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Deepwater Pacific Sand Lance (Ammodytes hexapterus) Habitat Evaluation and Prediction in the Northwest Straits Region

FINAL REPORT
May 1, 2010 – June 30, 2011
June 30, 2011

Prepared for:
Northwest Straits Commission
10441 Bayview Edison Rd
Mount Vernon, WA 98273
www.nwstraits.org

This report was funded in part through a cooperative agreement with the National Oceanic and Atmospheric Administration.

Deep-Water Pacific Sand Lance (*Ammodytes hexapterus*) Habitat Evaluation and Prediction for the Northwest Straits Region

Final Report

H. Gary Greene[1,2], Tina Wyllie-Echeverria[3], Don Gunderson[4], Joe Bizzarro[4], Vaughn Barrie[5], Kurt Fresh[6], Cliff Robinson[7], David Cacchione[8], Dan Penttila[9], Monty Hampton[8], and Adam Summers[1]

[1]University of Washington, Friday Harbor Laboratories, Friday Harbor, Washington
[2]Tombolo/SeaDoc Society, Orcas Island, Eastsound, Washington
[3]Wyllie-Echeverria Consulting, Shaw Island, Washington
[4]School of Fisheries and Aquatic Sciences, University of Washington
[5]Geological Survey of Canada, Sidney, BC
[6]NOAA, National Marine Fisheries Service, Seattle, Washington
[7]Parks Canada, Sidney, BC
[8]US Geological Survey, Emeritus, Menlo Park, California
[9]Washington State Department of Fish and Wildlife, Mill Creek, Washington

Summary

The goal of this project was to obtain new information on sub-tidal life history parameters of Pacific sand lance (PSL) (*Ammodytes hexapterus*) in the Northwest Straits region. We incorporated a unique rapid method to indentify and sample PSL habitat using acoustic multibeam echosounder (MBES) bathymetric and backscatter images, seafloor video and seafloor sediment samples. We quantified the seafloor physical characteristics in and around a dynamic bedform (sediment wave field) known to harbor PSL, and other sand wave fields and developed a predictive geomorphic model using GIS that can be used to identify potential PSL habitats in deep water within the Northwest Straits region. We directly investigated those sub-tidal substrate types where PSL were expected to be present based on our previous investigations. Since little is known about PSL deep-water habitats, we specifically focused on producing metrics (parameters) through which areas of significant PSL aggregation might be identified and investigated.

We compared sediment type and dynamic conditions in PSL habitats with other nearby areas where PSL do not reside and where known PSL occupied inter-tidal areas. Our aims were to:

1) Identify submarine sand fields that have the potential to harbor PSL in the Northwest Straits region using existing MBES images,
2) Sample selected potential PSL habitat in the Northwest Strait region identified through evaluation of the seafloor MBES bathymetric and backscatter images, existing videotapes, and experimental results,
3) Compare our *in situ* results with experimental work of PSL in tanks undertaken at Friday Harbor Labs (FHL), University of Washington to determine what sediment types they are capable of burrowing into and which substrate are preferred and,
4) Develop a geomorphic model to use for identifying and mapping potential PSL sub-tidal habitats and determine if habitat characteristics vary for different life stages of PSL

in various habitat types.

We were successful in accomplishing the above aims and were able to develop robust sampling protocols and metrics useful in developing a predictive model for identifying potential areas of PSL sub-tidal concentrations. Significant results of this study are presented below:

1. A total 38 dynamic bedforms or sediment wave fields were analyzed for wave amplitude, wavelength, orientation, slope, aspect and other parameters, which were used as metrics to develop a predictive model.
2. A total of 12 research cruises were completed on the R/V *Centennial* (5) and on the *Tombolo* (5) to collect sediment, fish and underwater video and on the Canadian Coast Guard vessel *Otter Bay* (2) to collect multibeam echosounder (MBES) bathymetry and backscatter data (northern Lopez Sound/Decatur Island, Sucia sediment wave field, San Juan Channel sediment wave field, Salmon Bank, and Iceberg Point, southern Lopez Island).
3. A total of 140 sediment samples were analyzed for grain size and combined with those analyses made by Blain (2006) to produce a sediment analyses data set of 180 samples.
4. The Van Veen sediment grab sampler was found to be a quick and dependable tool for collecting PSL in situ on the seafloor.
5. The USGS eyeball camera was successfully deployed to measure grain sizes in situ within the sand wave fields and proved to be a rapid sediment analyzer.
6. Over 1,060 PSL were captured with 1,027 fish used in the biological analyses.
7. One PSL egg was collected out of 60 samples obtained for egg counts suggesting that PSL sub-tidal habitats may act as recruitment areas.
8. The highest concentrations of PSL collected during the sampling cruises were found in sediment of medium- to coarse-grain sand (0.5-1.0 mm in size).
9. Tank experiments with captured PSL showed that the fish preferred medium- to coarse-grained sand (0.5-1.0 mm in size) although they would burrow into all unconsolidated sediment sizes.
10. In addition to the known PSL sub-tidal habitat, the San Juan Channel sand wave field (our prototypical PSL habitat type), five other locations were imaged and sampled (Iceberg Point sediment wave field, Sucia Island sediment wave field, Salmon Bank, Decatur Island, and northern San Juan Channel sediment wave field).
11. PSL were found in the sediment wave fields at Sucia and Iceberg Point and in the fine-grain sand sediment at Salmon Bank but not in the mud of Decatur Island or the very coarse-grain sediment in the northern San Juan Channels sediment wave field.
12. Regular periodic sampling of the San Juan Channel sediment wave field indicate that PSL increased in concentration from July to October 2010 and remained in the sediment wave field through June 2011, indicating that they may overwinter or rest in the sediment.
13. Stability of San Juan Channel sediment wave lies close to upper limit for maintenance of sediment type and morphology. However, uplift due to tectonic episodes and/or eustatic rebound could alter the current flow making it stronger and thus sweeping the sediment away. Conversely sea level rise could reduce current strength thus assuring stabilization.
14. All life stages after planktonic stage appear to use the San Juan Channel sediment

wave field.

15. The most favorable predictive potential PSL sub-tidal habitat type would be similar to the San Juan Channel sediment wave field with wave amplitudes of 3-5 m, wavelengths of ~100 m, aspect rations of and grain sizes ranging from 0.5-1.0 mm.
16.

Introduction

The PSL is an important forage fish along the coastal North Pacific Ocean from northern California to northern Hokkaido, Japan, and is one of six species in the genus *Ammodytes* (Robards *et al.*, 1999a,b). Although PSL is a key component in the Northwest Straits regional food web, very little is known of this species' biology. For example, only three peer-reviewed papers detail the biology of PSL in Puget Sound compared with 16 such manuscripts on Pacific herring (*Clupea harengus pallasi*). The burrowing behavior, recruitment rates and conditions, relative abundance and distribution, population structure, local spawning habits, and spawning and burial substrates remain largely unknown (Robards et al., 1999a, 2002; Tribble, 2000). The work that has been done on the biology and habitat of PSL has focused on the nearshore and shallow sub-tidal areas; little work has been done on the deep sub-tidal habitats. A disjunction occurs between the abundance of sand lance and the availability of known habitat and it is our hypothesis that predominant and important habitats exist in the deep sub-tidal areas.

In Northwest Straits region, PSL serve as the primary link between zooplankton and higher order predators, and are a vitally important food source for 29 species of birds, 10 species of marine mammals, and 30 species of commercial and sport fishes (Meyer et al., 1979; Auster and Stewart, 1986; Geiger, 1987; Robards *et al.*, 1999a,b; Tribble, 2000). Specifically, this species is a crucial component in the diet of common murres, rhinoceros auklets, tufted puffins, harbor seals, minke whales, salmon, lingcod, rockfish and other groundfishes (Geiger, 1987). The condition of the Northwest Straits region's ecosystem depends in large part on the large biomass of forage fish, including PSL, that transfer phytoplankton production to higher trophic levels (Fresh, 1979; Fresh et al., 1981; Duffy, 2003; Zamon, 2001, 2003; Johnson et al., 2008)

The Pacific sand lance, *Ammodytes hexapterus,* is known to deposit its spawn on sandy upper intertidal beaches throughout the Puget Sound Basin (Penttila 1995a, 2007). Roughly 10% of the shoreline of the Puget Sound basin comprised of fine-grained beaches has been found being used by spawning sand lance. It has also been hypothesized that sand lances might also use sub-tidal sandy substrates for spawn deposition, although no physical evidence of this has ever been reported.

The NWSC's deep-water sand lance project afforded the opportunity to sample potential sub-tidal sand lance spawning habitats for evidence of spawn deposition. Using the same VanVeen grab-sampler sediment volumes collected and searched for fish we also processed and inspected for evidence of eggs *in-situ.* So far as is known, no previous sub-tidal sediment sampling projects undertaken within the Puget Sound Basin have ever included sub-tidal searches for sand lance eggs, especially within specific habitat types known to be occupied by the fish during the potential spawning season. Documentation of sub-tidal sand lance spawning activity would expand the known habitat contexts used by the spawning fish, thus expanding the view of their "critical" habitat to which enhanced regulatory habitat management/protection might have to be extended for long-term conservation purposes.

Previous Work

Much of what is known about PSL benthic habitat comes from shallow water studies. Sand lance are dependent upon benthic sediment habitats to burrow into and, therefore, this species is most often associated with fine- to coarse-grain sand- or gravel-oxygenated sediments (Meyer et al., 1979; Auster and Stewart, 1986) in nearshore inter-tidal (-0.3m MLLW) and shallow (to 100 m) habitats (Wright et al., 2000; Pinto, 1984; Ostrand et al., 2005; Quinn, 1999; Robards et al., 1999a,b; Auster and Stewart, 1986). In the inter-tidal, sand lance were found to be buried 5.0 cm deep and to be oriented horizontally in the oxygenated sediment layer at densities of 5 fish per square meter and can remain buried in inter-tidal sediments during low tide exposure (Quinn, 1999). Sediment size conducive for sand lance to penetrate and burrow into ranged in size from 0.36 to 1.0 mm in diameter (Quinn, 1999). Inter-tidal beaches have been documented as habitat for PSL and their eggs (Moulton and Penttila, 2000). Sediments provide habitat for overwintering (Healy, 1984), to rest and conserve energy (Quinn, 1999), to avoid predation (Reay, 1970) and as spawning substrate where their adhesive eggs attach while incubating. When the fish emerge from the substrate they form large schools and feed on zooplankton in the water column during the day (Dick and Warner, 1982; Robards *et al.*, 1999a, b; Auster and Stewart, 1986; Geiger, 1987). They emerge from the sand at dawn and are vulnerable to predators as they enter the water column (Hobson, 1986).

Deposits of clean sand at the water depths where PSL reside in the sub-tidal environment (typically < 100 m) are common where relatively strong currents continuously sweep the seafloor. In order to maintain a deposit, a plentiful sand supply is necessary, although finer sediment might transit through the area and coarser sediment might be present as a lag. Sand-wave fields consisting of ripples, waves and dunes are common in such areas, and several fields have been mapped near the San Juan Islands (Barrie et al., 2009; Greene et al., 2011). One such sand-wave field was documented by Blaine (2006) in San Juan Channel of the San Juan Islands and was found to be a productive PSL habitat (Fig. 1). The aerial extent of this sand-wave field is delimited by a distinct boundary where the sand waves are in sharp contact with a relatively featureless surrounding seafloor. Such abrupt transitions have been reported in other nearby sand-wave fields (Barrie et al., 2009)

The central San Juan Channel sand wave field covers an area ~500,000 m^2 at depths of 60-80 m (Fig. 1). During a 2004 ROV video survey at this location, we observed PSL burrowing and emerging from the sediment. In October 2006 and 2008, we again discovered PSL in the sand waves and sampled them with a small Peterson grab, a grab sampler about the size of an adult male fist, which captured 1-2 live fish per grab. The sand wave field in San Juan Channel is a dynamic bedform that is influenced by a combination of strong currents, tides, and variable water flow. The significance of the sediment wave field as a PSL habitat was shown by the work of Blain (2006), who collected over 42 Van Veen sediment grab samples, 40 of which captured on average 10.5 live fish/grab (Fig. 2). Based on extrapolations from these collections, Blain (2006) estimated a total (night and day periods combined) average density of PSL at 84 fish/m^2, considerably more than the 5 fish/m^2 reported in inter-tidal sands by Quinn (1999), and surmised that the local population consisted of over 63 million individuals.

The relationship of sand lance to sediment that Blain (2006) found is shown in Figures 3, 4 and 5. The number of fish caught was compared to the percentages of gravel, sand, coarse grain sand, and medium grain sand present in the dark (night) and light (day) samples. Strong clustering was present in all correlations. Between gravel and sand, sand lance associated with low percent gravel composition and high percent sand composition (Figs. 4A, B). Similarly, between coarse grain and medium grain sand compositions, sand lance associated with low percent medium grain sand and high percent coarse grain sand (Fig. 5A, B)

Prior to this study we undertook cursory sub-tidal PSL habitat characterization and mapping, as well as initiated laboratory experiments to test the hypothesis that PSL prefer certain grain sizes and compaction and density levels (i.e., habitat types) when burrowing and to assess the range of habitat types that they are able to burrow into. This study was under the direction of Adam Summers and executed by Joseph Bizzarro.

Ten uniform grain sized sediments ranging from silt to coarse gravel were obtained and measured using a RoTap sediment analyzer on the field collected sediment samples. We used these experimental results to assist us in refining the substrate characteristics preferred by PSL in this study.

In addition, we built our PSL sampling design on previous nearshore and beach sampling projects that indicated areas of large concentrations of PSL in the past. PSL have consistently been found in the inter-tidal and pelagic areas (Fig. 6). Figure 6 shows where large catches of PSL were made in Haro and Rosario Straits and San Juan Channel. Sampling effort was widespread but the catches of PSL were localized. Tow net catches were predominatelyin April with 96% juvenile fish caught. The number of PSL sampled in San Juan County varied considerably through time (Table 1, Fig. 7).

Physical oceanographic data using a Hydrolab® were collected in Lopez Sound during 2009 and related to times when high concentrations of PSL were present (Fig. 8).

Methodology

Our research methods were unique, multidisciplinary and comprised of four phases designed to address the following null hypotheses:

H_o PSL do not inhabit unconsolidated sediment in deep sub-tidal areas
H_o All life stages of PSL occupy non-specific sediment types

Marine Geophysical Data

Marine geophysical survey data, primarily wide swath multibeam echosounder (MBES) bathymetry and backscatter acquired in cooperation with the Geological Survey of Canada, Canadian Hydrographic Service, Center for Habitat Studies, Moss Landing Marine Labs, and Tombolo/Sea Doc Society collected in the Northwest Straits region (southern Gulf Islands and the San Juan Archipelago) were used to produce the seafloor images of the sediment wave fields sampled in this investigation. These data along with side-scan sonar mosaics and 3.5 kHz sub-bottom seismic-reflection profiles were used to produce habitat types after Greene et al. (2007; Fig. 9) and published in a marine benthic habitat map series (Greene and Barrie, 2011). These data were used to identify and map dynamic bedforms and other sand fields in the region and to select the most promising sediment fields for sampling.

A pole-mounted Reson 8101 SeaBat™ (240 kHz) swath (150° swath coverage) MBES system mounted aboard a small boat was first used in 2000 to collect the data and initiate the mapping program. From 2001 through 2008 the Canadian Coast Guard vessels *Otter Bay*, *Revisor*, *Young* and *Vector*, and under the direction of the Canadian Hydrographic Service (CHS), acquired extensive high-resolution bathymetric datasets of the waterways surrounding the Southern Gulf Islands and the San Juan Archipelago. The MBES Simrad EM 1002 (95kHz frequency) and EM 3000-3002 (300 kHz frequency) systems were used for deep (>80 m) and shallow (<80 m) waters respectively. The dataset resolutions were 5 and 2 m respectively. In most of the areas, the tracks were positioned so as to insonify 100% of the seafloor with a 100% overlap, providing 200% coverage. Positioning was accomplished using a broadcast Differential Global Positioning System (DGPS) and MBES data were corrected for sound speed variations in the stratified water column using frequent sound speed casts.

In addition to bathymetric data, the MBES systems collected and recorded backscatter intensity. Backscatter intensity is a measure of sound that is scattered back toward the transmitter by acoustic reflection and scattering, both at the sediment-water interface and within the sediment (volume scattering). Many factors influence the intensity values, among them are: the angle of incidence of the beam, the volume scattered, the seabed slope and the surficial sediment type and roughness. With these factors in mind, backscatter strength datasets were used to determine relative sediment differences within one or many datasets and helped to interpret the benthic habitat types. To assure the best interpretation, backscatter images were used in conjunction with other multibeam bathymetry derivative datasets, such as seafloor shaded relief, slope analysis and bathymetric contours. The multibeam bathymetry and backscatter raster datasets, as well as the benthic habitat layer were processed using ESRI™ ArcGIS tools.

From the interpretation of the geophysical data, we selected three other dynamic bedforms (near Iceberg Point at southern Lopez Island, west of Sucia Island and off of Point Caution, central San Juan Channel; C, B, and F, respectively, in Fig. 10). Since dynamic bedforms are the product of sediment source (grain size), depth and seafloor energy (Southard and Bouguchwal, 1990), we compared these unique morphologies in relation to PSL habitat types such as shown Figure 1 and presence/absence of PSL. We then compared these bedform images and sampled them to produce a proto-typical morphologic predictive model.

Spatial Analyses Using GIS

The spatial analysis component of this project used GIS techniques, performed using ArcGIS software (ArcGIS Desktop, ESRI 10.0), to investigate and quantify geomorphic aspects of sand wave fields in the San Juan Island Archipelago. Based on previously collected bathymetric data and corresponding benthic habitat designations, thirty-five (35) sand wave fields were identified, in addition to the study site in San Juan Channel (Fig. 11). WGS 1984, UTM Zone 10 was chosen as the projection system for this project.

The following metrics were calculated for sand wave fields: area, maximum length and maximum width, aspect, amplitude, and wavelength.

Area and maximum linear dimensions were determined using the vector (polygon) file consisting of all 36 sand wave fields. Area (m^2) was calculated using the Calculate Areas feature present in the Utilities Toolbox of the Spatial Statistics Tools function of ArcToolbox. Maximum length and maximum width were measured directly on linear axes using the Measure tool in ArcMap. When the orientation of a sand wave field changed abruptly, a secondary line was drawn at the point of change in orientation and the total distance was summed between the initial and secondary lines (Fig. 12).

Aspect, wavelength, and amplitude also were calculated for the sand wave fields using the corresponding bathymetry layer and a derivative hillshade file. For fields with corresponding bathymetric data (n = 26), the outline of each sand wave polygon was depicted so that the underlying hillshade was visible. Profile lines oriented to the primary trend(s) in sand wave direction were drawn within each sand wave field. The Line of Sight feature in the Functional Surface Toolbox of the Spatial Analyst Tools function was then used to determine wavelength and amplitude along each designated line. Mean aspect also was determined along these profile lines using the Linear Directional Mean feature present in the Measuring Geographical Direction Toolbox of the Spatial Statistics Tools function of ArcToolbox. All lines were drawn from north to south; therefore, mean aspect results ranged from 90°-270°.

Tank Experiments

Laboratory studies were initiated to investigate the range of sediment types that PSL can penetrate, the selectivity of this species for different grain sizes and compaction levels, and the effect of compaction on burrowing force. All experiments were conducted under laboratory conditions in 20-gallon aquaria, with continuous seawater flow. Sediment was collected from beaches on San Juan Island, dried, and measured into eight uniform grain sizes ranging from silt (3.90 um) to pebble (4.75 cm) with a RoTap™ sediment analyzer. Only a subset of available grain sizes was used in these preliminary analyses. Pacific sand lance were collected during spring and summer months off Jackson Beach, San Juan Island, using a beach seine. Sizes were restricted to those corresponding to subadults (8.0-11.0 cm total length), the dominant life stage present at the time of surveys, to limit the effects of ontogeny on burrowing capability and selectivity.

A single, uniform grain size was used to test burrowing capability, whereas paired experiments were conducted to determine selectivity for different grain sizes and compaction levels. To assess the burrowing capability of PSL, 20 individuals were placed in an aquarium and observed until at least one incidence of burrowing was recorded or until four hours had elapsed. To investigate grain size selectivity, the bottom of each aquarium was divided into two compartments and filled with 15 cm of either medium sand (0.25-0.50 mm), coarse sand (0.50-1.00 mm), or very coarse sand (1.00-2.00 mm). Pacific sand lance (n = 50) were placed in an aquarium and allowed to acclimate for four hours. After four hours, each sediment compartment was sealed, all non-burrowed fish removed, and seawater was drained. The number of fish contained in each compartment was then counted. This process was repeated to obtain two trials for each grain size comparison. For compaction trials, sediments were compacted and tested with a cone penetrometer until maximum compaction was achieved. Six trials were then conducted as previously described using uncompacted and compacted coarse sand. To determine the effects of grain size and compaction on burrowing force, a resin model was created from the mold of a subadult Pacific sand lance. Using a materials testing machine, the first third of the body (shown from high speed video to be the portion that enters sediment upon initial penetration) was pressed into grain sizes corresponding to compacted and uncompacted medium sand, coarse sand, very coarse sand, and granule (2.00-4.00 mm) to determine the force required for burrowing. Chi-square statistics were used for comparisons of selectivity, whereas linear regression was used to evaluate the relationship between force and penetration depth for different grain sizes and compaction levels.

Sediment and In Situ Fish Sampling

Building upon the work of Blain (2006), we conducted a comprehensive sediment sampling effort of the central San Juan Channel sand wave field (see Appendix I), using Van Veen and Poner grab samplers and the USGS eyeball sediment camera in place of a video camera (SplashCam™) to selectively sample the crest, troughs, stoss and lee sides of the sand waves to determine grain size distribution throughout the sand waves themselves, and randomly sampled the field as a whole. Sediment analyses were undertaken using a RoTap sieving machine.

