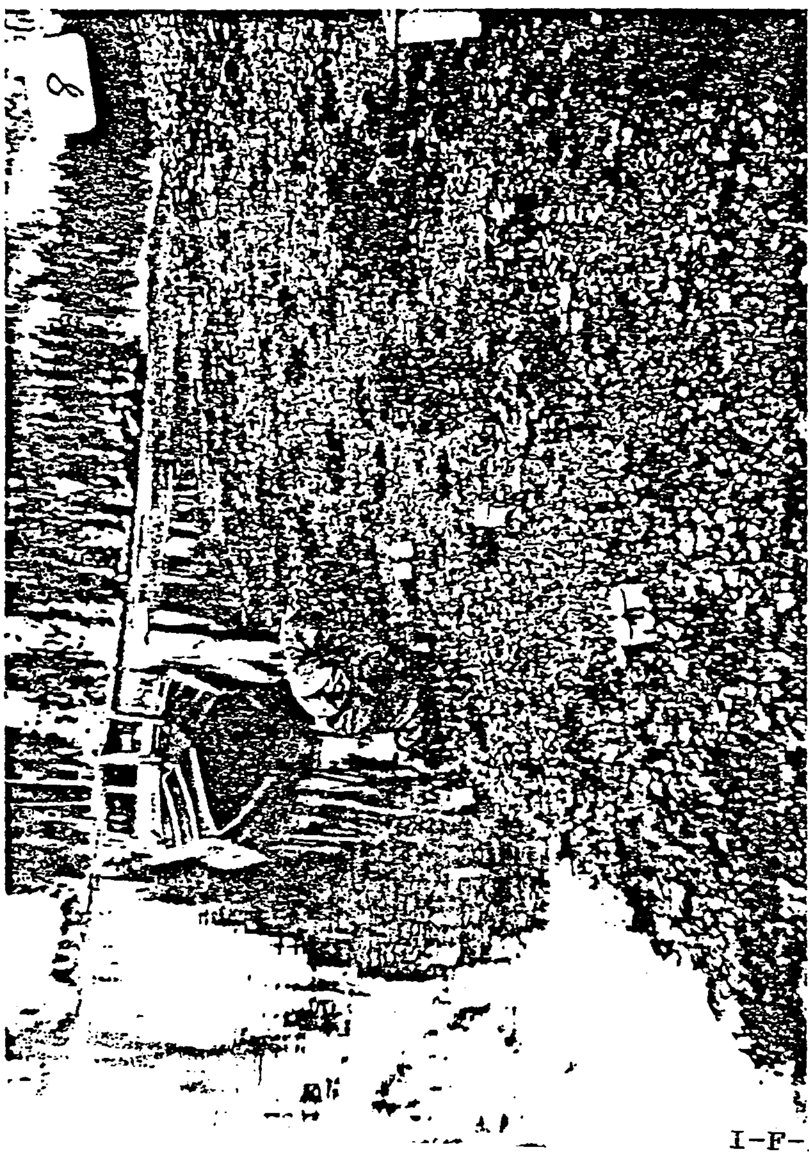
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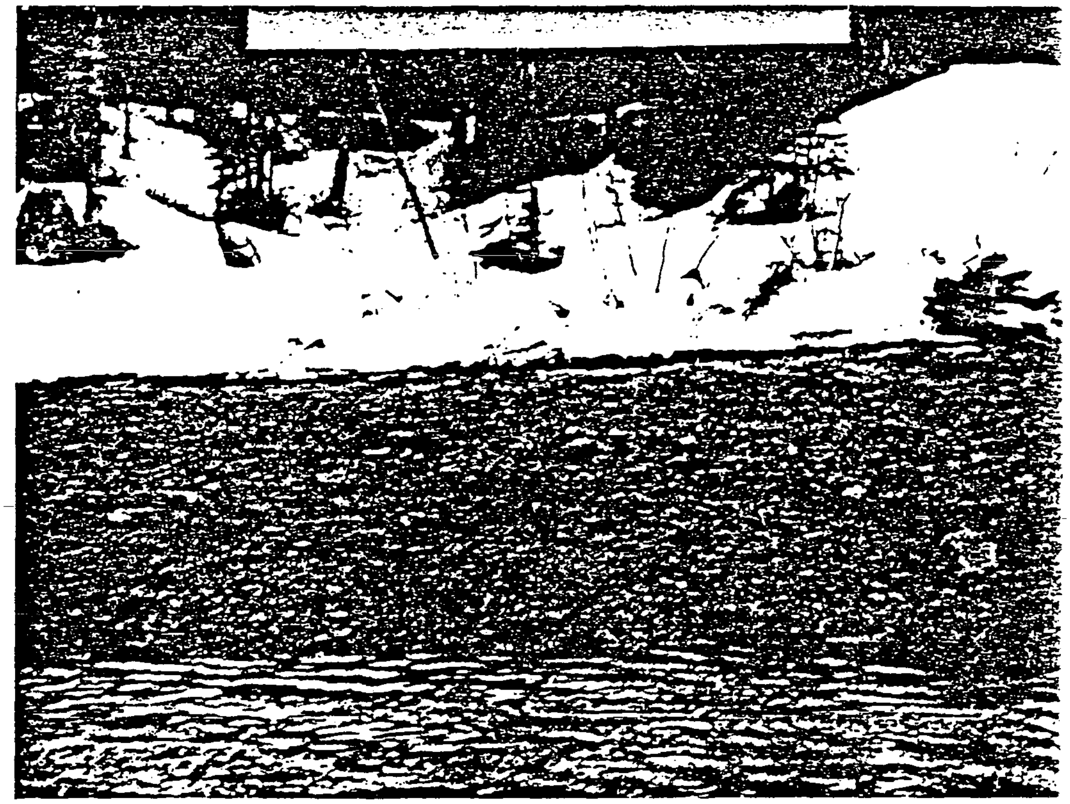

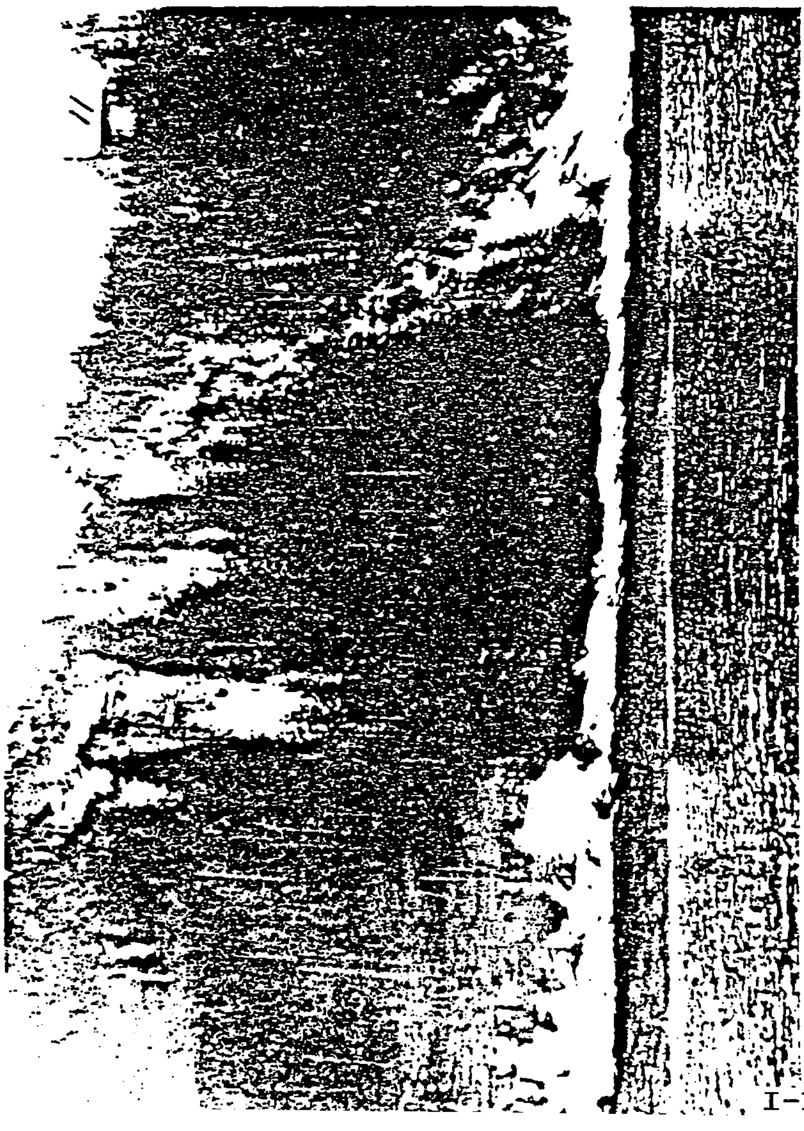