Our unique sampling method for the collection of PSL and sediment samples, although biologically unorthodox, has been extremely successful in obtaining significant numbers of live PSL. This method used a large Van Veen sediment grab sampler, a clamshell type sampler with long lever arms and sharp cutting edges on the bottom of the scoops that enabled us to cut deeply into unconsolidated sediment. The weighted jaws, chain suspension, and doors and screens allowed flow through during lowering, also allowing for vertical descent in a strong current regime. The sampler was allowed to free-fall from ~3 m above the seafloor, which caused it to suddenly, and rapidly, penetrate unconsolidated substrate. Once impact was made the lowering cable was swiftly raised, which exerts strong tension on the long arms extending beyond the jaws causing the jaws to dig deeper into the substrate and trap sediment and any burrowing fish, as the jaws rapidly and tightly close. The rather large surface area covered by the sampler and the strong closing mechanism allowed the jaws to excavate relatively undisturbed sediment. Upon retrieval the flaps on the top of the sampler close preventing flow through or any loss of sediment or fish. We used a 70 x 36 cm size sampler capable of retrieving 80 L of sediment. This sampling method has also been successfully used in the North Sea for recovery of sand eel (Freeman et al., 2004)

Our methods included collection and comparison of sediment samples throughout the sand-wave field, across individual sand waves, across the sand-wave field boundary, and outside the sand-wave field. These samples were augmented by the sediment information gathered by Blaine (2006; Figs. 4 and 5). Sediment grain sizes were measured and compared within and outside of PSL habitats in order to determine the range of size distributions favorable for their occupation (see Appendix II). In addition, we used the USGS eyeball camera to selectively sample various sand waves (see Appendix III). We duplicated Blaine's (2006) sediment analyses for the San Juan Channel sand wave field. A 400-600 mL sub-sample of the sediment collected at each site was used for grain size analyses to determine if the habitat is ephemeral (detected by changes in grain sizes from one sampling period to another). After being dried with flood lamps and weighed (in whole), the sub-samples were shaken through a series of sieves (2, 1, 0.5, 0.25, 0.125, 0.063, 0.038 mm) for 15 minutes on a Ro-Tap sieve shaker machine. Each size fraction preserved on each sieve was transferred to a pre-weighed weigh-boat and weighed in total. The proportions of gravel, sand, and silt of the samples, according to the Wentworth (1929) Grain-size Classification Scheme was calculated from the fraction weights and plotted as graphs and histograms.

To address the ephemeral nature of the pelagic life history we sampled one site (Jackson's Beach) intensely with tow-net and beach seines for pelagic habitat and with grab samplers for benthic habitat across two transects (see Fig. 13). This sampling coincided with sediment sampling of the San Juan Channel sand wave field. Intensive benthic habitat sampling and pelagic PSL sampling was used to develop our model. Transect One was from nearshore Jackson's Beach to the offshore sand field. Transect Two was over the sand wave field investigated by Blaine (2006). Nearshore beach seines were used to document nearshore presence of PSL in areas adjacent to the suitable benthic habitat.

We also sampled a well known inter-tidal and nearshore pelagic habitat that exists in northern Lopez Sound, near Decatur Island (Site 2, Fig. 6) where the largest (150 mm long) adult PSL have been captured in the past. In addition, we collected new MBES images of the sub-tidal area to determine the presence of promising sub-tidal habitat nearby.

Fish Analyses

All PSL captured in the grab sampler, trawl or seines were counted and bulk weighed. A sub-sample of up to 100 fish/habitat type were blotted dry, individually measured, weighed (± 0.01 gm), wrapped, labeled and frozen for laboratory analysis. In the laboratory each fish was partially thawed (this preserves tissue for maturity stage determination) and dissected. Sex was determined and gonads were examined for maturity stages (after Macer 1966; Nelson and Ross 1991) and included: immature, resting, developing, running ripe and spent stages. Sagittal otoliths were removed, mounted on slides and examined for annuli after Scott (1968) in order to determine age and growth. Age/length curves were constructed for each sex and length at maturity determined (see Appendix IV). All sediment samples were condensed and winnowed for PSL eggs, which were preserved in 70% alcohol following Moulton and Penttila (2000). Sediments were analyzed for eggs under a dissection microscope. Developmental stage was noted to estimate when spawning occurred.

Sampling in the inter-tidal and pelagic areas was done to determine presence and life stage of PSL in an attempt to correlate with presence and life stage of PSL in the sub-tidal areas. Measurements were made on the following: temperature, salinity, pH, turbidity, chlorophyll a, dissolved oxygen and depth down to ~8 m.

We did not propose to conduct genetic studies, however we did collect and preserve PSL tissue for eventual genetic analyses. The banking of these samples increased the sample size and distribution for future genetic studies without increasing the cost for the collection. The sand lance genetics work is being coordinated with Sharon Wildes and James Orr of NOAA/NMFS who are currently working on species level differences in sand lances of the North Pacific.

Egg Sampling/Analyses

Field survey protocols for the sampling of upper intertidal beaches for the spawn of intertidal-spawning marine forage fishes, the surf smelt, (*Hypomesus* spp.), and the PSL had been developed by the Washington Department of Fish and Wildlife (WDFW) in 1991 for use in a synoptic forage fish spawning habitat mapping survey of all fine-grained beaches in Puget Sound (Penttila 1995b, Moulton and Penttila 2001,(rev.) 2006). By these protocols, bulk samples of upper intertidal beach surface material were collected and processed in such a manner that forage fish eggs could be concentrated into preserved subsamples for microscopic inspection, with egg identification, enumeration and aging following. The protocols developed for intertidal beach sampling were presumed to be directly applicable to sub-tidal sediment samples produced by the planned Van-Veen grab sampling from sub-tidal bottomlands.

Sediment samples intended for sand lance egg inspections were obtained on-board the R/V *Centennial* during the course of its collection of Van-Veen grab samples of bottom sediments targeting the collection of the fish themselves from certain sub-tidal bed-forms. Typically, when the sediment-filled grab sampler was brought on-board and after a grain-size sub-samples had been collected from the undisturbed sediment sample, a sub-sample of several kilograms of sediment from the remaining catch volume was removed for egg-sample processing.

The sub-sample was wet-screened through a 2 mm mesh-size screen to remove large material. The material passing down through the 2 mm screen was collected in a 0.5mm screen, which presumably would retain any sand lance eggs in the subsample, a sand lance egg and its typical sand- grain coat being roughly 1 mm in diameter.

The material retained in the 0.5mm screen was placed in a plastic pan covered shallowly with water. The material was then “winnowed”, swirled and agitated in such a way as to allow the lightest fraction (=lowest density) of this material to concentrate on the surface of the deposit. A subsample of roughly 800 grams of the lightest material was then skimmed-off the surface few millimeters of the deposited into a wide-mouth jar, and labeled with the grab-sample number for the day and cruise number.

After disembarkation at the end of the cruise, each collected sample was preserved in Stockard’s Solution, a mix of 4% glacial acetic acid, 5% formaldehyde, and 6% glycerol in fresh water, for subsequent lab analysis. This solution has been the standard preservative used for forage fish spawn samples collected by WDFW since the early 1970s. It turns the embryos opaque white to ease in egg identification and aging of embryo developmental stages, while leaving the yolk sac a translucent color.

In preparation for lab inspections of the preserved material under magnification, the preserved samples were stirred and swirled in a manner to concentrate the lightest material on the surface of the deposit within the jar. This lightest material was then skimmed off into an oval lab dish. The sample was then re-stirred and re-skimmed additional times to accumulate about 100 grams of material in the lab dish.

A shallow layer of water was then added to the lab dish and tilted, swirled and agitated so as to concentrate the very lightest material on one feathered edge of the deposit (Moulton and Penttila, 2001, (rev.) 2006). This feathered- edge of lightest material was then inspected at 10X under a stereo-microscope. An egg observed was removed from the dish, identified, and aged into a series of embryo developmental stages. Any eggs that appeared a solid opaque white color, as an empty egg-shell without contents, or eggs that contained no discernable intact embryonic structures were listed as “dead”.

The above process was repeated at least twice for each dish of sample material before the sample would be considered “spawnless”. If eggs were observed to be present, after their removal, the dish-winnowing process was repeated until no additional eggs could be recovered. If only a single egg was found in the inspection of a first dish of material from a sample, a second additional dish of material would be collected from the jar of preserved material, and the process would be repeated.

At the completion of each NWSC/PSL project field trip, a “field report” was prepared, including a written summary of actions and observations, a field data sheet for beach substrate samples collected at Jackson Beach Park, and separate lab data sheets for the groups of samples collected at Jackson Beach Park or samples collected from subtidal bottoms aboard the RV Centennial (see Appendix V). These were routed to the project’s Principle Investigators for their information while the project was under way.

Geologic Setting

The San Juan Archipelago-Georgia Basin region is an active tectonic province whose physiography and geomorphology reflect both Mesozoic to Cenozoic convergent (subduction/accretion) plate tectonic processes and Pleistocene glaciation (glacial scouring/deposition). These processes have juxtaposed and deformed Jurassic-Cretaceous metamorphic rocks with Tertiary-Quaternary sedimentary rocks producing a complex of fjords, grooved and polished bedrock outcrops, and erratic boulders and moraines (Orr and Orr, 1996). Banks of till and glacial advance outwash deposits have also formed and contribute to the variety of relief within the region. Present day tidal action has fashioned much of the relic glacial-marine sediments into dynamic bedforms consisting of sand and gravel wave and dune fields (Fig. 14). Modern day sedimentary deposits (sand and mud banks) represent materials being supplied to the region by the Frazer River of British Columbia, Canada.

This tectonic province can be divided into two distinct zones based on bedrock types; a northern sedimentary bedrock zone and a southern metamorphic rock zone separated by the Haro Strait fault that cuts across northern Orcas Island and just north of San Juan Island. Both zones provide good hard bedrock exposures, however the sedimentary rock type is differentially eroded, thus forming ledges and overhangs while the metamorphic bedrock are highly fractured and faulted forming cracks, crevices, and blocky boulder aprons. The severity and variety of tectonic, geologic and physical processes active in the province are directly responsible for forming the large variety of potential marine benthic habitat types mapped in the region.

Results

The results of this study are far-reaching and multidisciplinary. Extensive sampling of the San Juan Channel sand wave field, our proto-typical PSL sub-tidal habitat type, on a regular basis through the summer, fall and winter seasons of 2010 and through the winter, fall and spring seasons of 2011 allowed for documentation of PSL occupancy and relative abundances. Comparative evaluation of the results from the tank experiment study and the in situ sampling confirmed our assumption that PSL prefer grain sizes of 0.5-1.0 mm (medium- to coarse-grain sand) to any other grain sizes and that dynamic bedforms can act as preferred habitats for the fish. All life stages of PSL after the larval stage were represented in the proto-typical habitat and one egg was recovered suggesting that recruitment may also occur in PSL sub-tidal habitats.

Study Areas

In addition to the proto-typical PSL habitat type in the San Juan Channel we selected five other areas to investigate in regard to potential PSL sub-tidal habitat types. These areas consist of the dynamic bedforms found offshore of Iceberg Point, southern Lopez Island, offshore, west, of Sucia Island and offshore Point Caution, northern San Juan Channel, Salmon Bank offshore southern San Juan Island and northern Lopez Sound offshore Decatur Island (Fig. 10).

GIS Analyses

From the larger San Juan Archipelago bathymetric dataset we selected 38 independent bedforms to analyze for wave amplitude (height) and length (periodicity). From this total we measured 36 sediment wave fields for length, width and area and 26 of these fields were analyzed for aspect (Table 2; Appendix VI). We drew bathymetric profiles across the four dynamic bedforms we investigated in order to obtain metrics for developing our predictive model (Figs. 15, 16, 17, and 18). We found we consistently obtained the highest amount of fish in the San Juan Channel sand wave field (see Figs. 1, 10, 15 and 19).

Sand wave fields were of highly variable sizes (Table 2, Appendix VI). Area ranged from 0.07 – 14.24 km^2 (mean ± SD), and averaged 2.74 ± 3.77 km^2. Half (n = 18) of the sand wave fields were < 1 km^2 in area, whereas three fields exceeded 10 km^2. Correspondingly, maximum length (0.51-8.61 km) and maximum width (0.12-4.98 km) also varied considerably. Shape also differed substantially among sand wave fields (Fig. 11) with the ratio of maximum length to maximum width ranging from 1.23 to 21.31. Boundary 2 and Boundary 8 sand wave fields were most similar to SJC 1 based on area, length, and width measurements (Table 2, Appendix VI).

Aspect of sand wave fields was also highly variable (Table 2). Aspect results encompassed nearly the full range of available values (Table 2), indicating that no single, uniform process was responsible for the creation of the delineated sand wave fields and that local physical conditions probably differed considerably among them. Mean aspect ranged from 155.0 ± 16.4° (Boundary, n = 9) to 184.1 ± 73.8° (JDF, n = 8), indicating that NW/N - SE/S was the general direction of orientation among regions. However, only four sand wave fields exhibited aspects within this range of values, further indicating the highly variable nature of sand wave fields in the San Juan Archipelago. The following sand wave fields have aspects within 5 of that determined for SJC 1: Rosario 2, JDF 9, and Boundary 2 (see Appendix VI).

Mean wavelength and amplitude at SJC 1 were 33.2 ± 13.9 m and 29.6 ± 13.7 m, respectively. These characteristics differed from those of most sand wave fields, which were typically of much lower amplitude and shorter wave length or else contained a few, high-amplitude waves of high wavelength. Six sand wave fields, however, were of similar characteristics: JDF 9, Rosario 1, Rosario 2, Boundary 1, Boundary 2, and Boundary 8 (see Appendix VI). Of these, all but Boundary 1 and Rosario 1 (which were considerably larger) were also of similar dimensions. These sand wave fields therefore represent promising habitats for PSL and warrant further investigation.

Depth also was considered in determining metrics and for those field of those most promising fields identified above Boundary 8 at 32-39 m deep, JDF 9 at 77-82 m deep and Rosario 2 at 43-52 m deep fit within the depth range of our proto-typical PSL habitat type (see Appendix VI). Fish (PSL) were consistently found in the San Juan Channel sand wave field at a depth of between 70 and 80 m and the fish are know to exist in waters shallower than 80 m.

Tank Experiment

Subadult PSL were capable of penetrating all grain sizes ranging from silt to pebble. However, among the grain sizes that were tested, this species exhibited significant selectivity for coarse sand (Table 3). No significant difference was detected in selectivity for medium sand or very coarse sand, although the majority of fishes burrowed into medium sand (71.9%, N = 32).

No significant difference was detected in selectivity for uncompacted or compacted coarse sand (F = 0.052, P = 0.824), although compacted sediment of all grain sizes required significantly more force to penetrate (Table 4). In comparisons of burrowing force among grain sizes, larger grain sizes generally required more force to penetrate regardless of compaction level (Table 5).

Based on these preliminary results, it appears that medium sand is the preferred grain size for PSL, even though smaller grain sizes require less force to penetrate. In addition, although compacted sediment is more difficult to penetrate, a lack of selectivity between uncompacted and compacted coarse sand indicates that PSL prefer this grain size regardless of compaction level.

Dynamics and Sediment Stability of PSL Habitat

Our intent was to establish a catalog of dynamic bedforms and sand wave field types that may be potential PSL habitats (see Appendix VI). Therefore, we investigated the dynamics and stability of the sand-wave field in central San Juan Channel. Our major objectives were to compare sediment types and dynamic conditions in a known PSL sub-tidal deep-water benthic habitat (San Juan Channel sand wave field) with other nearby areas where PSL do not reside. The basic questions concern: 1) the stability of the sand-wave field (and therefore the PSL habitat) in response to variations in current strength as a function of atmospheric and tidal conditions, 2) the nature and variability of the transition across the sand-wave field boundaries in terms of sediment type and current velocity, and 3) the impact of changes in sediment supply (size and amount) on the viability of the habitat.

The first objective of this part of the project was to characterize the grain-size distributions of sediment within and outside the identified sand-lance habitats and compare with sediment in other habitats globally. The second objective was to evaluate the dynamics and stability of the sand-wave fields, where virtually all of the PSL have been recovered for this project.

We have documented that PSL bury themselves to depths of a few cm in the seabed within the sand-wave fields we investigated. The fish are rare to absent in samples taken outside the fields. According to studies conducted elsewhere PSL prefer areas where the sediment has a high proportion of sand-size grains (~2 -0.062 mm) and a near absence of fines (silt and clay sizes, < 0.062 mm), because this type of sediment (clean sand) is easily penetrated and has an adequate supply of oxygen (Wright et al., 2000; Holland et al., 2005; Pinto et al., 1984; Haynes et al., 2007).

Grain Size Measurements

Grain-size measurements of our samples are presented in Table 6 and Figures 20, 21, 22, 23, 24, 25 and 16 (Appendix II). Using the San Juan Channel sand wave field as the proto-type geomorphology and sediment type for the preferred PSL sub-tidal habitat we see that from the results of grain size analyses all other field we sampled were composed of different grain sizes than the proto-type habitat, which is well-sorted, little skewness and a mean near 1 phi (Figure 21). Sucia sand wave field contains grain sizes coarser than the San Juan field with a mean of approximately 0 phi and well-sorted (Figure 22). Median grain size at Iceberg Point is finer grain than at the San Juan field at 2 phi, well-sorted and little skewness (Figure 23). At Salmon Bank median grain size is 2 phi, well sorted (Figure 24) while off Decatur Island the sampled area there is very fine-grain with sediment sizes ranging from 2 to >6 phi (Figure 25). Although the sampling of the sediment wave field off Point Caution was not concentrated within the sediment wave field, we did obtain sizes that in the field that are very coarse-grain at ~-2 phi surrounded by sediment with an approximate mean of 2 phi, fine-grained (Figure 26).

Sand-Waves Stability

Sand-wave fields are dynamic features, affected by strong, temporally and spatially variable currents. In order to maintain the sand-wave deposit, a plentiful presence of sand is necessary; finer sediment might transit through the area and coarser sediment might be present as a lag. Several sand-wave fields have been mapped near the San Juan Islands (Barrie et al., 2009). The San Juan Channel sand wave field (Fig. 1) as documented by Blaine (2006) is a prolific sand-lance habitat. The areal extent of this sand-wave field is delimited by a distinct boundary where the sand waves are in sharp contact with relatively featureless sea floor. Such abrupt transitions have been reported in other nearby sand-wave fields within the Salish Sea (Barrie et al., 2009).

The San Juan Channel field contains sand waves with wavelengths up to ~100 meters and ~3-5 m in height within the central area. Smaller sand waves are superimposed upon the large ones. Furthermore, two major crest orientations can be detected (Fig. 27). We recorded video transects across portions of the field and then mosaicked the video frames along the track lines (Fig. 28, courtesy of Peter Dartnell, USGS), for future high-resolution analysis of sand-wave geometry and spacing. We used a unique shipboard digital underwater camera developed by H. Chezar and D. Rubin, USGS Pacific Science Center, Santa Cruz, CA, (referred to as the “eyeball” camera) (Fig. 29, courtesy of Hank Chezar, USGS) to take close-up high-resolution photographs of the sea floor that subsequently were used to calculate median grain size (Rubin, et al, 2007). The advantage of the eyeball camera is that extensive coverage can be obtained across the sea floor (~3 images/minute) to rapidly obtain numerous estimates of sediment grain size and its areal variation. The locations of two photographs, one in a sand-wave trough and the other on the adjacent crest, are shown in Figure 30, and the actual photographs in Figure 31. Notice that the grain size at the crest of a wave is significantly finer (median ~0.5 mm) than that in the trough (~2.0 mm), a typical occurrence. Furthermore, biogenic shell material dominates in the trough whereas siliclastic grains dominate on the crest. We used these grain sizes to evaluate the stability of this sand wave for an indication of the stability of the entire field.

A record from the nearby tidal station (South San Juan Channel Entrance, NOAA Tide Tables) is shown in Figure 32 at about the time the eyeball photos were taken. The maximum tidal current velocities for each ebb and flood tide are indicated. These velocities, along with grain size and measured water depth at the site, can be used for a preliminary evaluation of the stability of the sand-wave field. This is done using “bed-phase diagrams”, which show the stable seabed configuration over a range of flow velocity, water depth, and grain size (Southard, 1971). Two such diagrams, derived from observations in flumes (small water depths) and in San Francisco Bay (greater water depths) (Rubin and McCulloch, 1979, 1980) where conditions are similar to those in the San Juan Channel, are shown in Figure 33. A plot of the grain sizes derived from Figure 31, the maximum current velocity from Figure 32, and water depth obtained from a shipboard depth sounder show that conditions are near the upper level of stability for the observed sand waves.

An implication of these plots is that a modest increase in current velocity would move the sea floor into the upper flat-bed regime, where sediment-transport rates increase rapidly, possibly leading to removal of the sand and destruction of the habitat. This result suggests that measurements of currents at the sand wave field are needed to make more accurate predictions of habitat stability. These measurements can be made on different stages of the tides (spring-neap; flood-ebb) to improve our understanding of sand wave stability.

A rough estimate of sand wave migration can be made using available information. Sediment transport rate (Qb) is proportional to a power of the bed shear velocity (u*b). There are several equations available that relate these two dynamic parameters, and we have chosen a formulation (Van Rijn, 1993) that is commonly applied to sand waves in a tidally dominated environment. This formulation is used in the Delft 3-D Sediment transport Model. The results are shown in Figure 34. For the two median sand sizes at the crest (0.5 mm) and in the trough (2.0 mm), Qb is plotted against u*b. Based on the maximum tidal currents taken from the NOAA tables, we estimated u*b to be about 0.06 m/s. For this bed shear velocity, sand waves with 3 m height will migrate at about 0.8 m/hr for the smaller sediment size.

This result must be considered only a rough estimate because of the lack of measured current data. However, it does indicate that the sand waves are likely active during maximum flows, and more study is needed to obtain more accurate predictions of their mobility.

It is important to note that the above analysis only addresses half of the habitat-stability problem. Smaller current velocities also would pose a risk to the health of the habitat. In this case, the sand waves would transition to ripples then to no sediment movement, which would lead to mud deposition on the sea floor. This condition makes the seabed uninhabitable by sand lance. This issue will be addressed in future studies.

Biology of PSL

This part of the results focuses on the biological metrics that we determined were necessary to collect in order to understand the population of PSL that inhabits deep-water sand wave fields (Table 7).

Beach Seines and Tow Nets

To address the ephemeral nature of the pelagic life history stage of PSL our strategy was to sample one site (Jackson's Beach) with tow-net and beach seines for pelagic habitat and with grab samplers for benthic habitat across two transects. These samples were taken during the cruise of July 13, 2010. Two transects were sampled with a tow net. The tow net samples surface waters down to 4.57 m (15 feet) with a small mesh net that is towed between two boats. Both transect 1 (perpendicular to Jackson's Beach; Figure 13) and transect 2, the surface waters over the sand wave field, did not catch sand lance.

Near shore beach seining was used to document near shore presence of PSL in areas adjacent to suitable benthic habitat. A large beach seine, as used by Skagit River System Cooperative, that is 36.58 m (120 feet) long and 3.66 m (12 feet) deep with uniform small mesh was set at two places along Jackson's Beach. No fish were caught in tow #1 and 84 PSL were caught in tow #2. The size range of the captured fish was 61-138 mm FL (Fig. 35), a similar size range as was caught in our benthic grab samples later in the year (Fig. 36). This seems to represent the size range of PSL in San Juan County. To complete the comparison of pelagic and benthic sand lance we proceeded to use the Van Veen grab sampler on the San Juan Channel sand wave field. Out of 15 grabs we only caught two fish in one of the grabs (Fig. 37).