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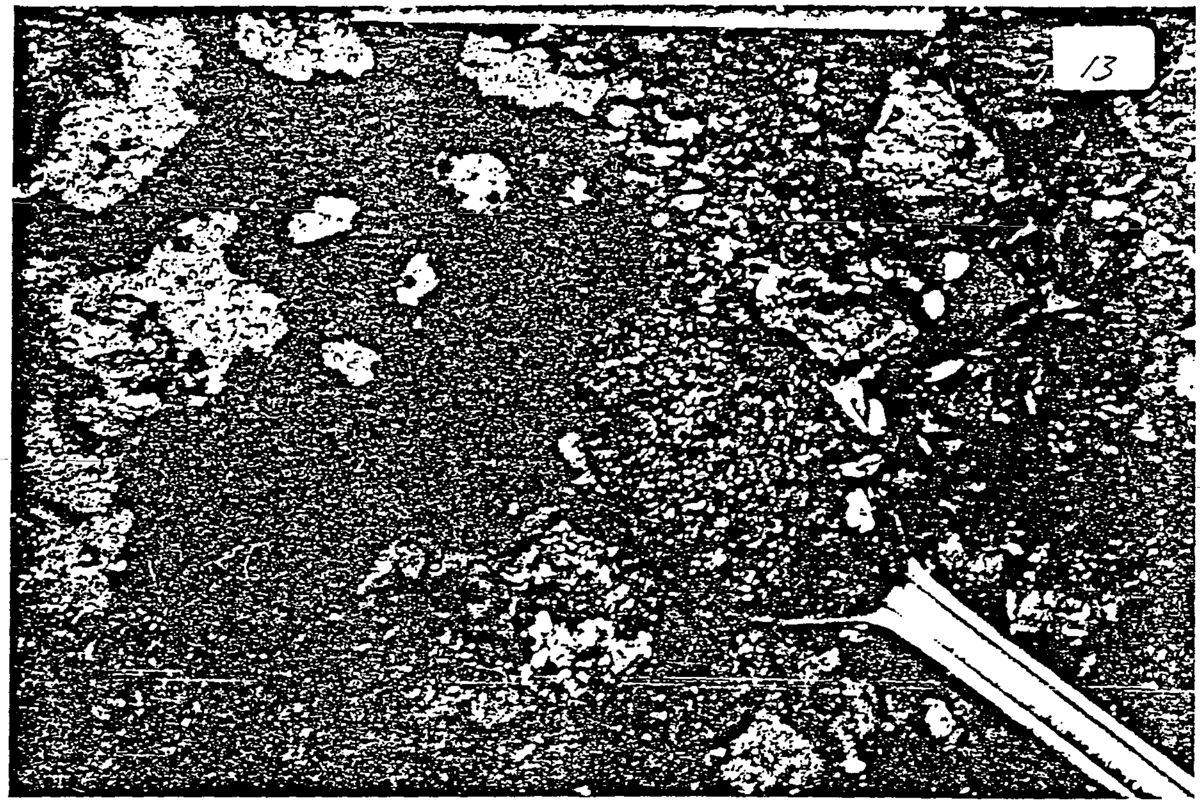
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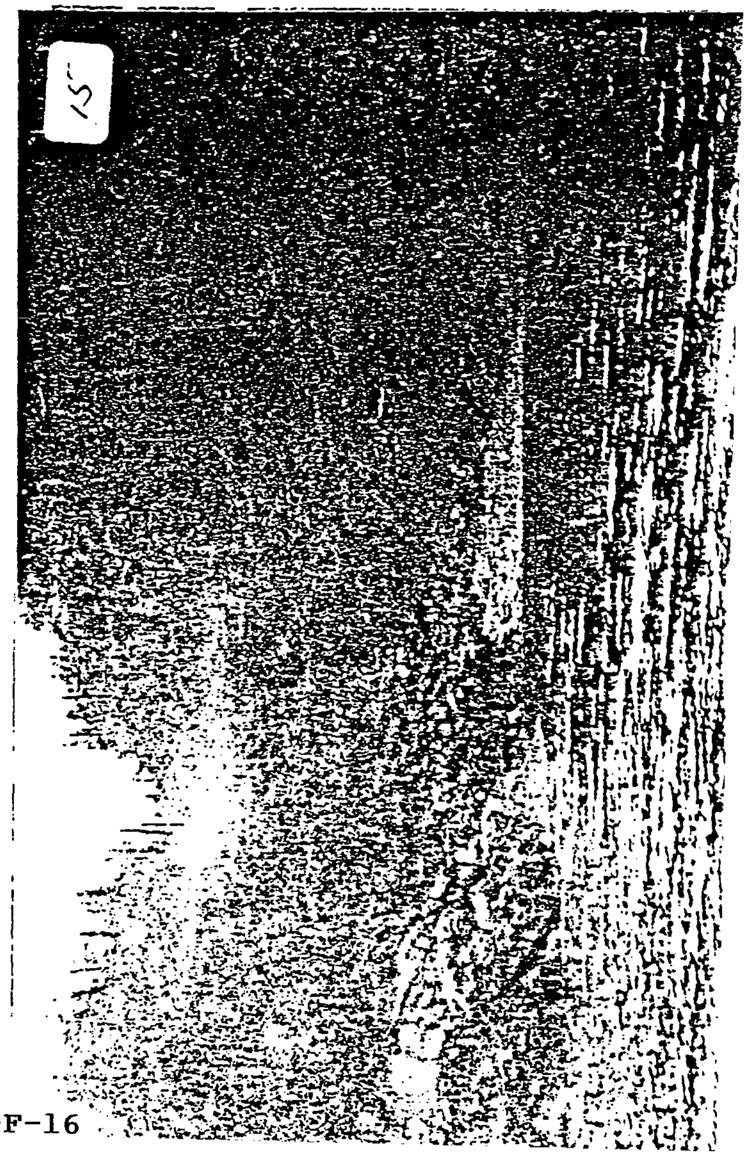
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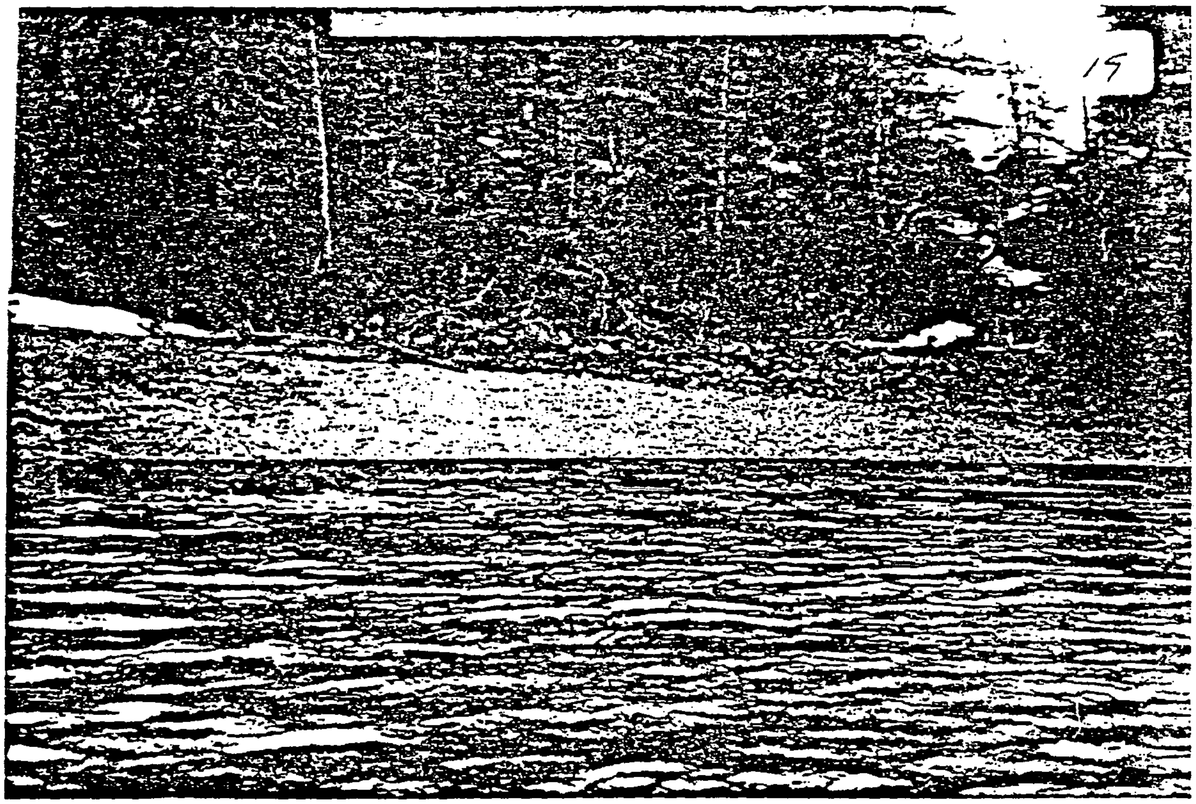
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