Biological Metrics of Sand Wave Fields

A total of 1,060 PSL were collected with a Van Veen Grab Sampler during 7 cruises conducted in San Juan County for this investigation. A sample of 1,027 fish was measured for length and weight. A sub-sample of fish was dissected to gather biological metrics of age (443 fish), sex and maturity stage (389 fish each). The goal was to dissect up to 10 fish per grab sample, as the grabs obtained up to 76 fish in a single sample. A few fish were caught in the jaws of the Van Veen grab and not whole, so were not counted. These fish totaled 33 of the 1,060 fish, or 3%. This number could be used as the expected mortality for this type of sampling gear.

Fish Numbers

Comparison of the number PSL caught from the San Juan Channel sand wave field can be made by standardizing the sampling effort for each cruise. This is accomplished by adding the total number of fish captured by the Van Veen grab and then dividing this by the total number of grab samples taken on a particular sampling effort. This catch per unit effort (CPUE) is a technique for standardizing sampling data. In Figure 38 the variation in seasonal abundance of sand lance that are buried during daylight hours is apparent. From October through February the densities are highest, peaking in November. Densities are dramatically lower from April through July.

Density

Blaine (2006) estimated the population of PSL on the San Juan Channel deep-water sand wave field to range between 40-60 million fish. In this study we have a seasonal time series to assess population density and to determine how the sand wave fields are being used by PSL. To obtain population estimates we take the CPUE of each cruise (Fig. 38) and multiply by the volume the fish could occupy in the sand wave field. Area of the field is 500,000 m^2 and we assume the fish can penetrate the sediment for one body length or about 150 mm maximum. To compare the numbers with those obtained by Blaine (2006) we also calculated the population only using the area of the field ($P = 500000/0.25 \times CPUE$). Area is used to estimate population with the understanding that the fish are buried in the sediment and not lying on top of the waves. It is unknown if the fish orient themselves in a single layer or bury and rest in overlapping layers. We feel that these estimates will provide a minimum population density.

In Table 8 we can see the seasonal variability of PSL density in a deep-water sand wave field. Population densities during winter are much higher when more of the population is quiescent. A maximum estimate of population densities in the sand-wave field was 72 million fish in November 2010.

Length-Weight of PSL

Length and weight of fish were measured for 1,027 individuals. All intact fish were measured. The curve in Figure 36 is a power curve but not the typical one of $L=W^3$ because these fish are long and thin. The smallest fish caught was 58 mm Fork length indicating that this species begins to settle out of the planktonic stage around that size and assumes the adult habitat of burying in sediment. It is worth noting that the entire size range of PSL known to reside in sub-tidal benthic habitats in San Juan County waters were sampled using the VanVeen Grab method.

Age-Length Relationships

Age0 or young-of-the-year fish settle into the sub-tidal sand wave fields when they are close to 60 mm FL. A few smaller, pigmented fish were collected in the wave fields, but nothing larval or post-larval (unpigmented). Poikilotherm is dependent on food availability and ambient temperature, thus individual fish grow at varying rates and overlap in length at age curves is inherent. The same is true for PSL (Fig. 37) where the average length of an age0 fish is 70 mm FL, an age1 fish is 78 mm FL; an age2 fish is 98 mm FL and an age3 fish is 120 mm FL. Birthdates of PSL are taken to be January 1. The first annulus is taken to be the end of the first “summer” mark since these fish spawn in winter. The size ranges at each age are also shown in Figure 37 and illustrate the amount of overlap. For example, an 80 mm FL fish could be age 0, 1 or 2. We note that while it is useful to think a particular size of fish is representative of a given age, it is a fuzzy number and it is wise to remember that any conclusion on age at maturity, or other biological relationship, is also fuzzy.

Sex and Maturity

The ratio of males to females approached 1:1 so the population seems to be fairly equally distributed between the sexes (Fig. 39). Of the 389 fish that were studied three stages of sexual maturity were determined: immature, resting and ripe with eggs or sperm. The majority of fish were immature (60% females; 78% males), the rest of the fish were mature with 3 females (2%) and 13 (8%) males in ripe condition, ready to spawn (Figs. 40 and 41). It is necessary to note that since Pacific sand lance are broadcast spawners perhaps more males are necessary to ensure fertilization of eggs. Also, since only 5% of the total fish sampled were in a ripe stage perhaps the concentration of mature fish are elsewhere, in areas other than those that we sampled.

Age at Maturity

The Pacific sand lance that populated the sand wave fields, primarily the San Juan Channel sand wave field, were dominated by immature males and female fish that were Age0 and Age1. A few fish were still immature at age 2, but the transition from immature to mature occurs at age1 to age2 (Fig. 42). By Age 3 both males and females were sexually mature. Sexual maturity can be seen in age1 fish when fish are at least 79 mm FL (Fig. 23) as documented by ripe gonads (Table 9).

Spawning

A few PSL were found in spawning condition, with either eggs or sperm filling their gonads. Fifteen, or 8% of the males and 2% of the females were in the “ripe” stage (Table 9, Figs. 40 and 41). From our samples males appear mature at Age1 while females were mature at Age2. Males were ripe as small as 79 mm FL while females were at least 93 mm FL when ripe. All ripe fish were sampled in January (during cruise C1-11) and February (during cruise C2-11) of 2011. We observed that males are maturing younger and smaller than females and more males were ripe than females. Spawning time coincides with spawning season for beach spawning PSL of winter months (see section on eggs below).

Physical Conditions

Physical parameters of temperature, salinity, Chlorophyll a (fluorescence), dissolved oxygen and density were collected with the shipboard CTD (Table 10). Sampling protocol followed standards employed aboard the R/V *Centennial*. The CTD was lowered for a count of 10 to saturate the sensors while the readings were monitored on the shipboard computer. When the readings were stable the CTD was raised to just below the surface and then lowered at a constant rate to within 1 meter of the bottom and then immediately raised to the surface. Each set took approximately 10 minutes. Conditions on the bottom, next to the sand wave fields are shown in Table 10. Temperatures from June-October were around 10°C and were around 7.5°C from February through April. Lower chlorophyll a values also occurred in February. Dissolved Oxygen ranged between 5.6-8.4 mg/L with low levels during October and higher levels during April**.**

Light

Light levels at depths where PSL were buried in the San Juan Channel sub-tidal sand wave field were collected during daytime (2052 hr) and nighttime (2305 hr). Light levels were measured to determine if light might be a cue to the fish to rise from within the sediment to the water column. It appears that during daylight and nighttime hours (Figs. 43 and 44) the light level at 60-80 meters depth is not detectable by the light meter. Light levels for daylight hours were around 200 lumin/ft^2 at the surface and at night around 18 lumins/ft^2, a higher level than expected probably the cause of the deck lights being on. Sunset was at 2108 hr. A new moon occurred on July 11, 2010, two days prior to our July 13 cruise when we measure light. It appears that because of the absence of light at depth PSL do not receive light cues when buried in the San Juan Channel sand wave field. This conclusion supports Blaine's (2006) findings that similar numbers of sand lance were sampled during daytime and nighttime surveys.

Canadian Fish

VanVeen grab samples were obtained from 6 sites in Canada: Sandy Isle Marine Park (Jan. 2010), Sidney Channel (Feb. 2010), Cordova Channel (Feb. 2010), Deep Bay (Mar. 2010), Balcome Inlet (Jul. 2010), and Scott Island (Aug. 2010). Twenty-five PSL were collected and frozen by Dr. Cliff Robinson and processed by Dr. Wyllie Echeverria. Sample sizes were small compared with the San Juan Archipelago samples and they were dominated by mature fish (with only 4 immature fish of 78-87 mm FL) in contrast to the San Juan samples being dominated by immature, young fish. Gonads of PSL sampled January-March 2010 were ripening with one 3-year old male in Ripe condition in February (Sidney Channel). Different areas appear to have different size ranges of the PSL population (Fig. 45). Balcome Inlet (purple in Fig. 45) had smaller, immature fish while Sidney Channel (red in Fig. 45) had larger, mature fish. It must be noted here that these data from Canadian sand wave fields and sandy bottom areas are too small to make any inferences as far as maturity is concern, but it is interesting that all Canadian fish were in the larger size range than we sampled in the San Juan Archipelago.

Eggs

The search for sub-tidal sand lance eggs by the NWSC's deep-water sand lance project involved participation in four Van Veen grab-sampling cruises on the RV *Centennial*: November 17, 2010 (17 samples), January 22, 2011 (15), February 16, 2011 (15), and April 15, 2011 (12), for a total of 59 sub-tidal substrate samples (see Appendix VII). The documented sand lance spawning site at Jackson Beach Park was also visited on the latter three survey days for a total of 8 substrate samples, from which no sand lances were recovered.

During the course of the sampling of sub-tidal substrates, it is estimated that about 236 kg of original Van Veen sample material was screened and processed to generate the 59 "winnowed light fraction" preserved lab sub-samples, which in turn comprised about 47 kg of material. Further processing steps in the lab generated a total about 5 kg of the lowest-density material of inspection under magnification.

No evidence of subtidal sand lance spawning was found in any of the grab-sample sediments collected from November through February, a period of the year when sand lance eggs were expected to be encountered at their intertidal spawning beaches in the Puget Sound Basin.

The only evidence of sub-tidal sand lance spawning discovered during this project was the recovery of a single sand lance egg from Van Veen grab-site #5 on the San Juan Channel sand wave field, SE of Turn Point (our proto-typical PSL habitat) on April 15, 2011. The egg resembled outwardly the many tens of thousands of such eggs recovered from Puget Sound intertidal-beach samples dating back to late 1989, in size, shape and the presence of adherent sand grains scattered on the egg-shell. The egg was "late-eyed" in embryo developmental stage (photo to be supplied at a later date). Estimating a one-month incubation period in keeping with that of intertidal sand lance eggs, the egg is estimated to have been about 3 weeks old, and was thus deposited in late March. It should be noted that the latest sand lance eggs ever found on an intertidal beach in Puget Sound were in similar late embryo developmental stages, but were found in mid-March and thus had been deposited in late February (Penttila, WDFW, unpub. data).
The egg had considerable time to disperse from its original deposition site, but it is not considered un-likely that it dispersed off of any intertidal beach in the vicinity. It is conceivable that subtidal sand lance eggs, not being exposed to warmer ambient terrestrial temperatures during part of every intertidal tide cycle, would have a longer incubation period, and thus the egg is somewhat older.

Little of a definitive nature can be deduced from the discovery of a single sub-tidal sand lance egg during the course of this study. On one side of the argument for sub-tidal sand lance spawning, it is possible that the focus of the projects' Van Veen sampling on current-swept sand wave fields missed functional spawning sites elsewhere in a different sub-tidal habitat context. On the other hand, the observation of only a single sub-tidal sand lance egg in all the volume of "likely-looking", fine-grained material searched, at a time when it was well-populated by sand lances, including a small proportion judged to be ripe adults, during a likely spawning period, may be a true reflection of the relative intensity of sub-tidal spawning as compared to intertidal sand lance spawn deposition.
If sub-tidal sand lance spawning activity resembles that known to occur on intertidal beaches, initial spawn deposits may be quite limited in areal extent. The sand lances' "preferred" intertidal spawning substrate is a fine- to medium-sized, silt-free sand with the bulk of the material being in the range of 0.2-0.4 mm in diameter (Penttila 2007). Material of this grain-size composition might be of limited areal occurrence in the sub-tidal zone. It is presently thought that very silty sites with anoxic conditions near the surface, comprising perhaps the vast majority of total bottomland acreage in Puget Sound, would be avoided by burrowing or spawning sand lances.

It was consistently noted while processing Van Veen grab-samples from the San Juan Channel wave field that the material was significantly coarser than typical intertidal sand lance spawning substrate. It may be that sub-tidal sand lance spawning activity would only occur on the finest-grained portions of the sand wave fields, which might comprise so small a proportion of the total target area that any "blind" grab-sampling through 80 meters of water would be unlikely to encounter such sites or spawn deposits in a fresh condition.

Conclusions

Our tentative conclusions are that PSL prefer to burrow into medium- to coarse-grain (~0.5-1 mm) size sand in dynamic bedforms that have a wave amplitude of 3-5 m and wavelength of ~100 m, the seafloor conditions found at our proto-typical habitat type in San Juan Channel where we found the highest concentrations of PSL during our investigation (Fig. 19). Mature fish found in the San Juan Channel sand wave field were primarily caught in the northern and southern part of the bedform where sediment is smaller in size (Fig. 46). From our sampling exercise we found that more fish burrow into the sediment during winter months than during summer months (Figs. 47 and 48). In contrast few fish were collected from the Sucia field (Fig. 49), Iceberg Point field (Fig. 50) and Salmon Bank (Fig. 51).

Although our conclusions are preliminary at this time, we have prepared a predictive promising potential PSL sub-tidal habitat model that would have a geomorphology similar to the San Juan Channel sand wave field (Fig. 52). Metrics for this predictive model include grain size (0.5-1.0 mm, ~1 phi), depth (30-80 m), wave amplitude (3-5 m), wavelength (50-100 m), and current strengths of ~0.06 m/sec. However, smaller concentrations of fish occur at different types of fields or sand flats and further study needs to be undertaken to place limits and threshold conditions for the habitat attraction for these smaller concentrations of fish within the Salish Sea.

New MBES bathymetric and backscatter data collected for this investigation at Salmon Bank and offshore of Decatur Island, in areas where PSL have been reported to be presence on beaches and in the nearshore areas, show that no dynamic bedforms exist in these areas. Also, sampling of the seafloor near Jackson’s Beach, where prolific concentrations of PSL are often seined from the beach, revealed mud and no presence of sand lance. Consequently we tentatively conclude that PSL can travel a fair distance from their egg laying sites to sub-tidal habitats, however this relationship needs further investigation.

We concluded that any program established for mentoring the population condition of PSL should occur during winter months when the fish are buried and the sampling can be accomplished with a day cruise. We sampled through one year and to determine the annual variability of PSL within the Salish Sea multiyear sampling needs to be undertaken. Temperatures of the waters in the San Juan Archipelago are lowest during winter when the highest number of fish were found in our investigation and may be a cue for fish to enter a quiescent stage and remain in the sediment for much of the winter months. Dissolved oxygen is another important variable, as fish buried in the sediment must continue to absorb oxygen across their gill membranes. Levels just above the sediment wave field are at an adequate level (Palsson et al., 2006)

Our light measurements indicate that no light exists at the depth where PSL were found in the San Juan Channel field and therefore, we surmise that the PSL move out of the sediment into the pelagic environment irrespective of light conditions. There are probably other cues that trigger their exit from the sediment.

All life stages of PSL following the larval stage were found in the sediments of the San Juan Channel sand wave field indicating that such a sub-tidal habitat hosts multiple life stages including egg laying and therefore could also could act as recruitment habitats.

Stability of the dynamic bedforms that may be promising sub-tidal habitats in the Salish Sea appear to be near the threshold of stability. Any changes in current strength could upset this stability and such change could come about from continued tectonic uplift or eustatic rebound (possibly leading to increase in current strength) or sea level rise (possibly leading to decrease in current strength). More physical oceanographic measurements need to be made in order to better understand this process.

This investigation has yielded an extensive dataset that will take time to digest. Our reported results and conclusions here are tentative and based on only the most cursory examination. We placed a great deal of emphasis on the collection of data and less so on interpretation because we understood we had only one shot at collecting data. We will continue to evaluate the dataset and intend to publish several papers on our results in peer-reviewed journals. This report in all likelihood will be revised and submitted to the funding agencies as our continued interpretations take place. However, this investigation represents the most comprehensive study of PSL sub-tidal habitat to date, not to suggest that followup work should not be undertaken.

Recommendations

Based on our metrics examination of the following dynamic bedforms are recommended for future: Boundary 2, 3, 4, 6, and 9, Haro 4, 8, 10, and 12, JDF 3, 4, 9 and Rosario 2. The high priority fields should be Boundary 2, Haro 8, JDF 9 and Rosario 2 (see Appendix VI).

Continued monitoring of the San Juan Channel, Sucia and Iceberg Point sediment wave fields for presence/absence of PSL through several annual cycles is recommended for understanding the annual variability of the fish.

It is recommended that the search for sub-tidal sand lance spawning habitat based on our constructed tentative predictive model continue, perhaps aided by both wide-scale bottom photography, to look for fresh sand lance “spawn-pit” disturbances, and extreme close-up “Eye-ball Camera” photography, which may be able to detect individual sand lance eggs in-situ under certain conditions.

Additional MBES multibeam surveys and sediment/fish sampling is recommended to take place in other areas of the Salish Sea that are data poor. Further evaluation of existing MBES data and new data slated to be collected is recommended in order to better refine the predictive model and geographic locations of promising potential PSL sub-tidal habitats.

Relationship of sub-tidal habitats to PSL beach recruitment areas should be investigated. In addition, contribution of sediment, such as from feeder banks, that is needed to keep sub-tidal PSL habitats healthy need to be studied along with the physical oceanographic dynamics that are necessary for the structuring and oxygenation of sediment wave fields.

Other recommendations will be forthcoming as we continue to reduce and interpret the dataset.

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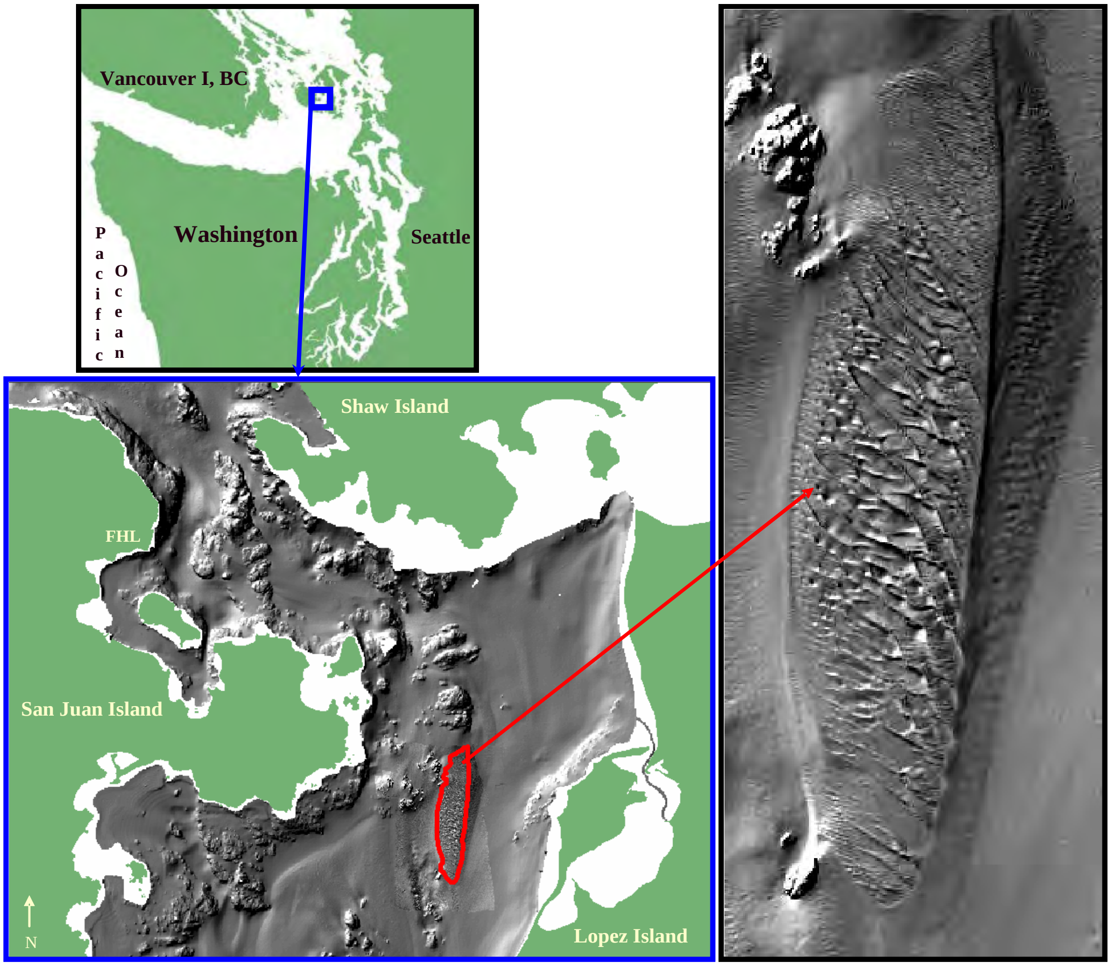


Figure 1. Sand wave field in San Juan Channel of the San Juan Archipelago where considerable amount of PSL were collected in the autumn of 2006. Simrad EM 3001 bathymetric image on left shows the high-resolution details of this dynamic bedform, after Blain (2006).