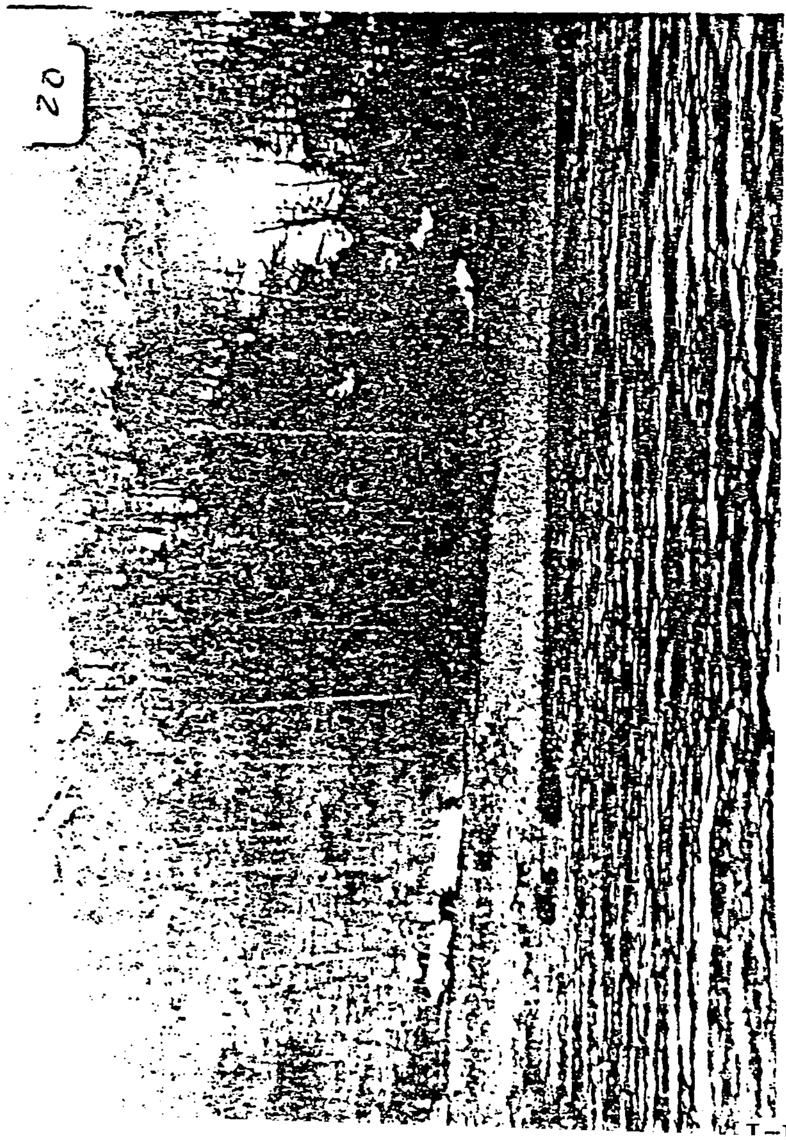
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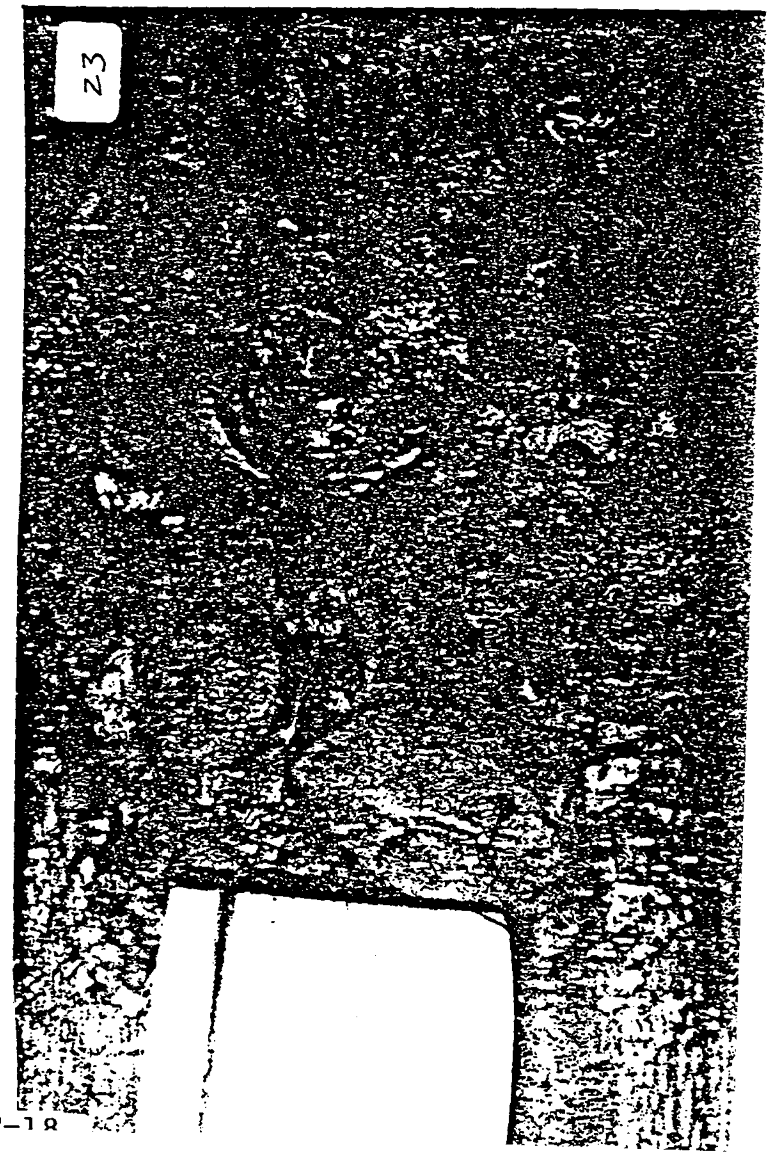
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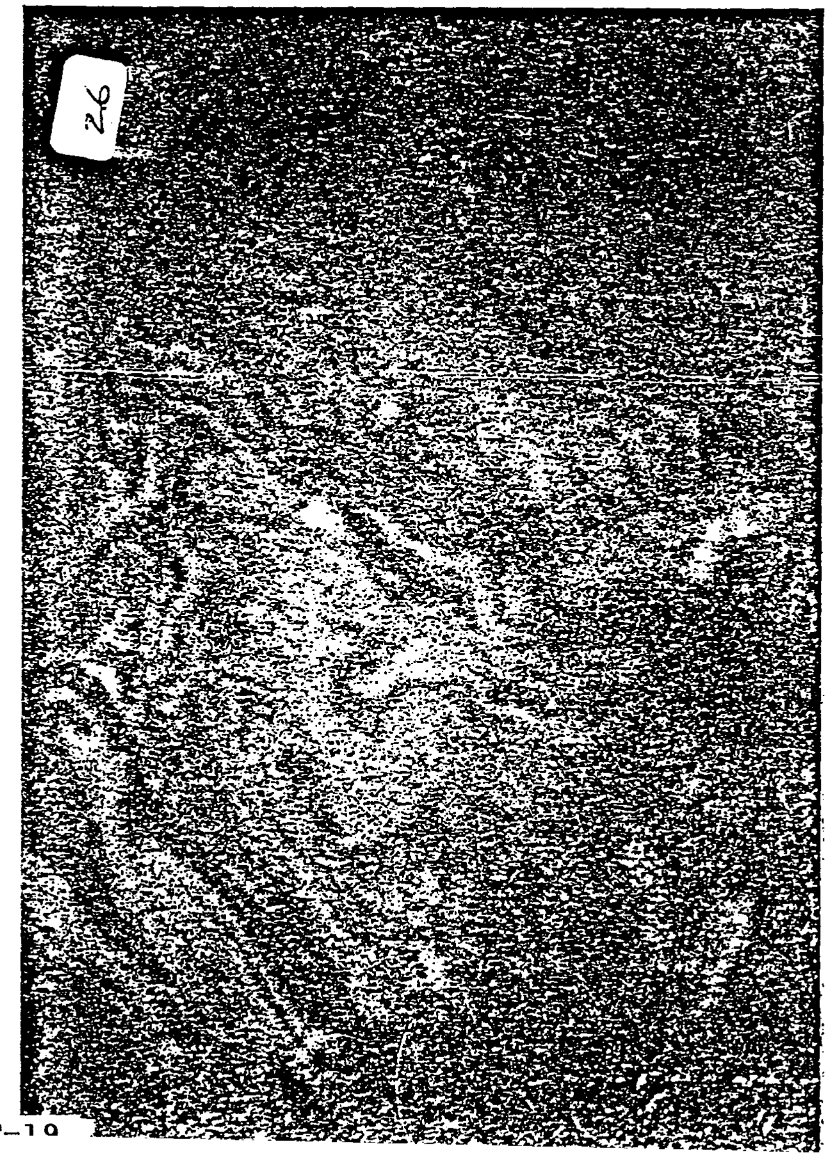
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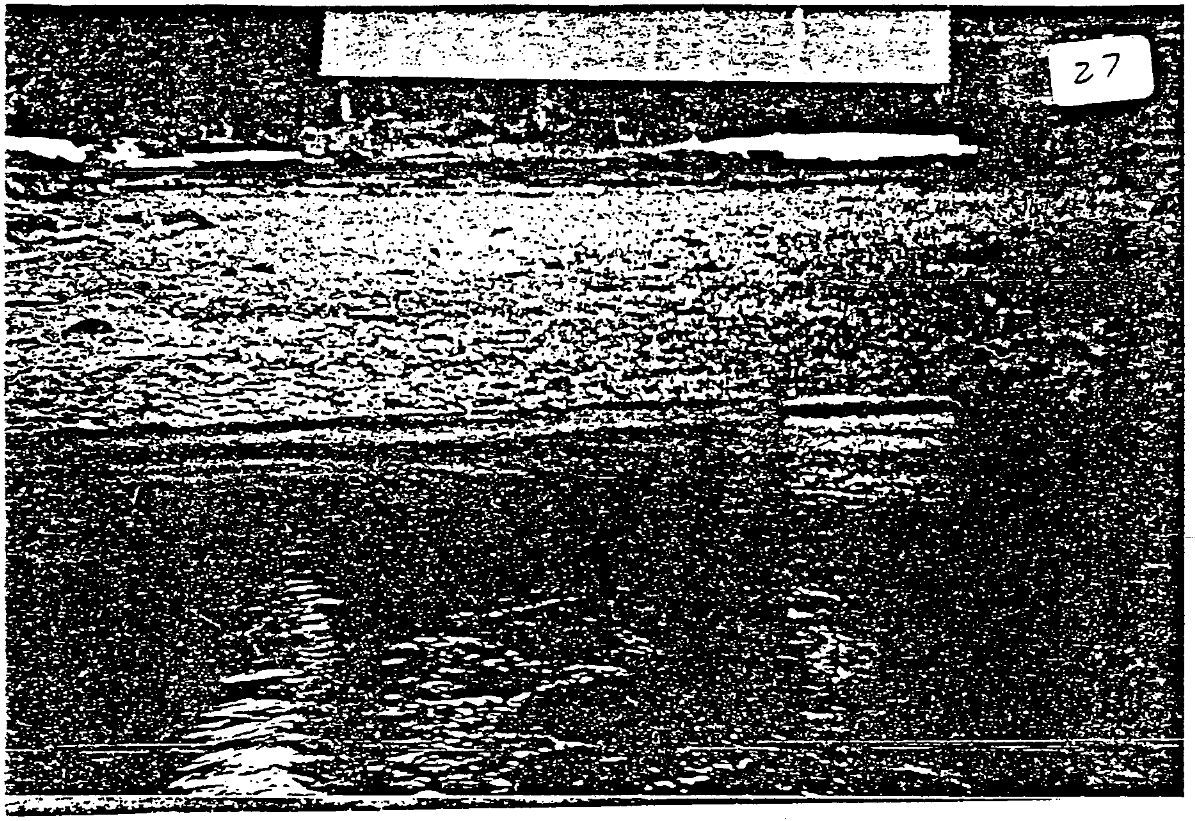