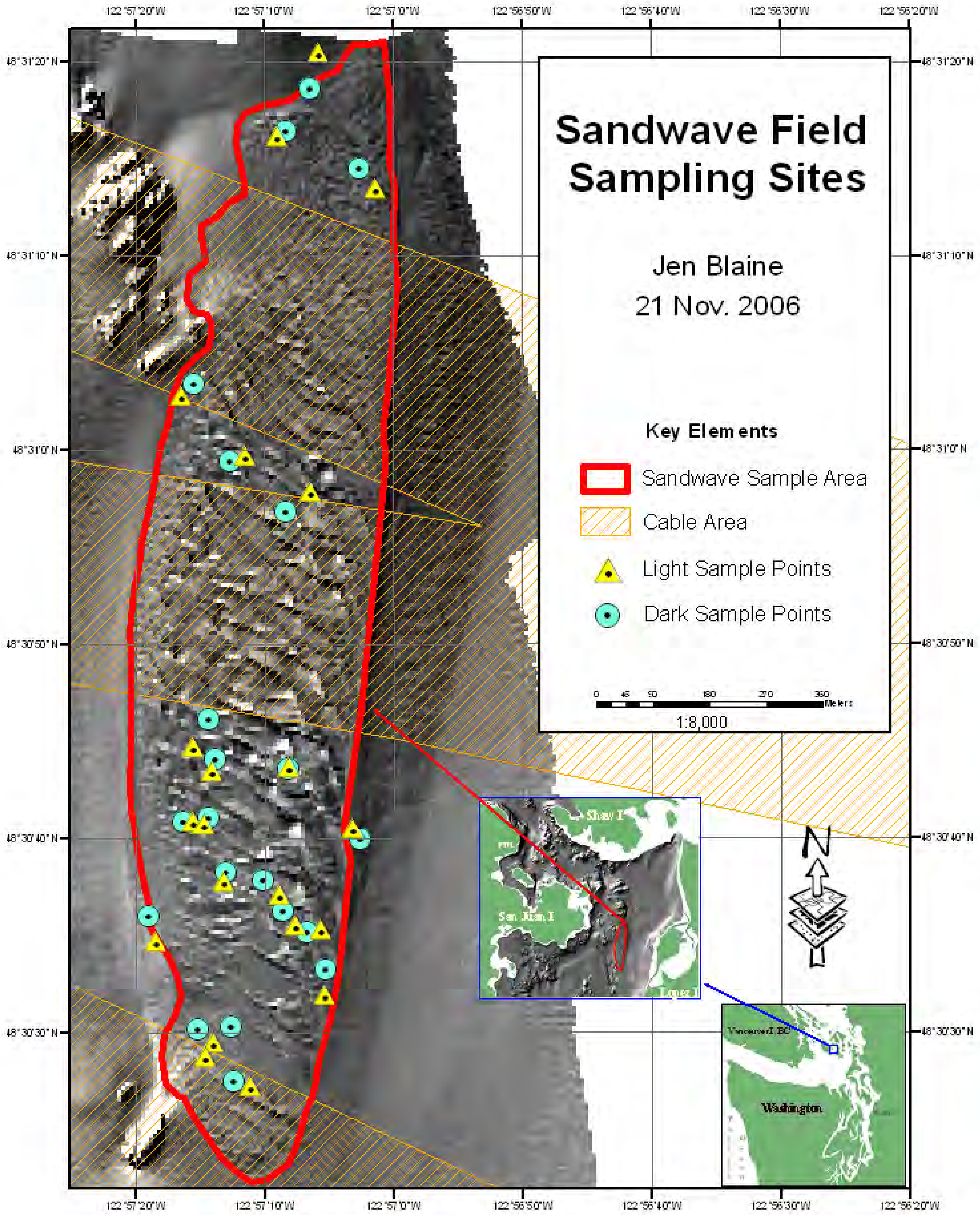
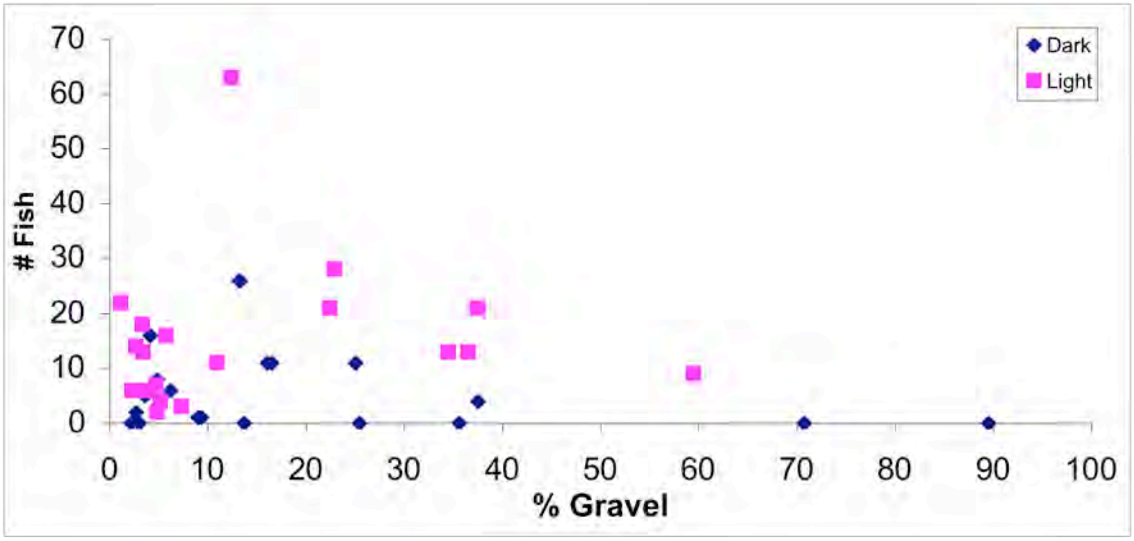


Figure 2. Sand wave field sampled from central San Juan Channel, November 21, 2006 (Blaine 2006). Sampling was done during both daylight and nighttime periods to investigate diurnal burial behavior.

Figure 3. A 150 mmFL sand lance captured with a beach seine in April 2009 and displayed on a bed of sediment dredged up from the sand wave field in central San Juan Channel in October 2007 that was studied by Blain (2006). Sediment size is ~1 mm and is a mix of sand and shell hash.

A



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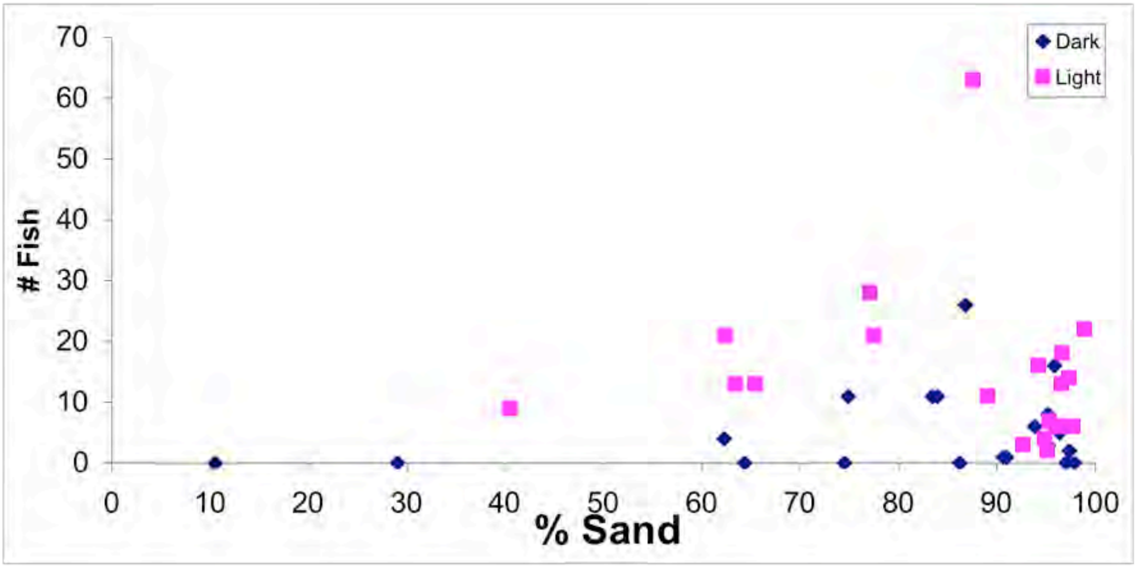
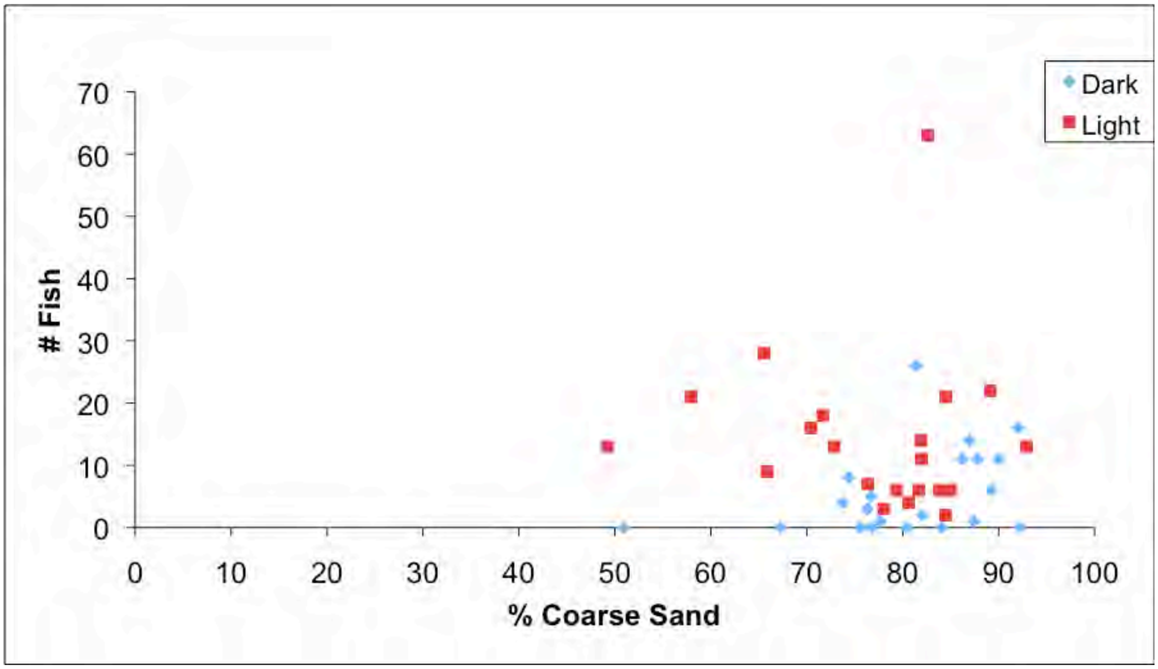


Figure 4. Grain size analyses of sediment collected in central San Juan Channel sand wave field showing concentration of sand lance in relationship to grain size. Dark and light samples represent samples collected at night and during the day. Graph A is for gravel and graph B is for sand; x-axis=number of fish collected and y-axis=percent of gravel or sand; after Blain (2006).

A



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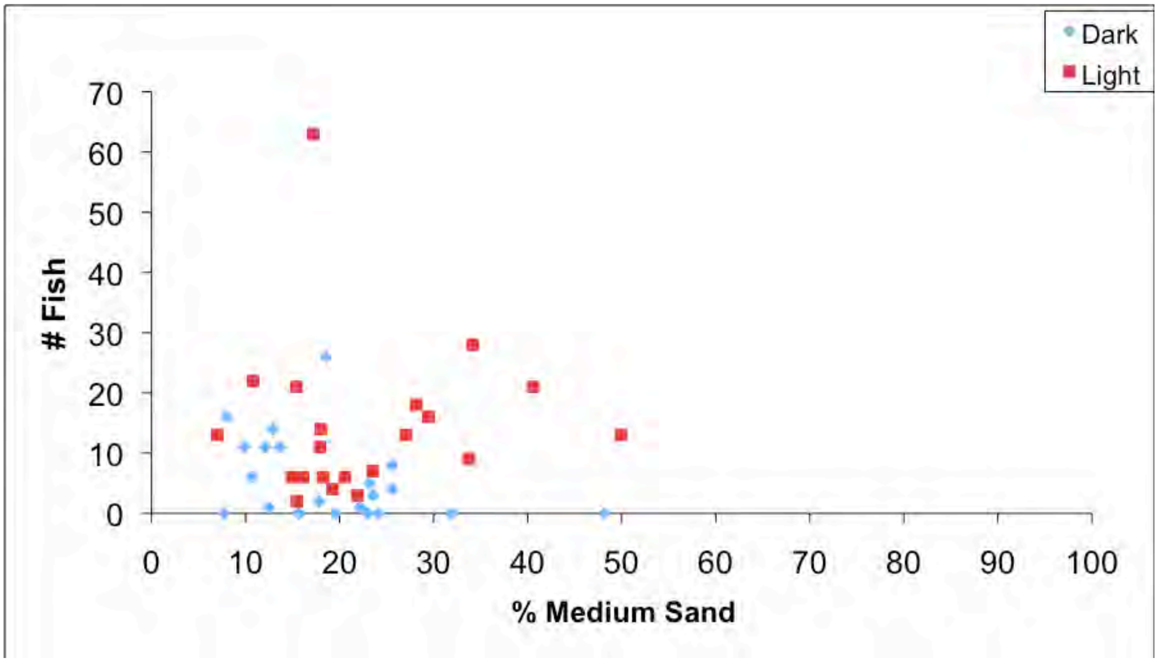


Figure 5. Grain size analyses of sediment samples collected in the central San Juan Channel sand wave field showing comparison of the number of fish in each sample with percent of coarse grain sand (A) and percent medium grain sand (B) compositions of each sample; x-axis=number of fish collected and y-axis=percent coarse- or medium-size sand; after Blain (2006).

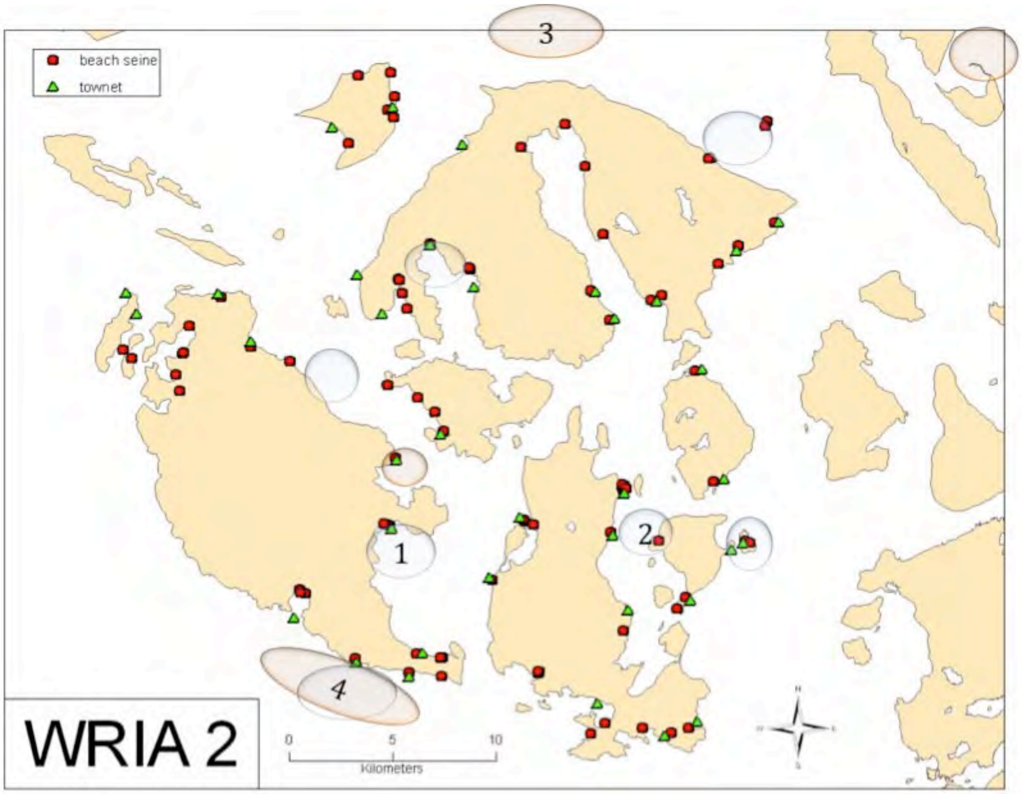


Figure 6. San Juan Archipelago beach seine (red dots) and tow net (green triangles) sampling sites from March-Oct during 2008 and 2009. Blue circles indicate large catches of sand lance (100-30,000/tow or seine), brown circles represent sampling logs and published reports showing large catches near Sucia Island and in Bellingham Bay (Borton and Miller 1982). Blue circles indicate large catches of sand lance (100-30,000/tow or seine), brown circles represent sampling logs and published reports showing large catches near Sucia Island and in Bellingham Bay (Borton and Miller 1982). Four sites are noted for future work (two to be undertaken during this project): Site 1 includes the sand wave field investigated by Blaine (2006), Site 2 is where larger PSL were captured in beach seines but MBES data needs to be collected (scheduled for fall 2010), Site 3 is where historic catches of PSL were reported and a sand wave field is identified from the MBES data and Site 4 where large catches of PSL were found in 2009 but no MBES data currently exists.

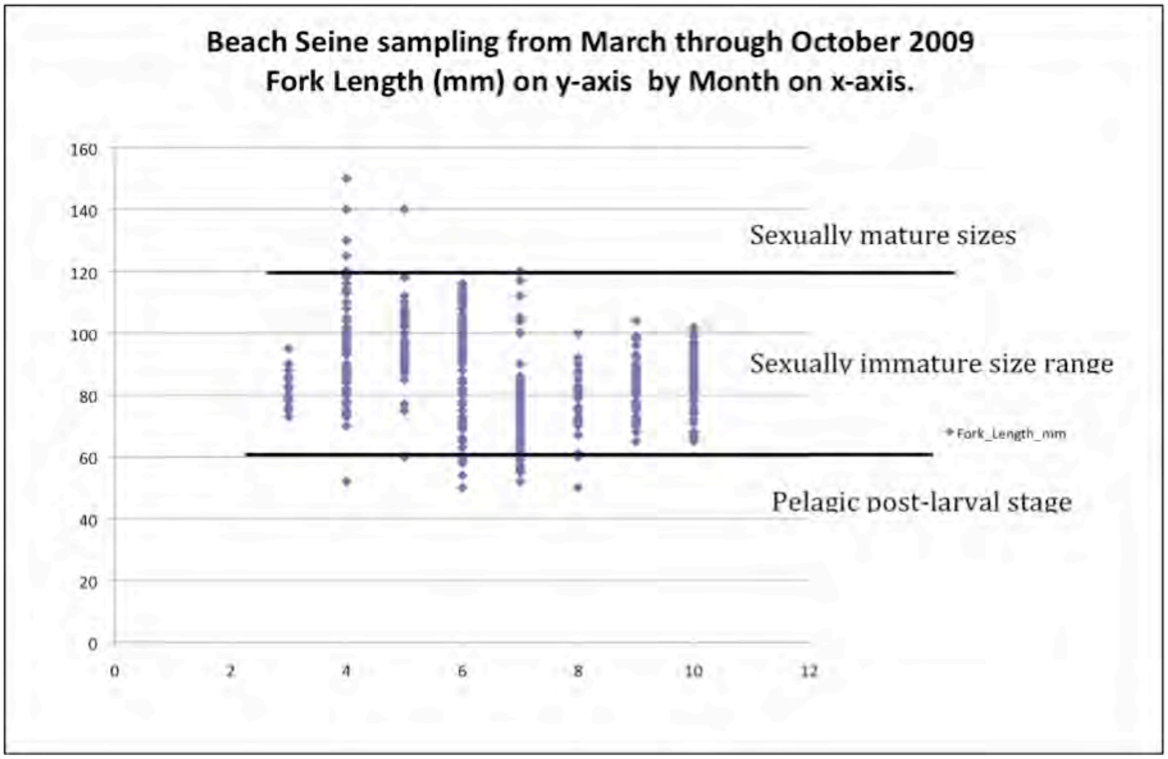


Figure 7. Lengths of PSL and time of year sampled in the inter-tidal and pelagic environment with beach seines. Largest fish were found in April and May- Mature fish (<110mm) were found from April-July, primarily at Decatur NW and Jackson's Beach). Maximum size is around 200mm FL in NE Pacific (Bering Sea up to 260 mm), 95,000 PSL were caught in 2009. Sexual maturity is reached around 120 mm FL (Robarts et al. 2002).

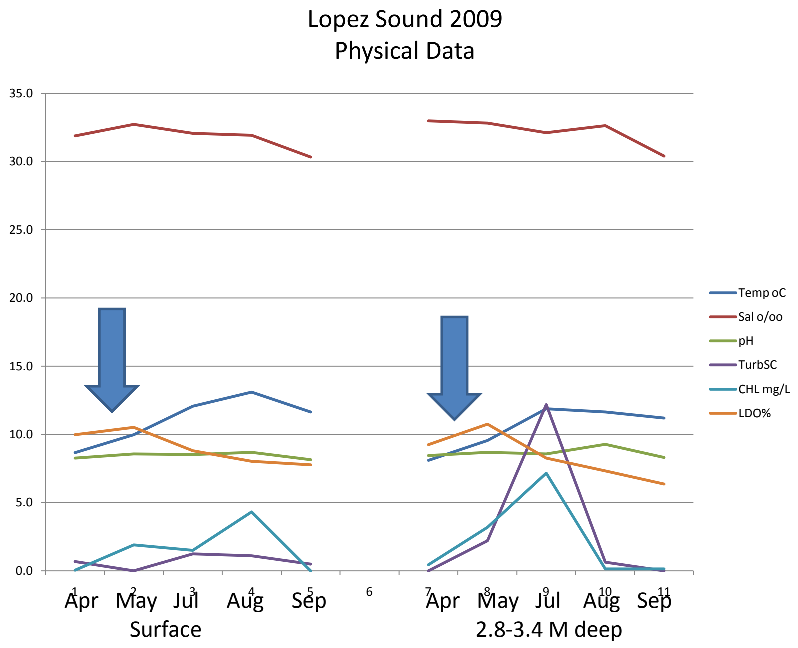


Figure 8. An example of the physical parameters measured monthly at each beach-seining site in San Juan Archipelago in 2009. Measurements were taken with a Hydrolab. Physical parameters for surface and bottom depths are shown; the blue arrow indicates a catch of large sand lance. Sample data for Decatur NE shows that PSL were captured primarily when dO2 and salinity were higher and Chl a and temperature were lower.

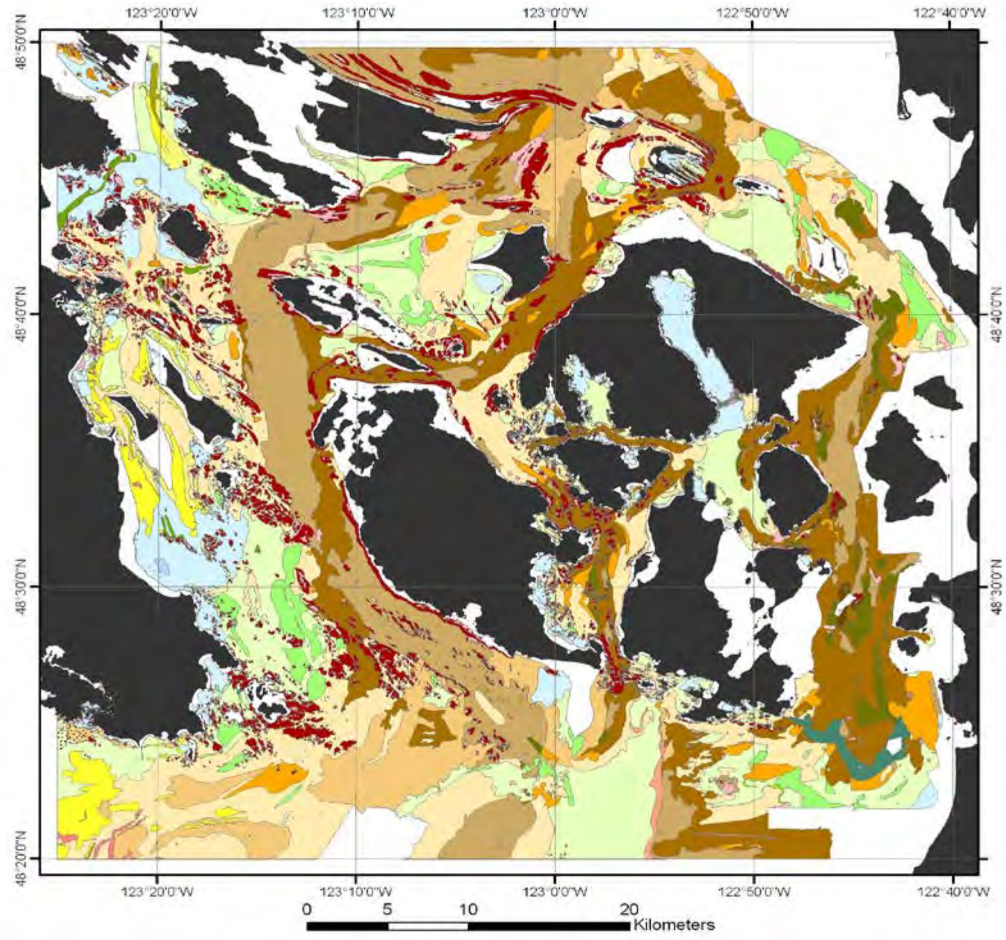


Figure 9. Example of potential habitat map constructed for the San Juan Islands that will be used to select sand wave fields to investigate. Warm colors (orange, brown, tan) represent areas of unconsolidated sediments that have bedforms diagnostic of potential sand lance habitats.