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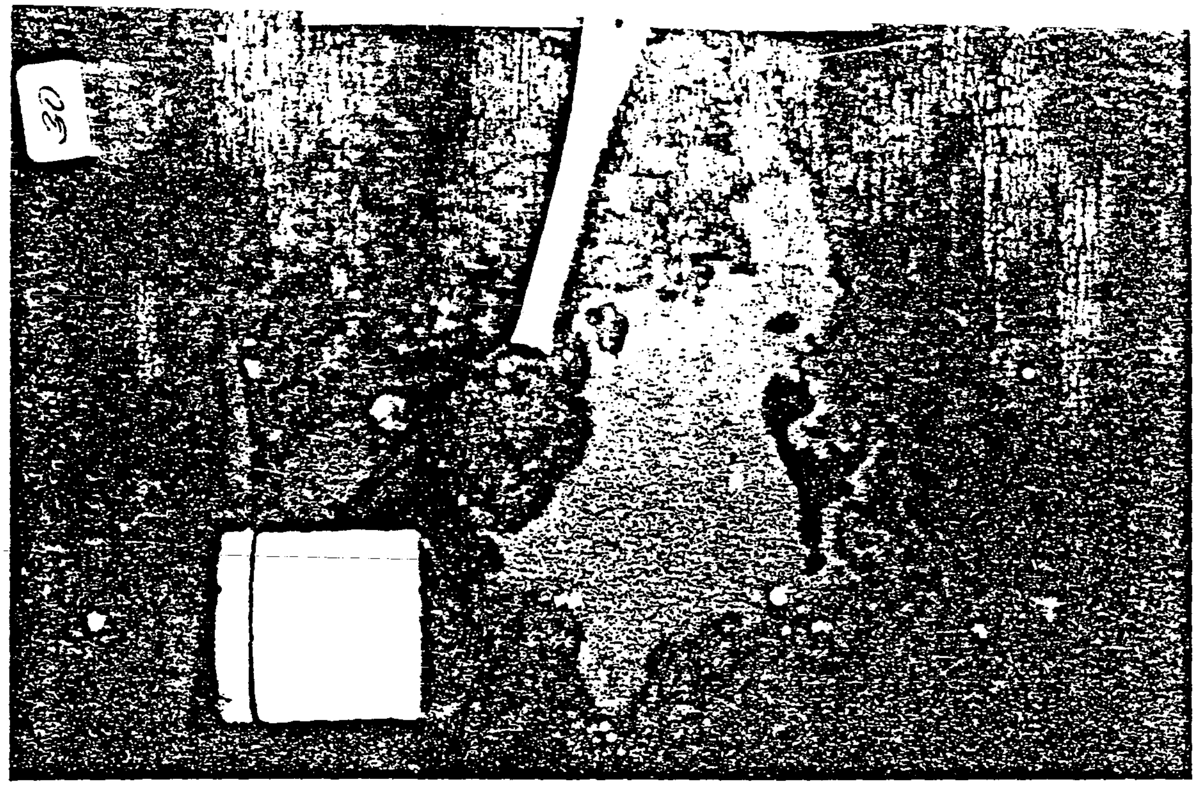
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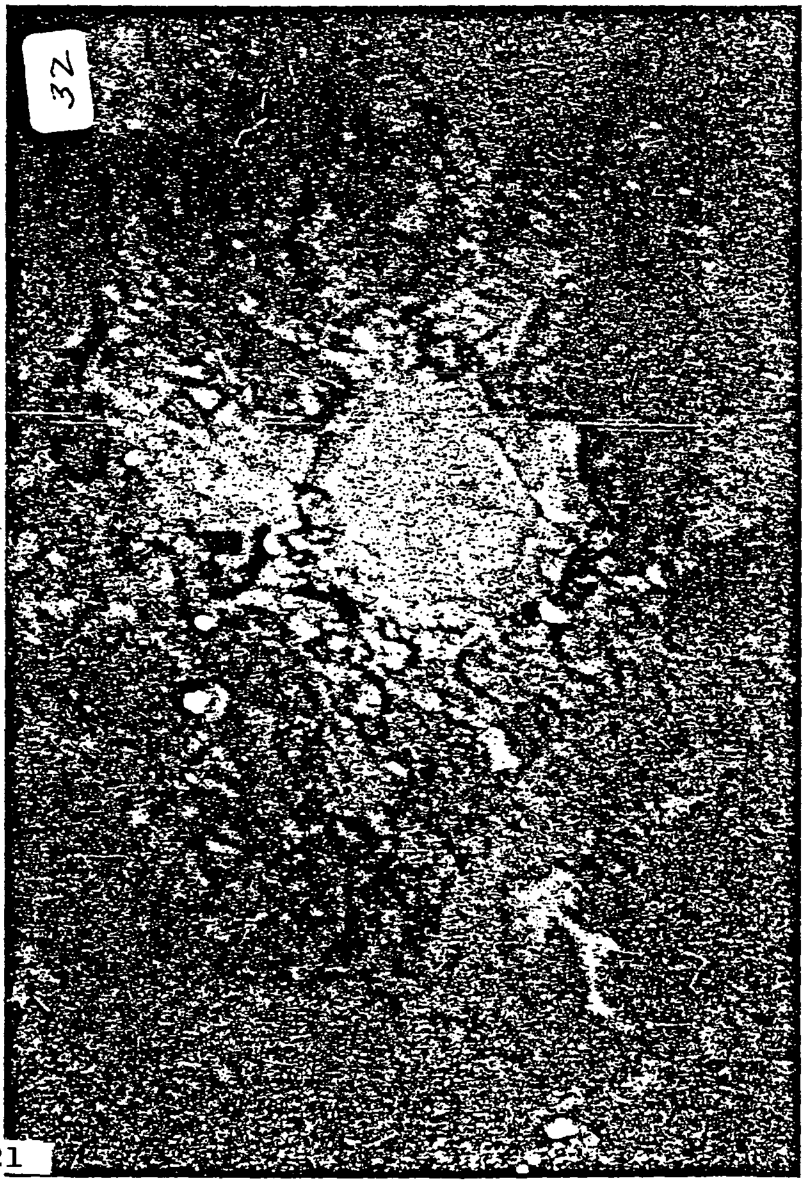
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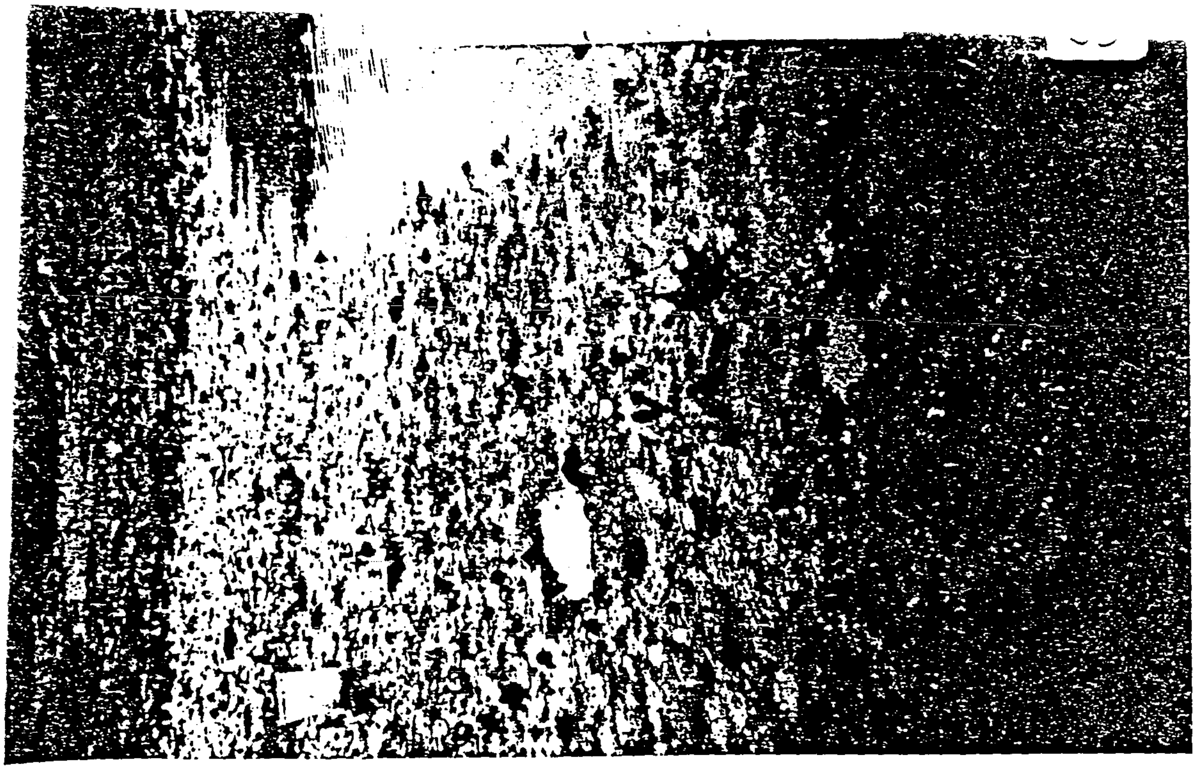

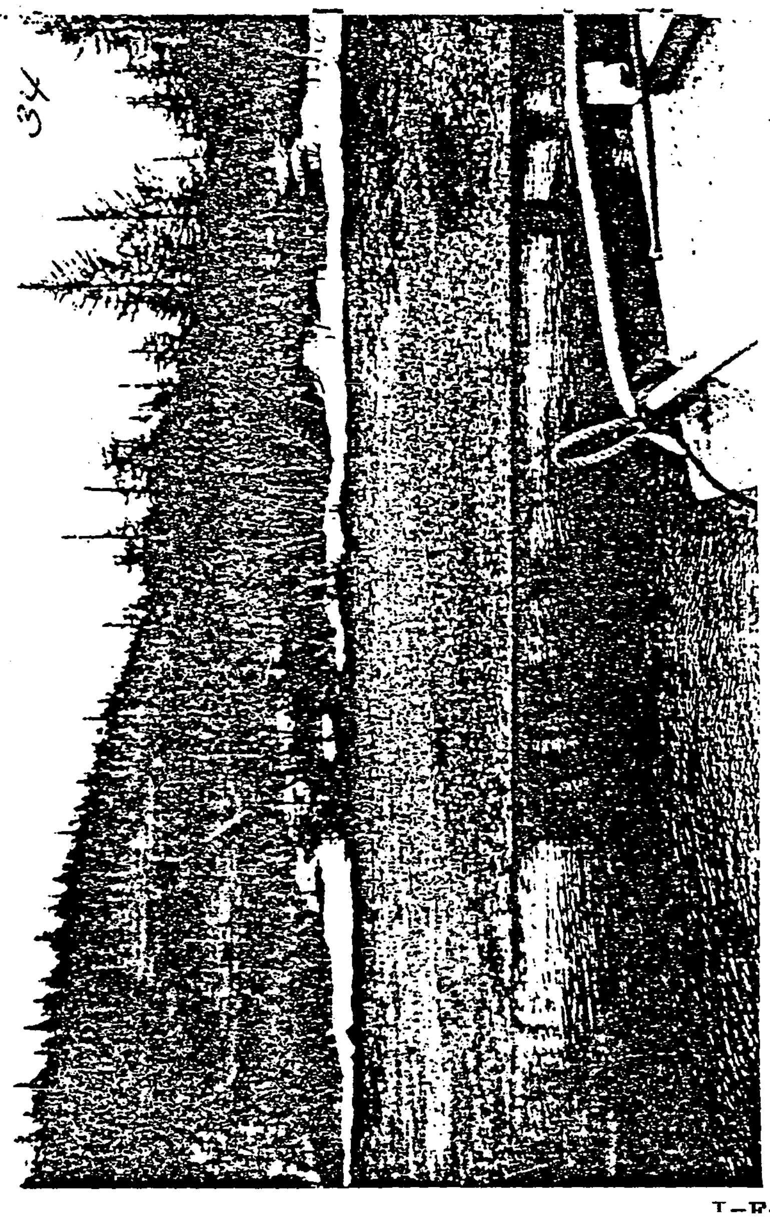