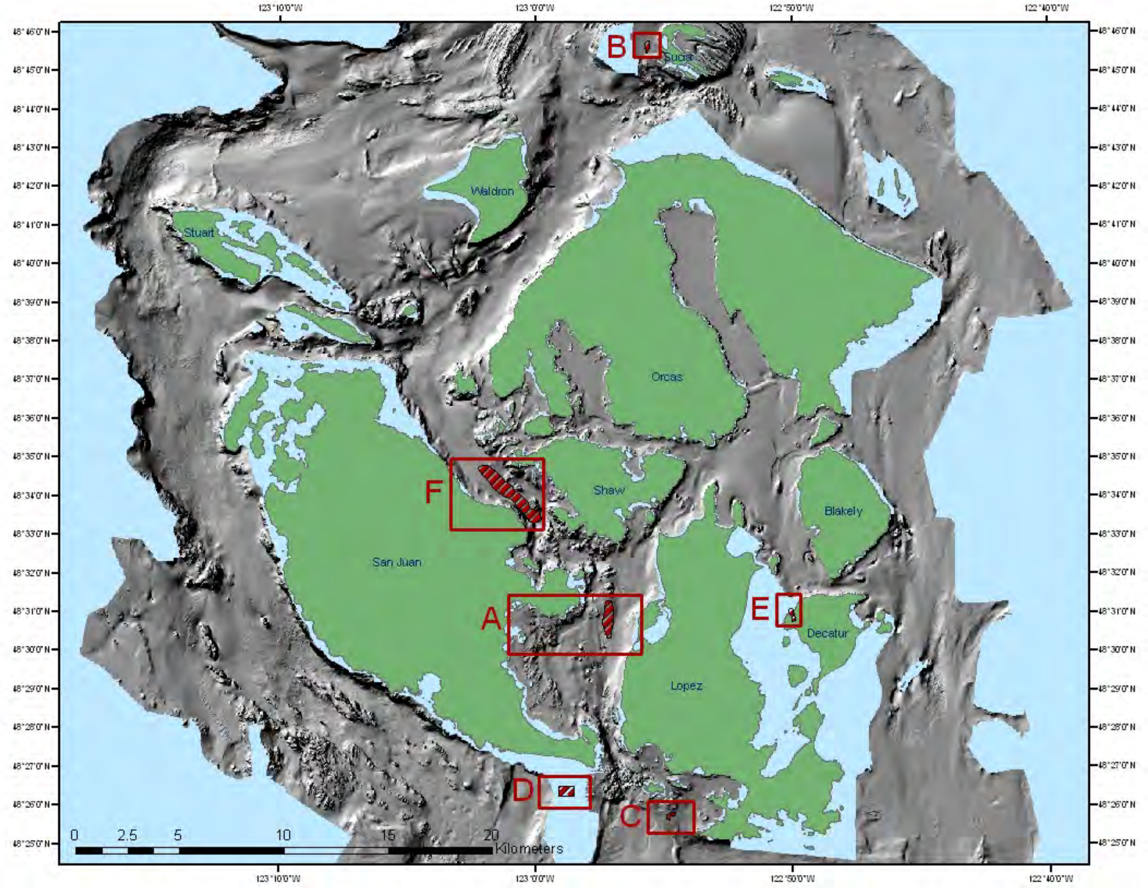


Figure 10. Index map of the San Juan Archipelago showing locations of study sites. Letters represent site location in order of PSL preferences for occupation.

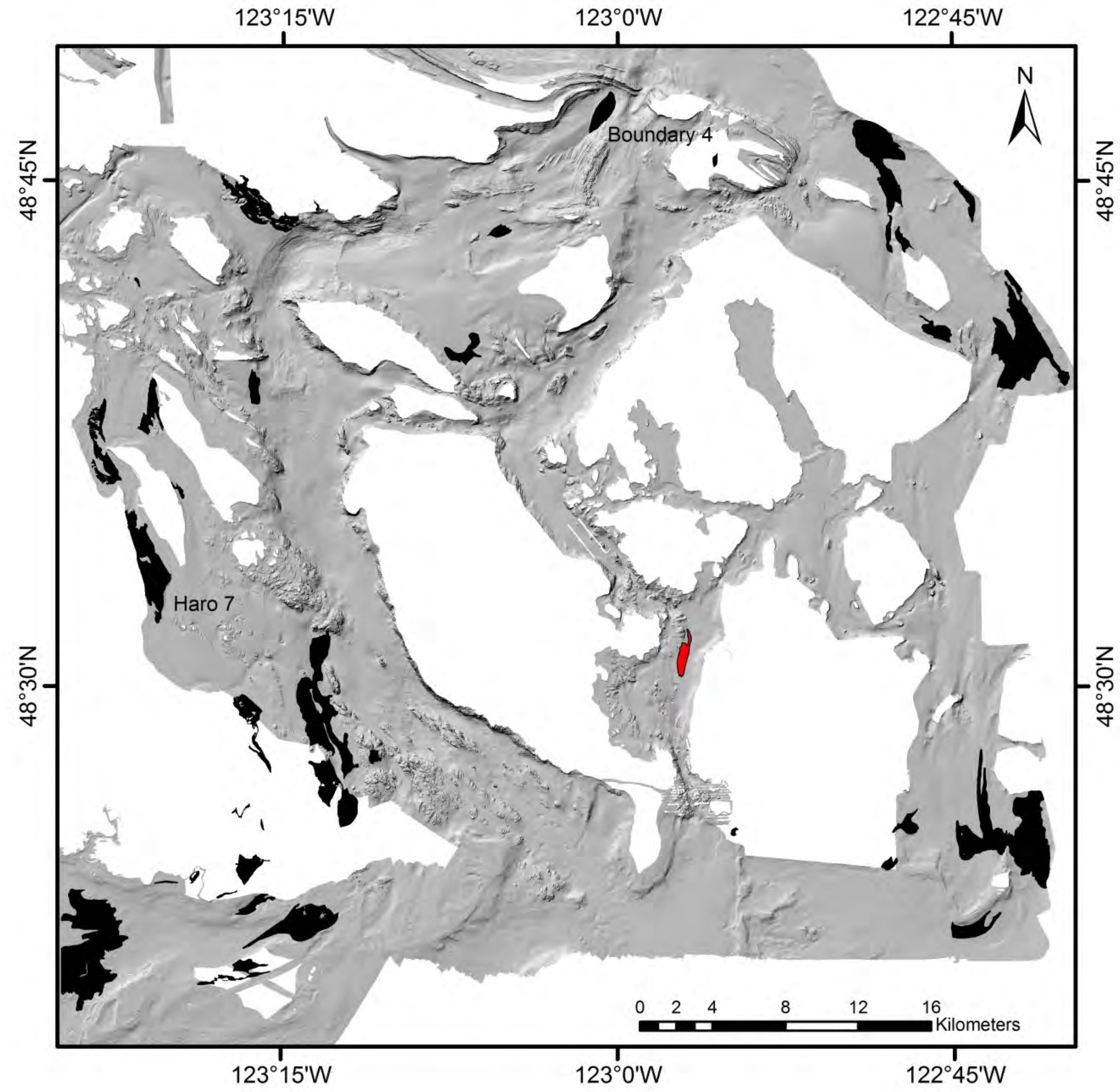


Figure 11. Location of thirty-five sand wave fields identified in the San Juan Island Archipelago (black polygons) and the primary study site in San Juan Channel (red polygon). The two sand wave fields (Boundary 4 and Haro 7) that are referred to in this report are labeled for reference. Baseline files consist of: 1) a hillshade that was created from the bathymetry grid that was used for all calculations, and a benthic habitat interpretation polygon file from which the depicted sand wave fields were extracted.

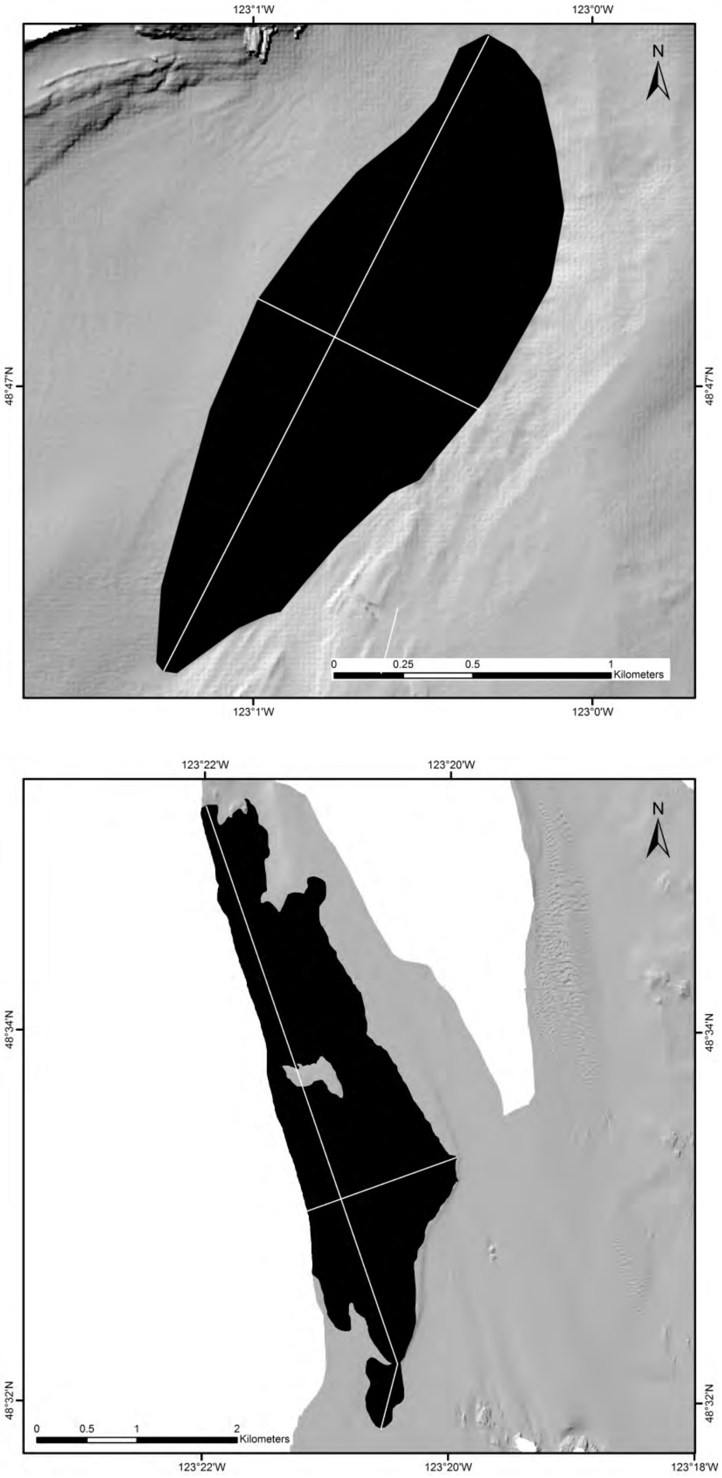


Figure 12. Maximum length and width measurements, as depicted for the Boundary 4 (A.) and Haro 7 (B.) sand wave fields.

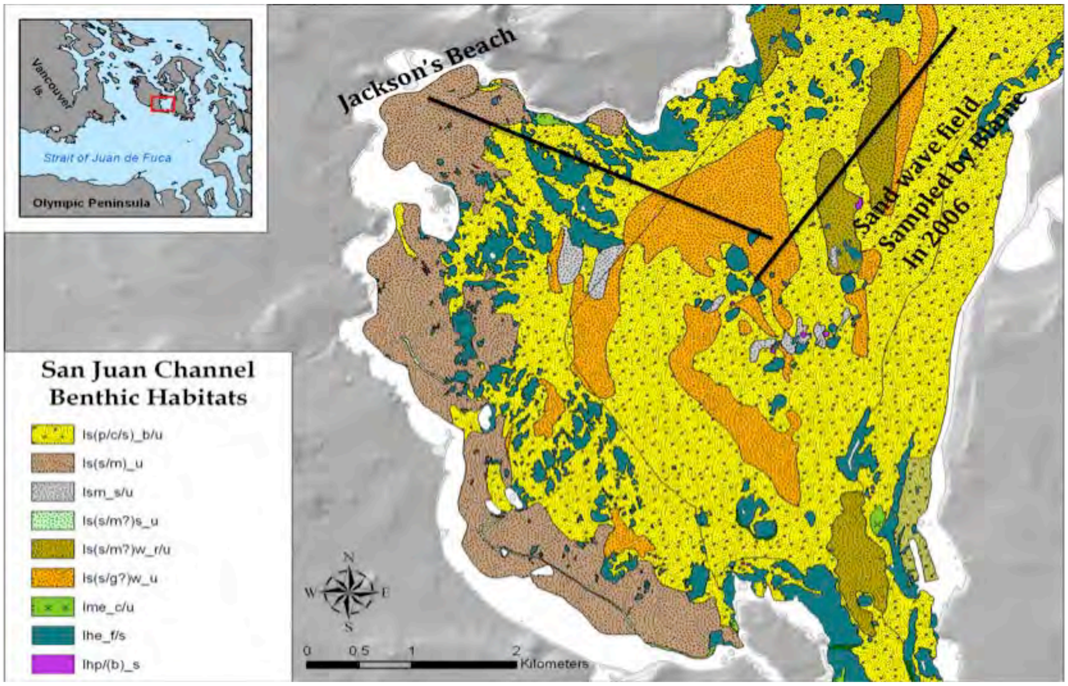


Figure 13. Habitat map constructed from high-resolution MBES data for Griffin Bay, San Juan Island showing potential marine benthic habitat types (substrate) and proposed transects to be run to develop our geomorphic model for PSL habitat prediction. We will also evaluate the relationship between inter-tidal PSL habitat with sub-tidal habitats and possible migration pathways.

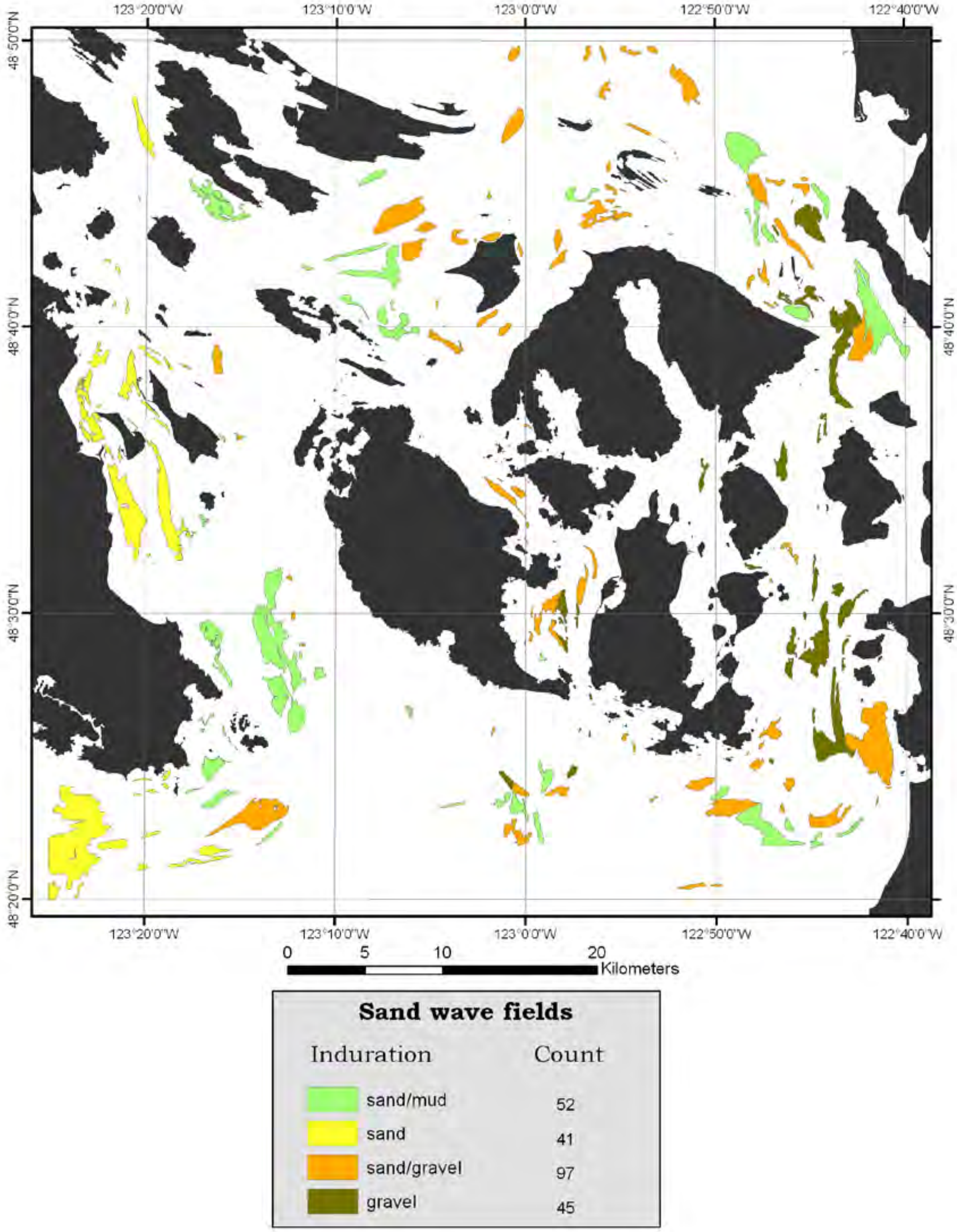


Figure 14. Locations of dynamic bedforms or sediment wave and dune fields that may act as potential forage fish habitat providing prey for rockfish. These morphologic features are based on multibeam echosounder bathymetry and backscatter data.

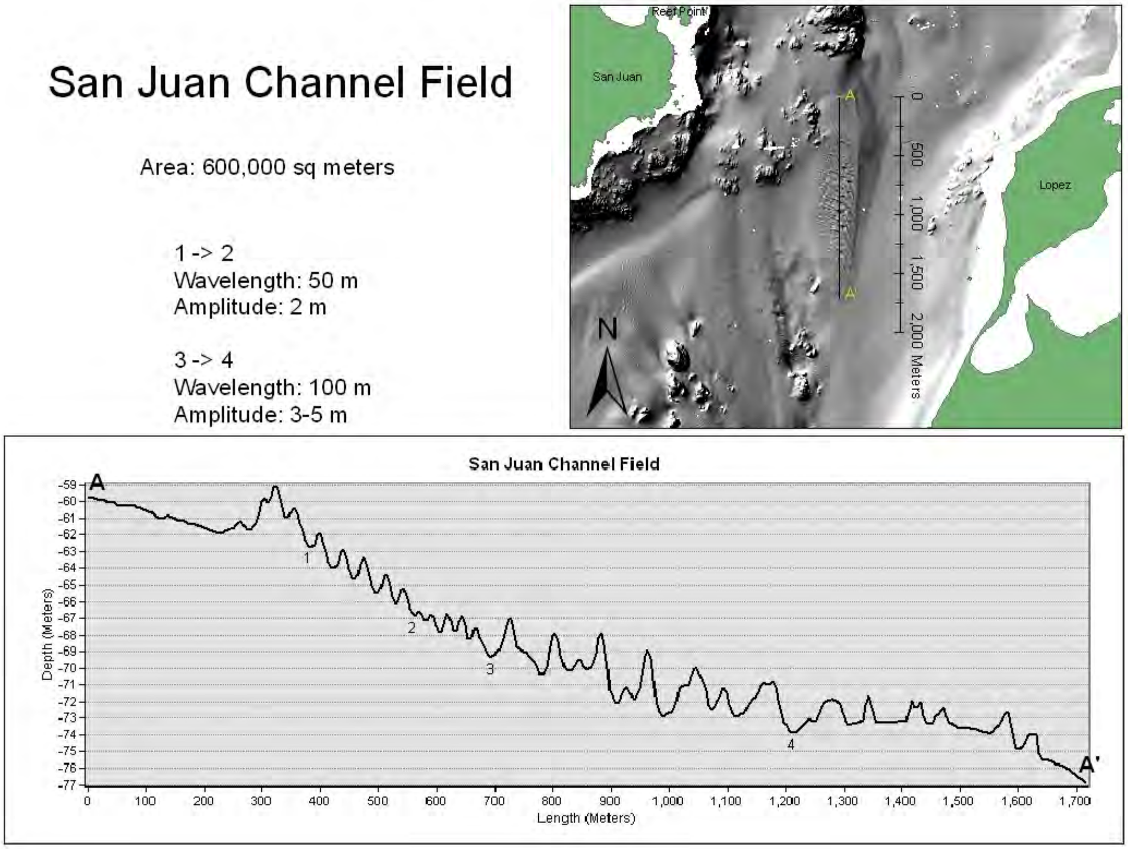


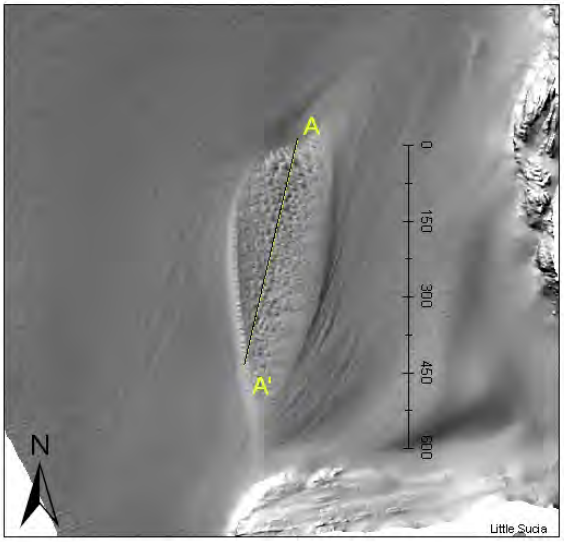
Figure 15. Length and amplitude of sand waves obtained from a bathymetric profile across the San Juan Channel sand wave field, the proto-typical PSL habitat type in the San Juan Archipelago (considerable fish caught here).