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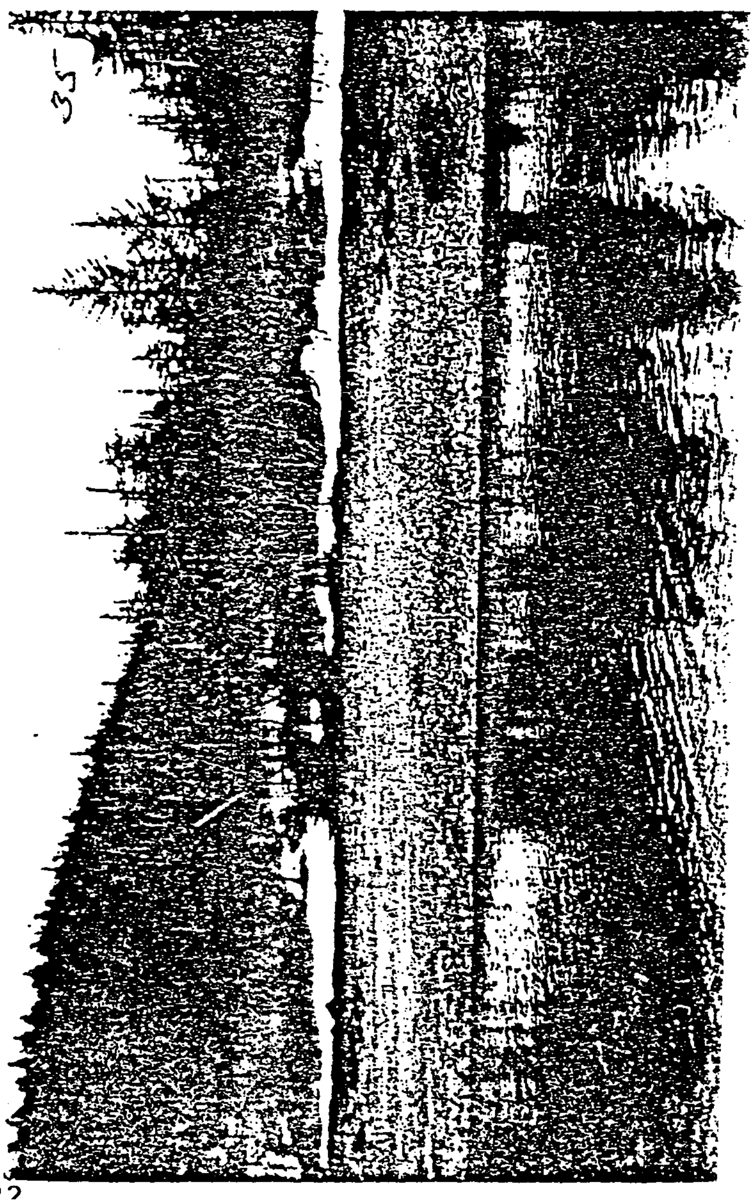
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