Sucia Field

Area: 80,000 sq. meters

1 -> 2
Wavelength: 10-15 m
Amplitude: .3 - .5 m

3 -> 4
Wavelength: 10-15 m
Amplitude: .1 - .5 m



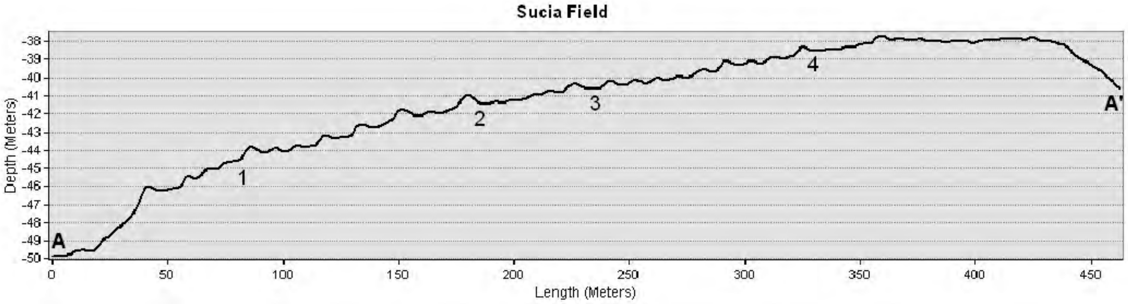


Figure 16. Lengths and amplitudes of sediment waves in the Sucia Island dynamic bedform obtained from a bathymetric profile across the feature (some fish caught here).

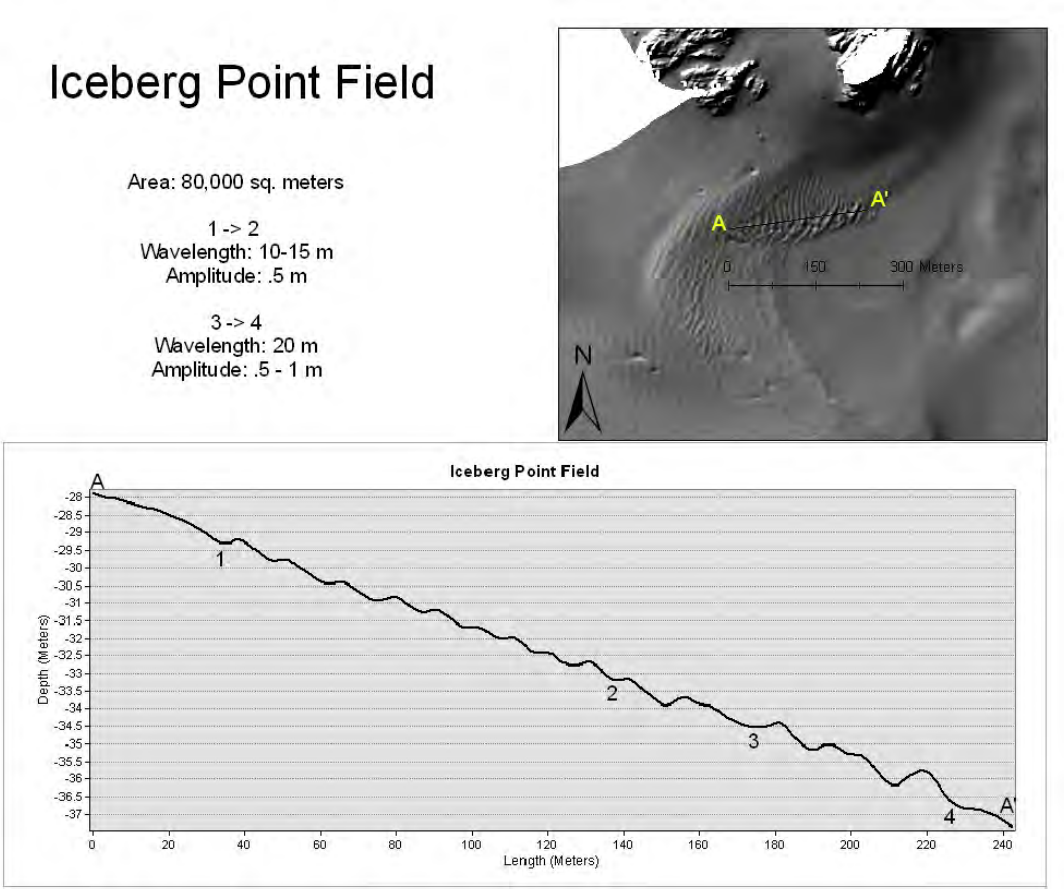


Figure 17. Lengths and amplitudes of sediment waves in the dynamic bedform off Iceberg Point, southern Lopez Island obtained from a bathymetric profile drawn along the field (few fish caught here).

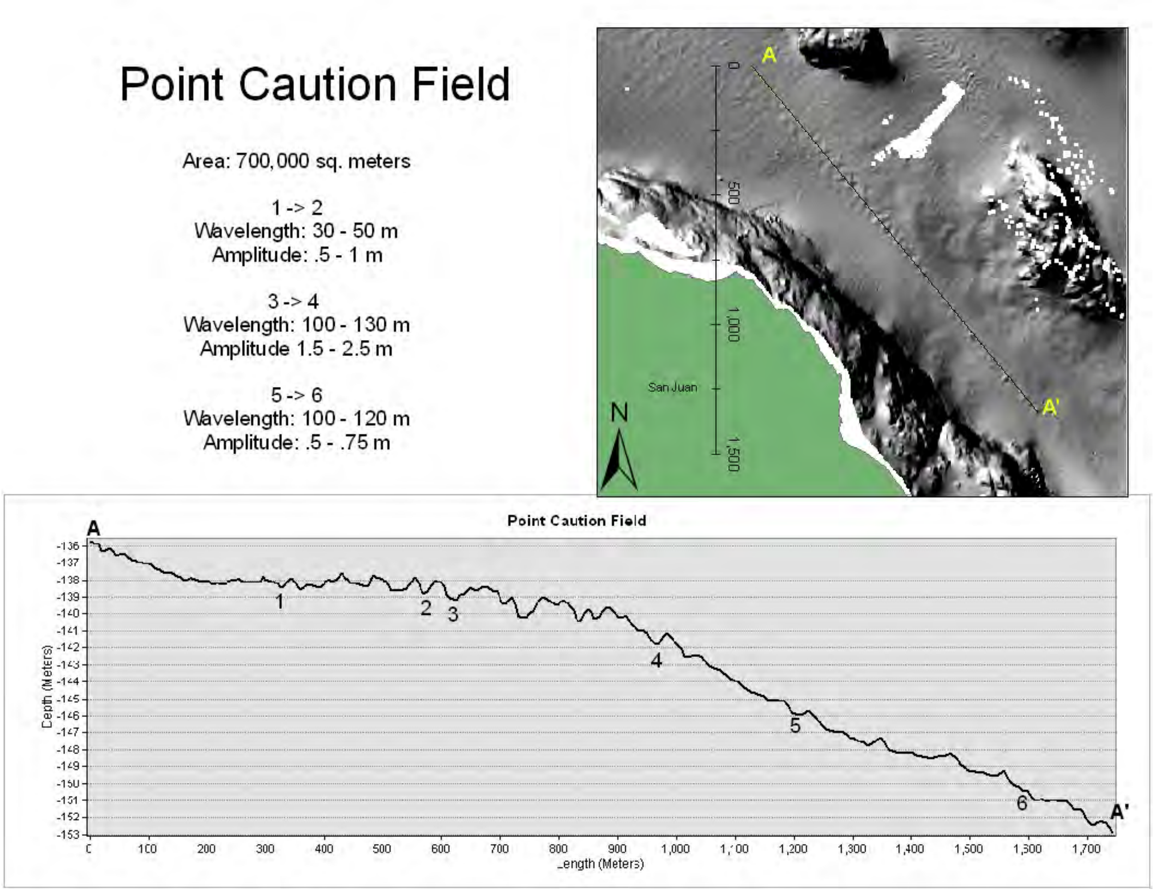


Figure 18. Lengths and amplitudes of sediment waves in the dynamic bedform off Poitn Caution, central San Juan Channel obtained from a bathymetric profile (no fish caught here).

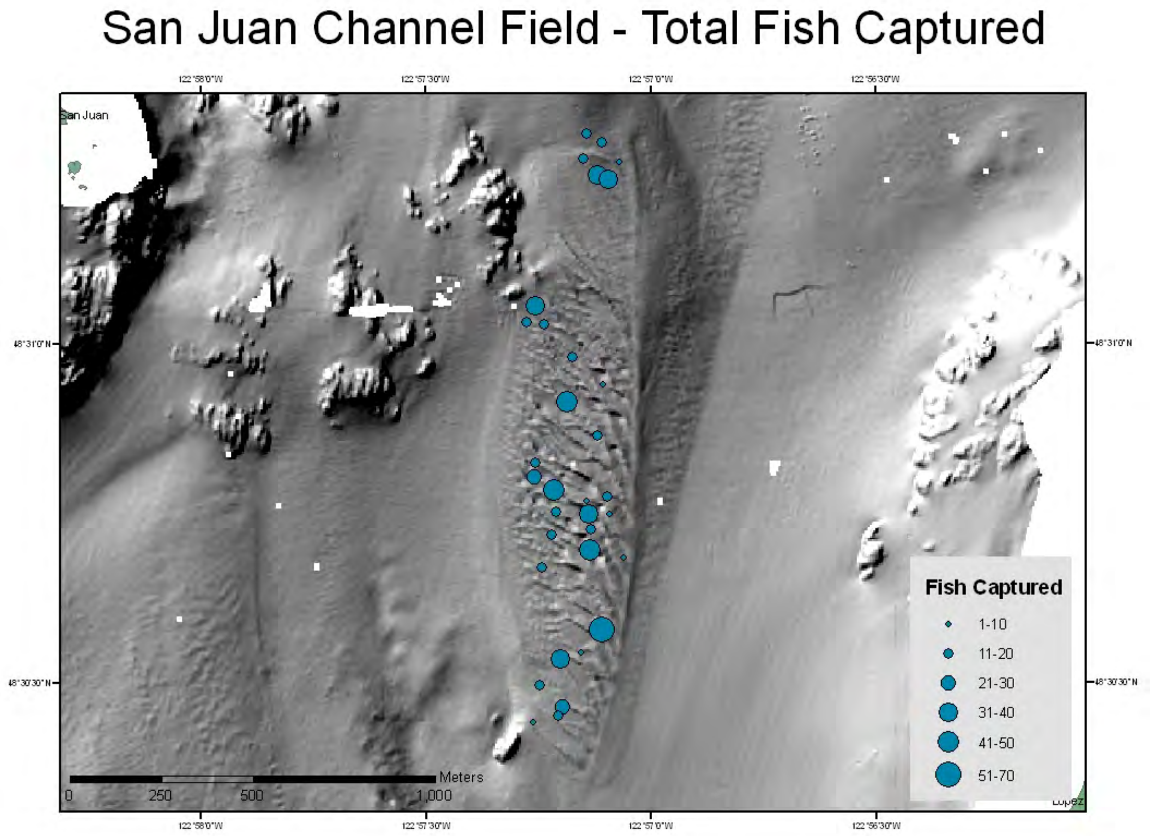


Figure 19. Locations and total numbers of PSL caught in a single grab sample for the San Juan Channel sand wave field during the period of July 2010-June2011.

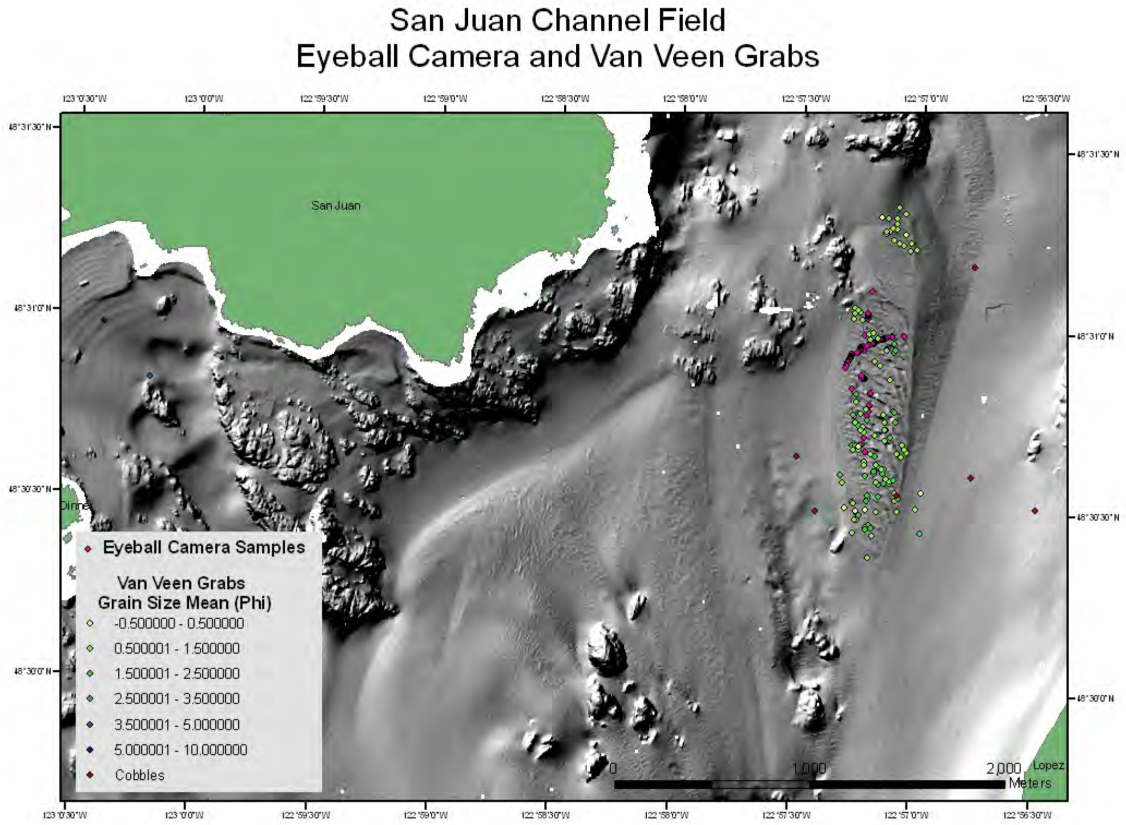


Figure 20. Locations and grain sizes of Van Veen sediment grab samples and USGS eyeball camera drop locations at the San Juan Channel sand wave field, the proto-typical sub-tidal PSL habitat.

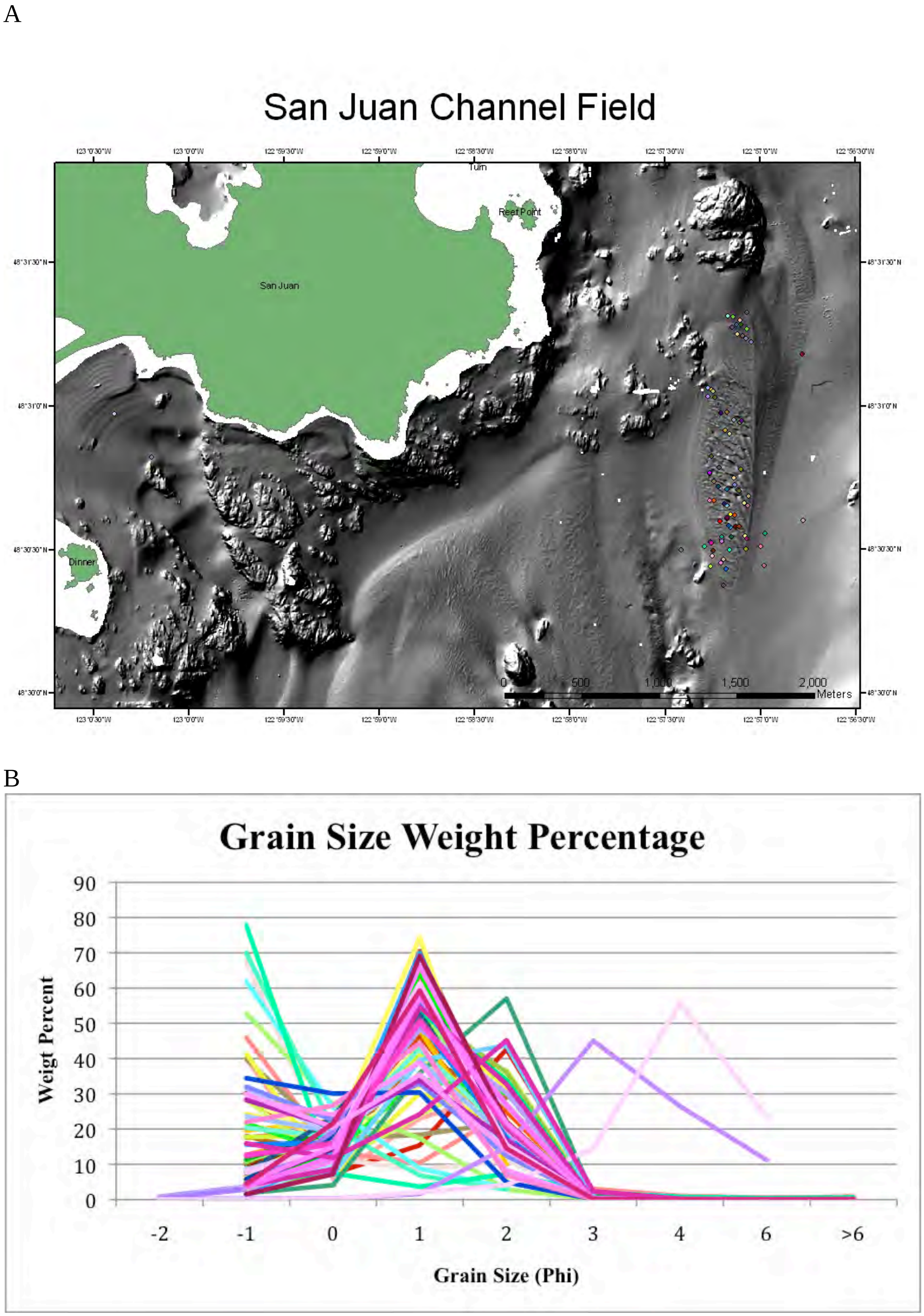


Figure 21. Sample locations(A) and grain sizes (B) from San Juan Channel field.

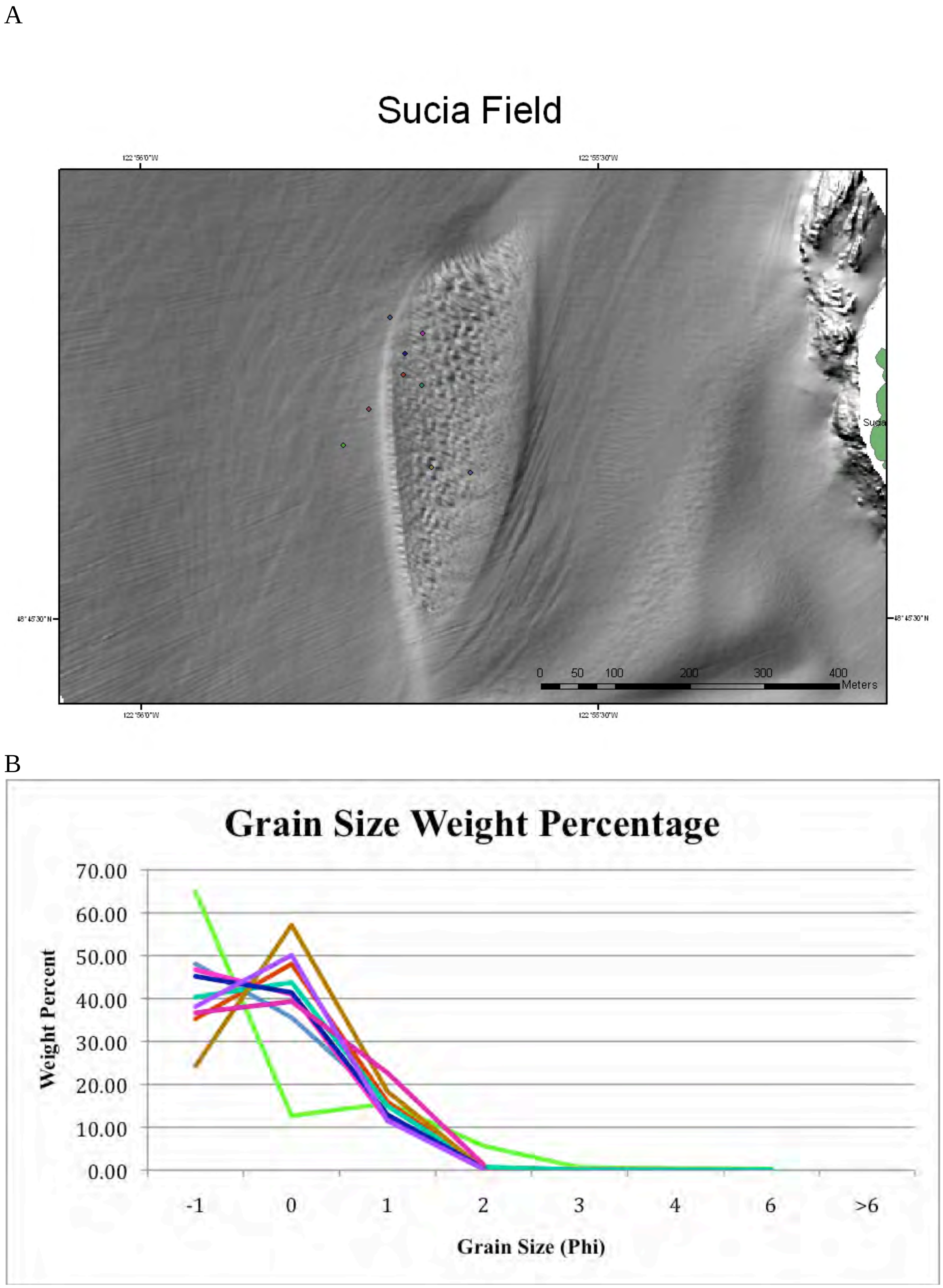


Figure 22. Locations (A) and grain sizes of Van Veen grab samples collected from the Sucia Island sediment wave field.

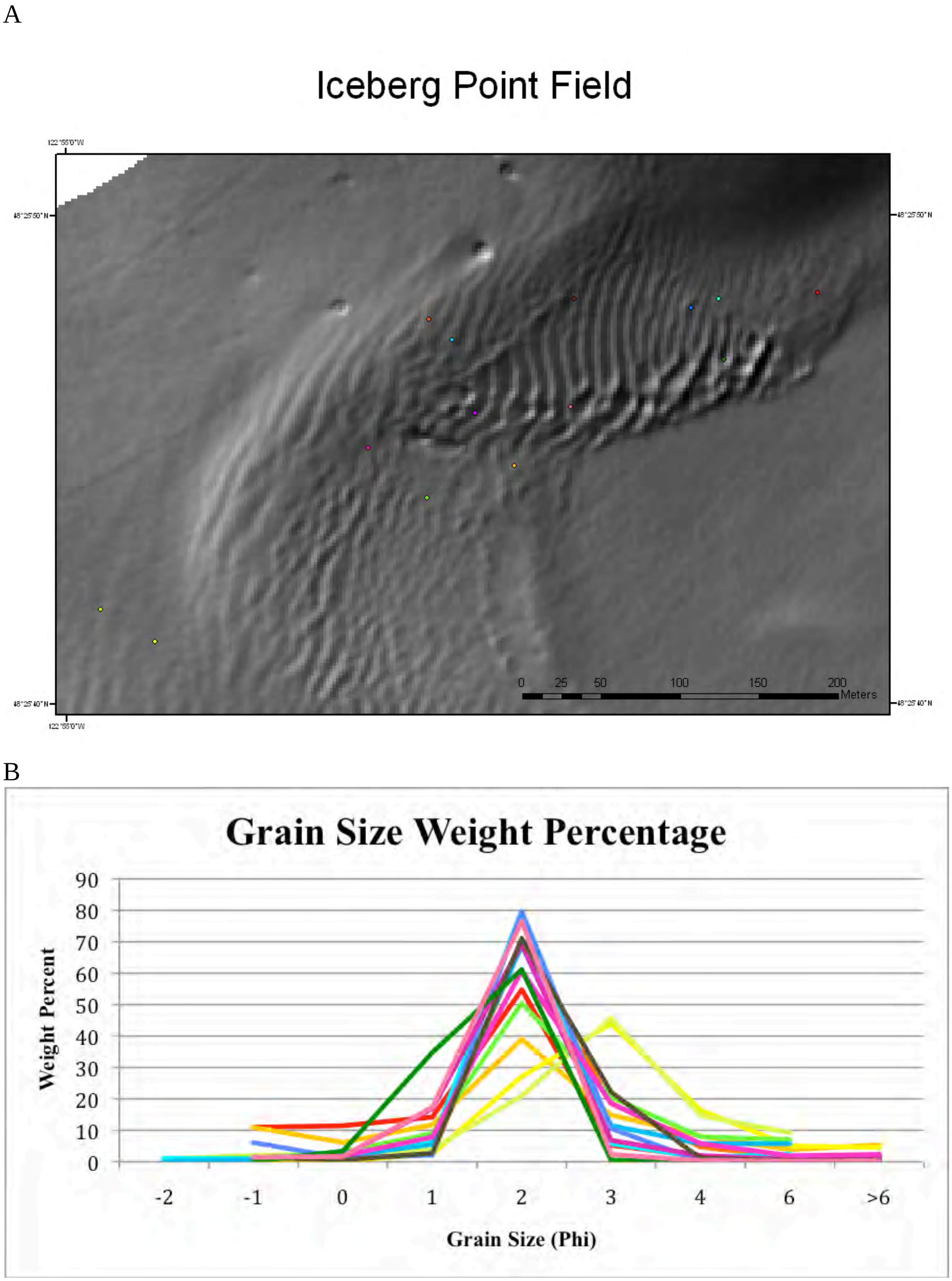


Figure 23. Locations (A) and grain sizes (B) of Van Veen grab samples collected from the sediment wave field off of Iceberg Point, southern Lopez Island.

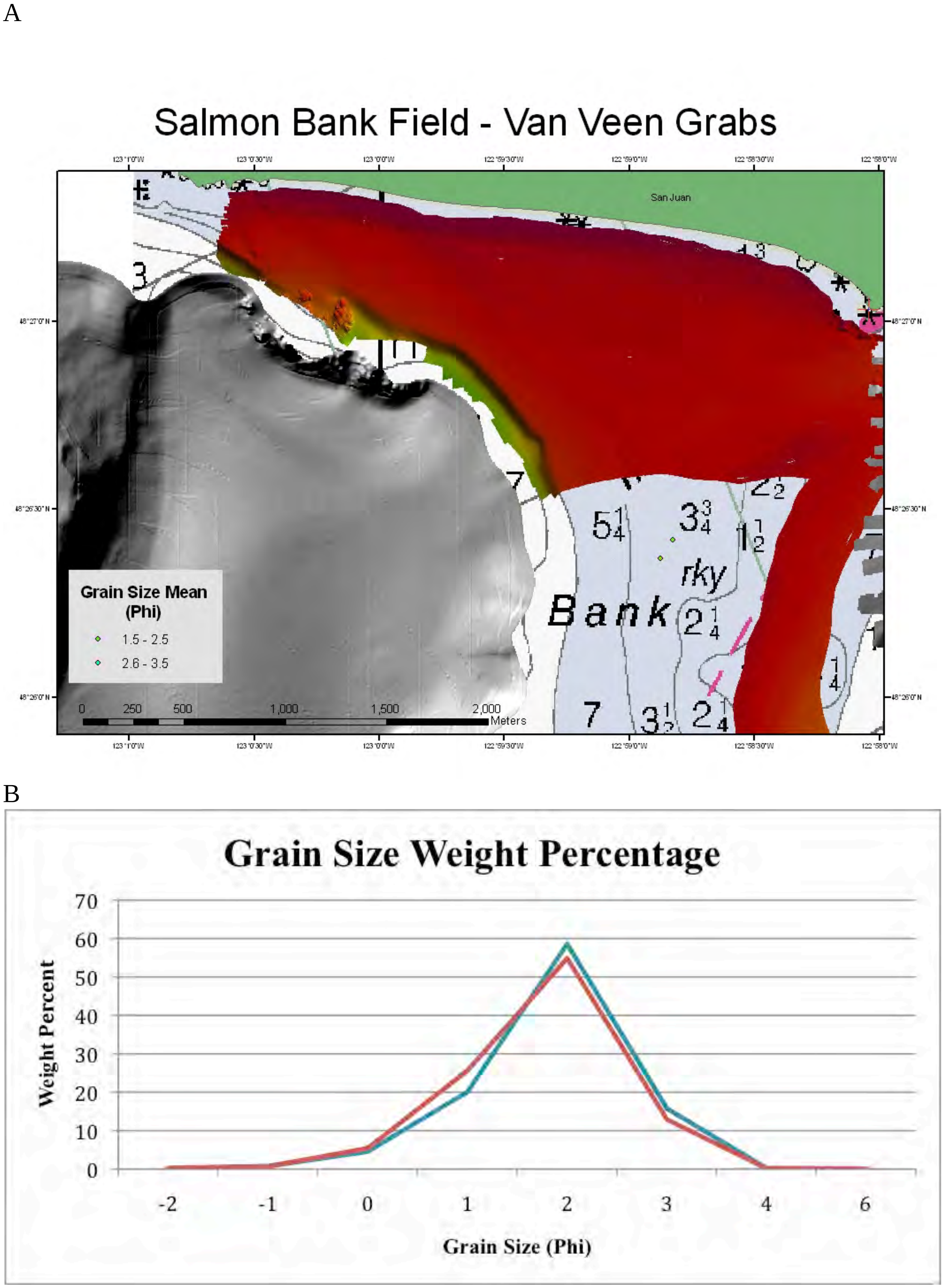


Figure 24. Locations (A) and grain sizes (B) of Van Veen grab samples collected on Salmon Bank.

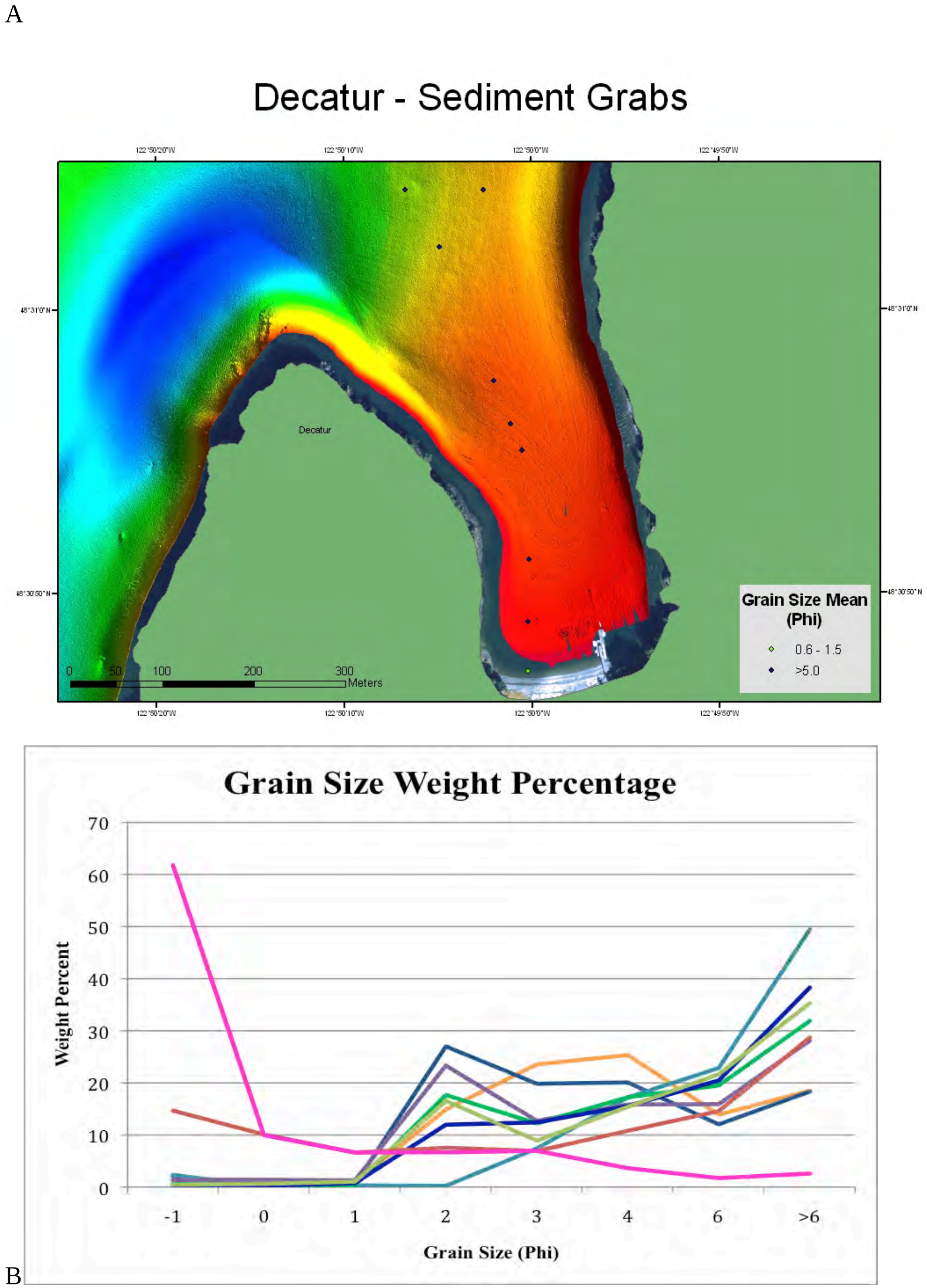


Figure 25. Locations (A) and grain sizes of Ponar grab sediment samples collected off Decatur Island.

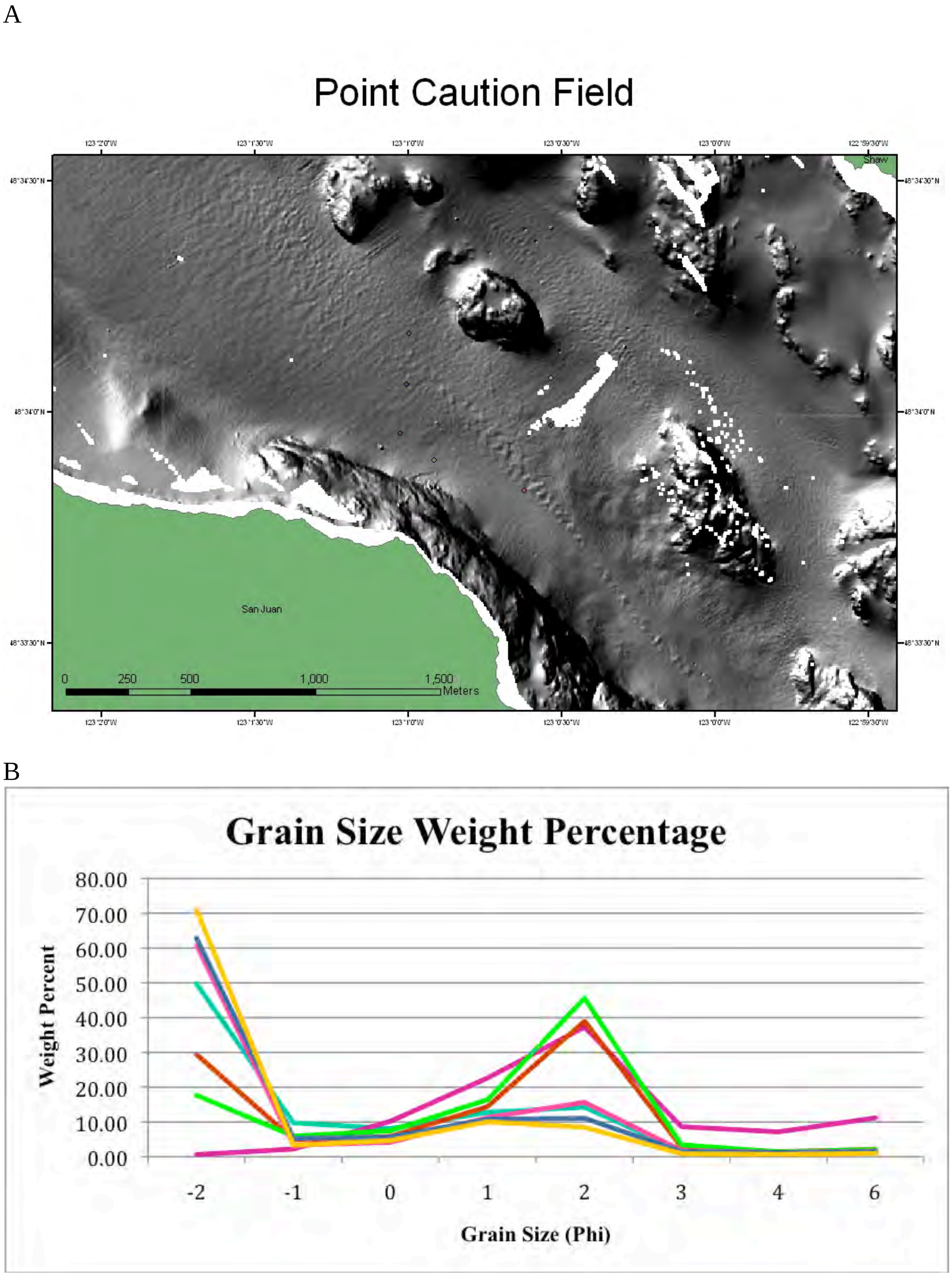


Figure 26. Locations (A) and grain sizes of Van Veen sediment grab samples collected from the Point Caution, central San Juan Channel, sediment wave field.

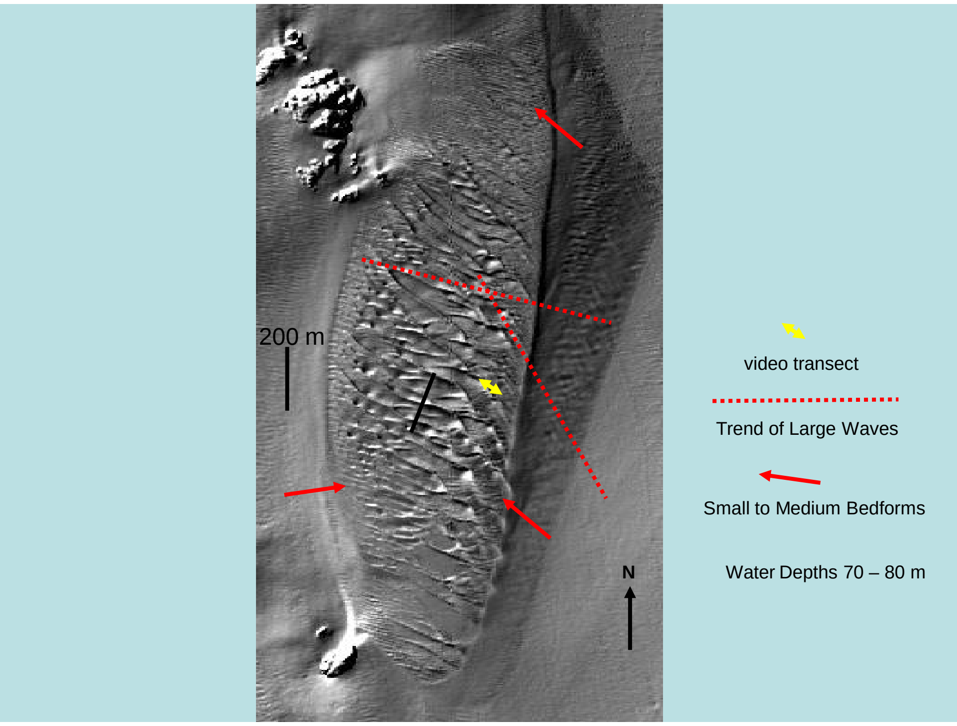


Figure 27. MBES image of San Juan Channel sand wave field showing different size bedforms and trend of large waves.

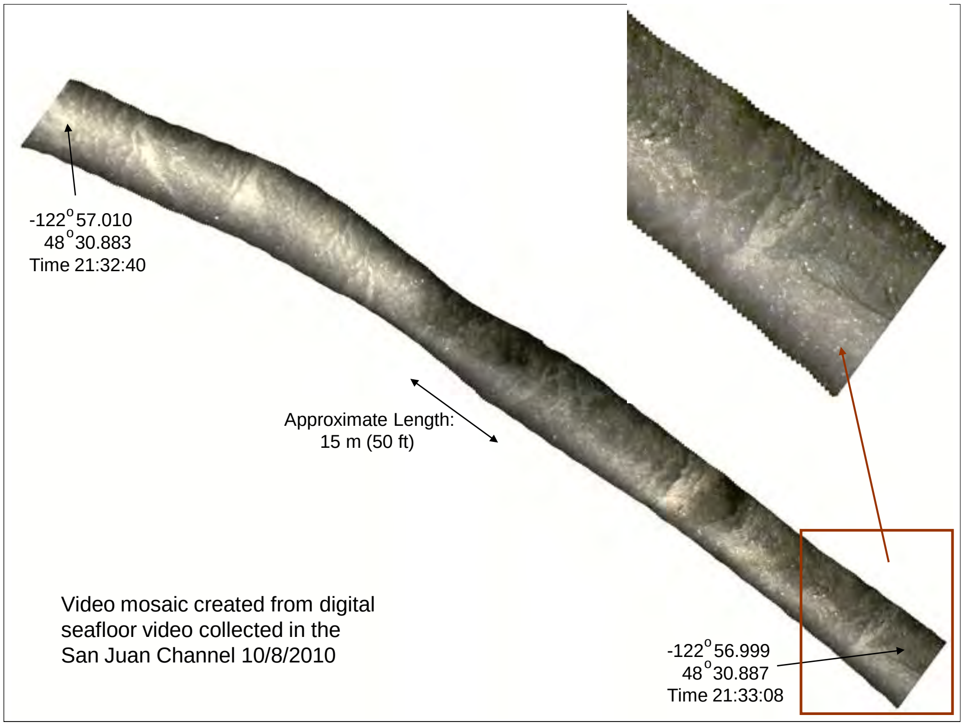
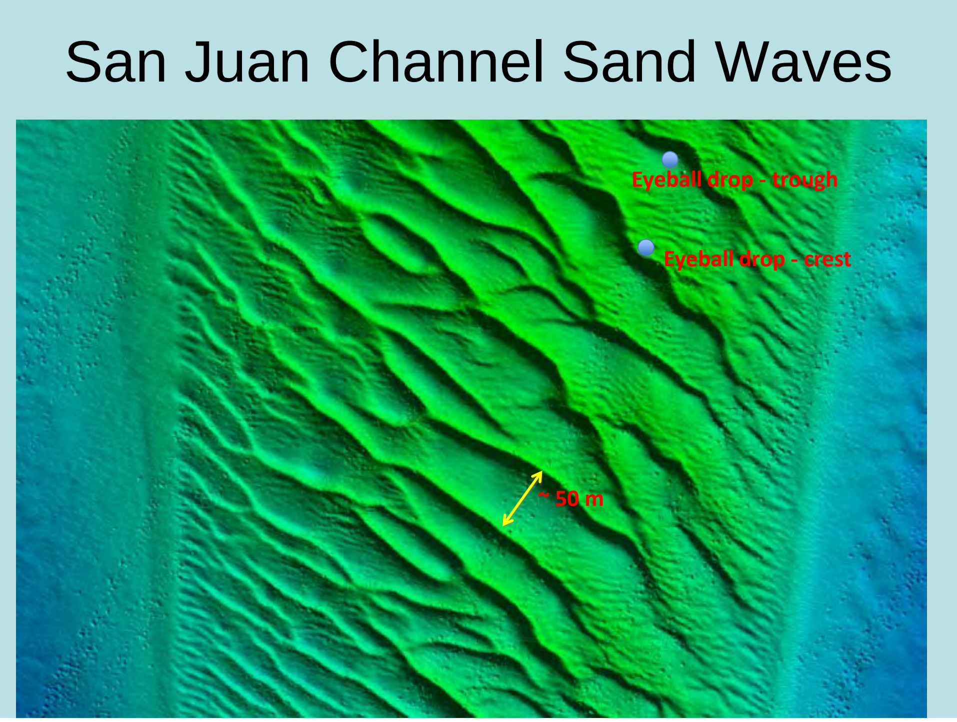


Figure 28. Video mosaic created by Pete Dartnell of the USGS in Menlo Park of a vidio transect made across the San Juan Channel sand wave field.

Eyeball camera in box
(arrow)

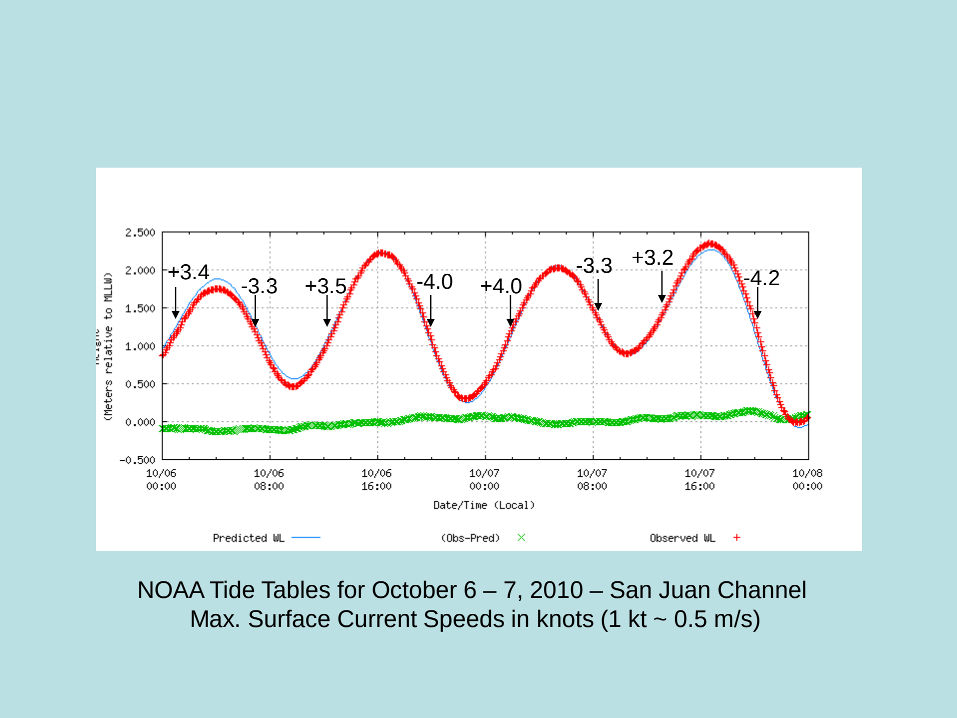
Figure 29. Photo of USGS eyeball camera.



San Juan Channel Sand Waves

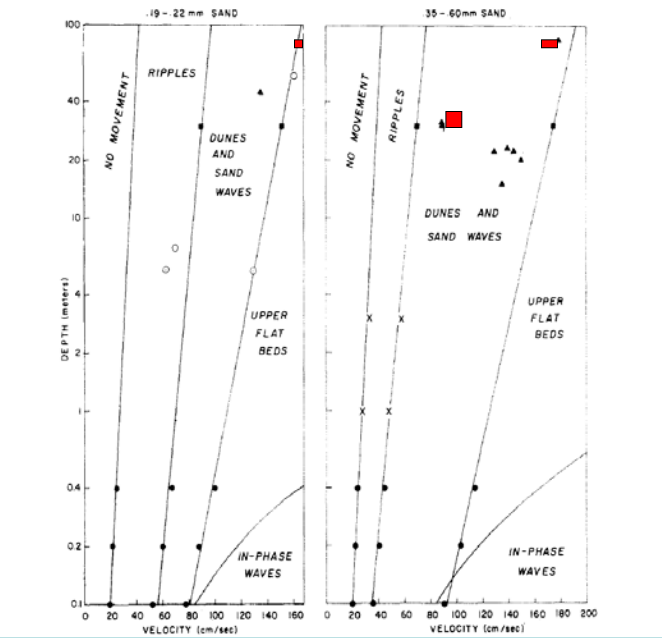
Figure 30. High resolution MBES bathymetry showing locations of eyeball camera drops in the San Juan Channel sand wave field.

Figure 31. Photos of sediment taken with eyeball camera on the crest (upper) and trough (lower) of sand waves in the San Juan Channel sand wave field.



NOAA Tide Tables for October 6 – 7, 2010 – San Juan Channel
Max. Surface Current Speeds in knots (1 kt ~ 0.5 m/s)

Figure 32. Tidal curves showing current speeds within the San Juan Channel that affect sediment distribution in the San Juan Channel sand wave field (After NOAA, Oct. 6-7, 2010)



Bed Phase Diagram from Rubin and McCulloch, 1980, Single and Superimposed Bedforms:
A Synthesis of San Francisco Bay and Flume Observations. Sedimentary Geology, 26, 207-231.

Figure 33. Bed phase diagram that show stability of the seabed and sediment wave fields in San Francisco Bay, California and mimic the conditions observed at the San Juan Channel sand wave field (after Rubin and McColloch, 1980).

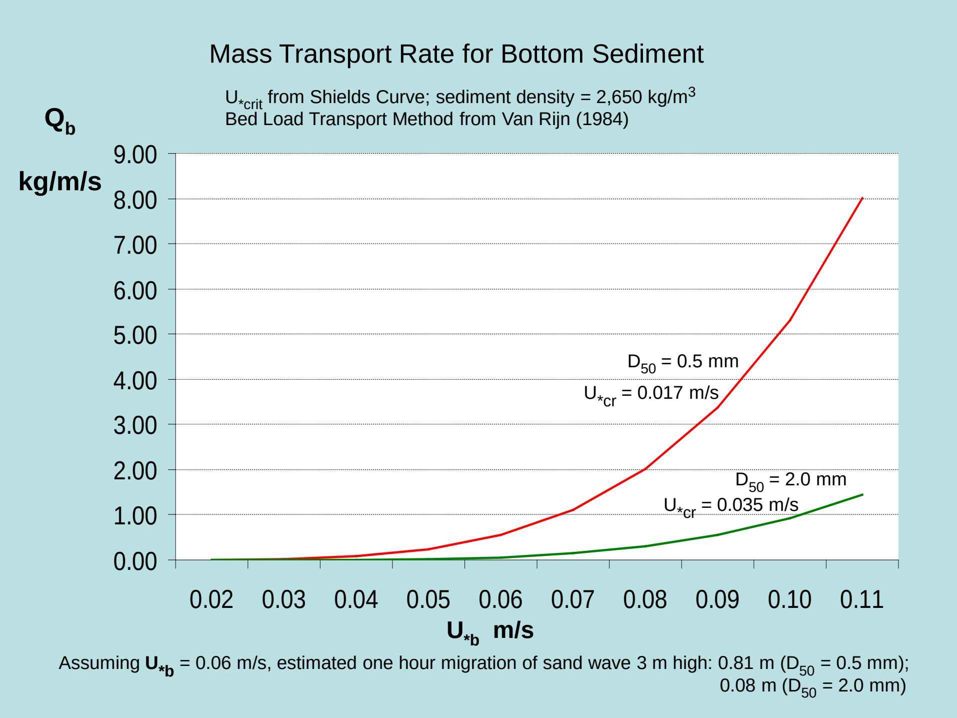


Figure 34. Graph showing mass transport rate of bottom sediment for the San Juan Channel sand wave field based on methods of Van Rijn (1984).

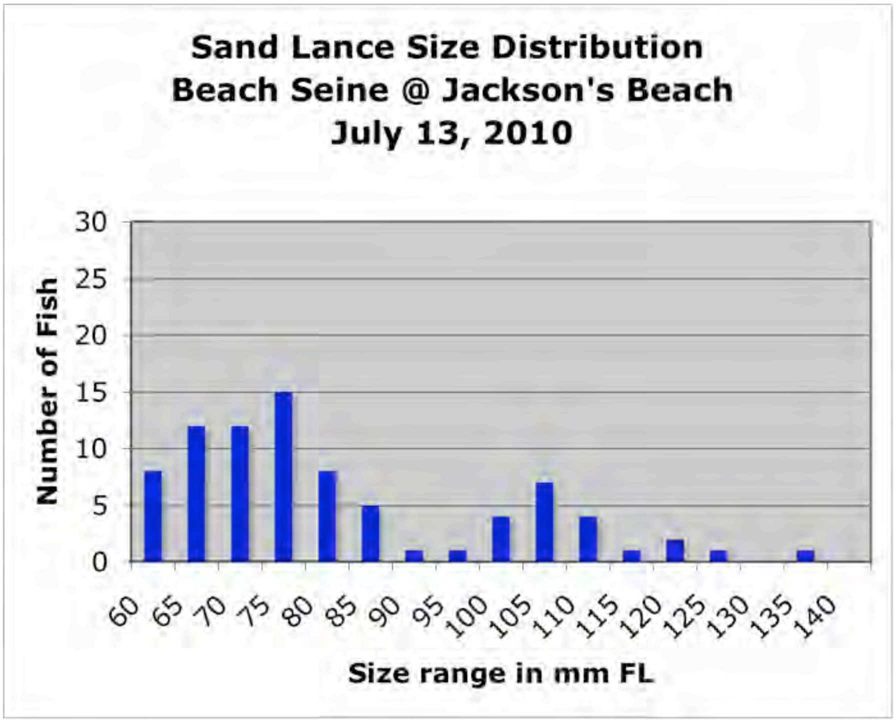


Figure 35. Size distribution of PSL captured by beach seine at Jackson's Beach on 13 July, 2010.

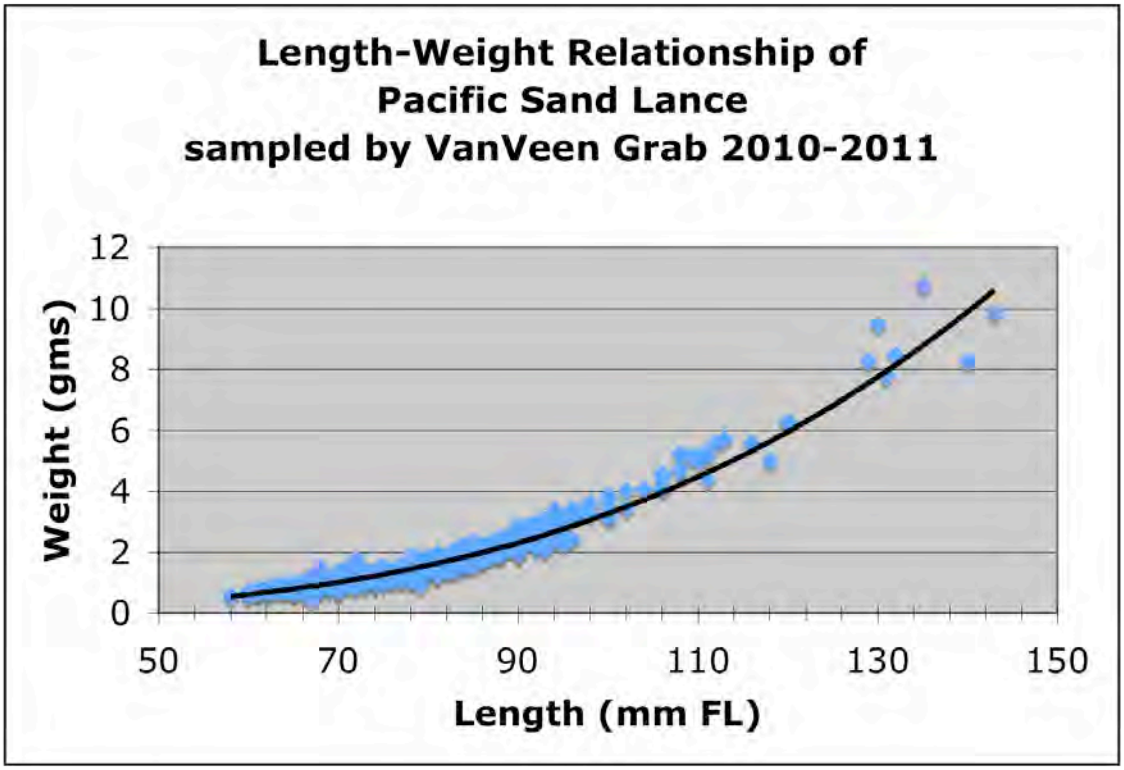


Figure 36. Length and weight relationship for all PSL sampled during 2010-2011 in San Juan County.

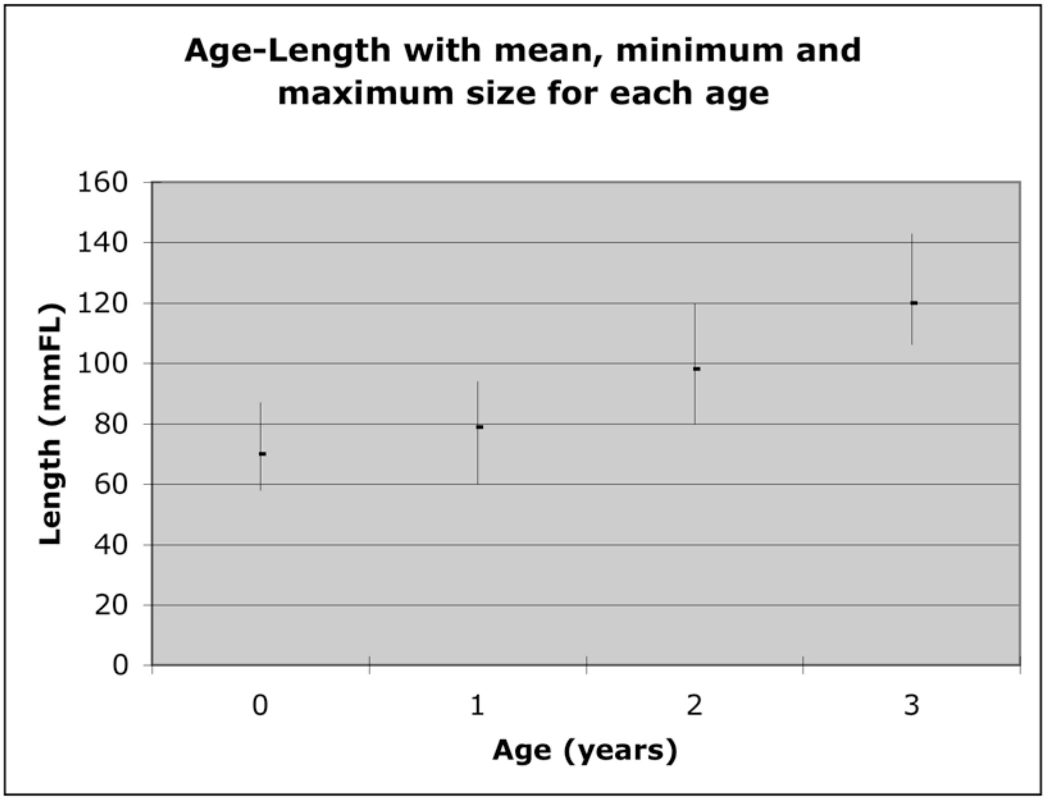


Figure 37. Length at age relationship for PSL sampled during 2010-2011 in the San Juan Archipelago. The majority of fish were captured in the San Juan Channel sand wave field.

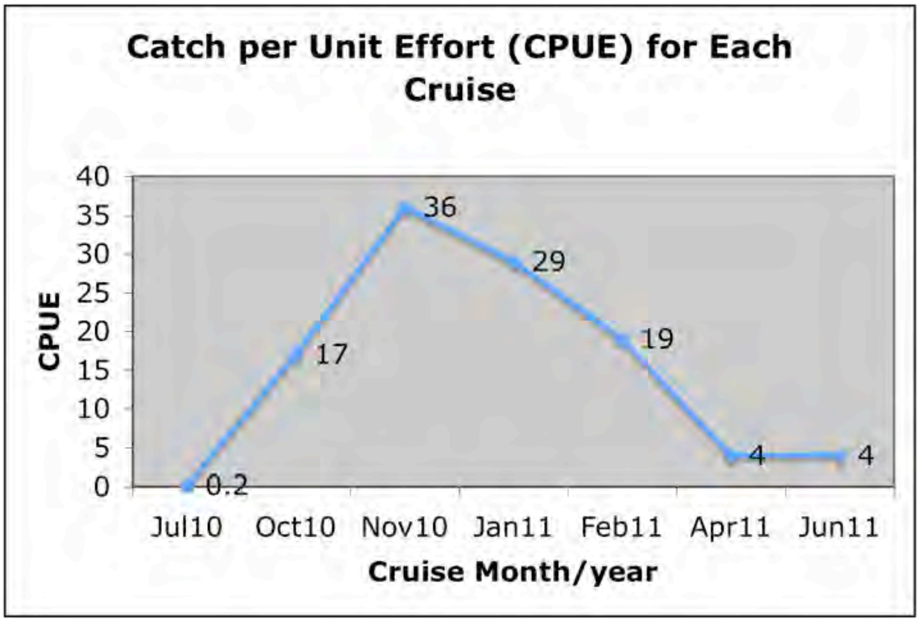


Figure 38. Average number of fish captured in each grab sample taken on the San Juan Channel deep-water sand wave field by sampling effort.

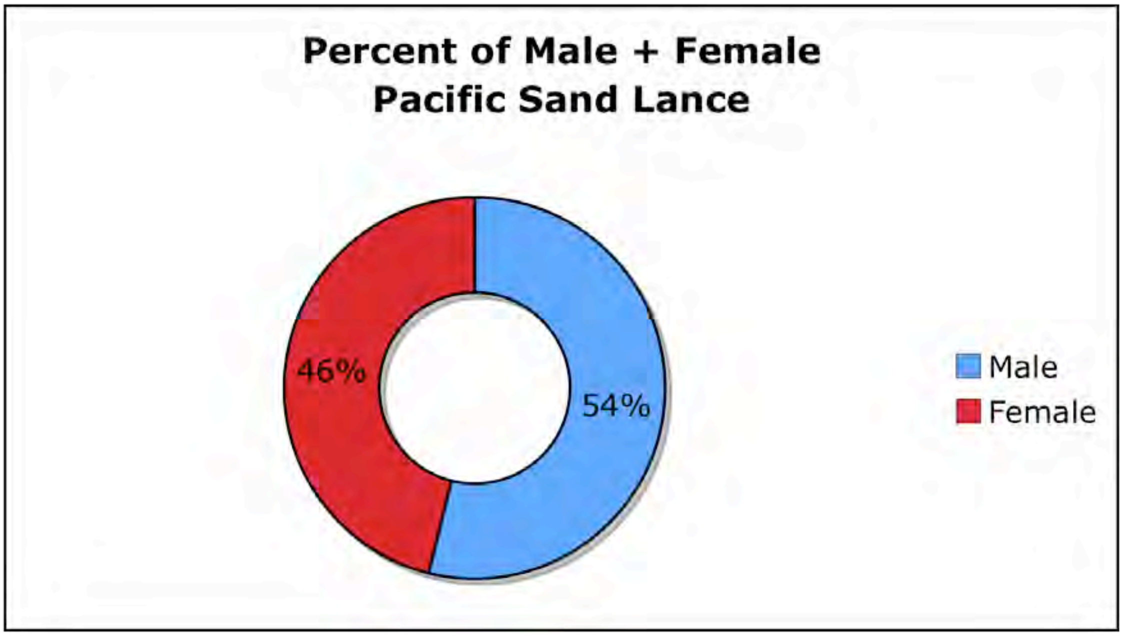


Figure 39. Percent of male and female PSL sampled from San Juan County during 2010-2011.

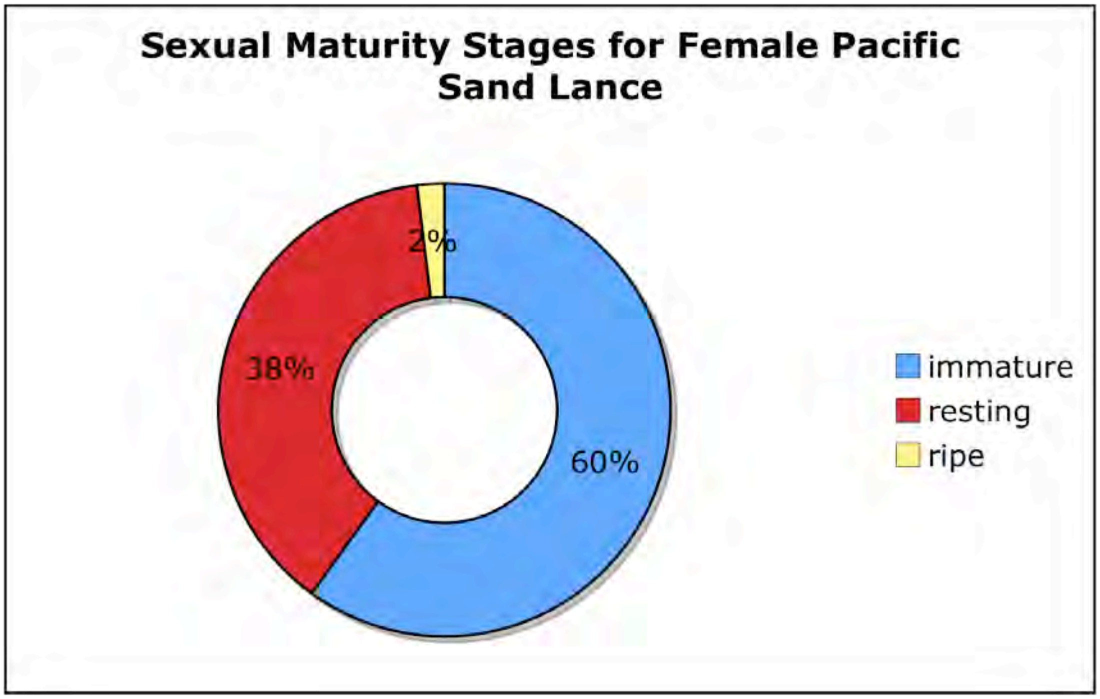


Figure 40. Percentage of female PSL at varying stages of sexual maturity.

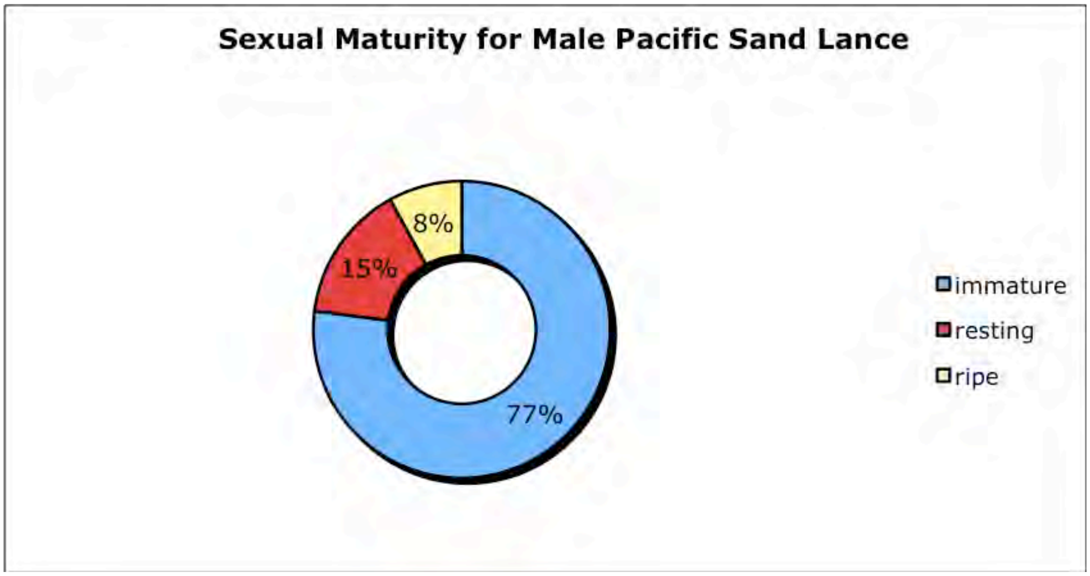


Figure 41. Percentage of male PSL at varying stages of sexual maturity.

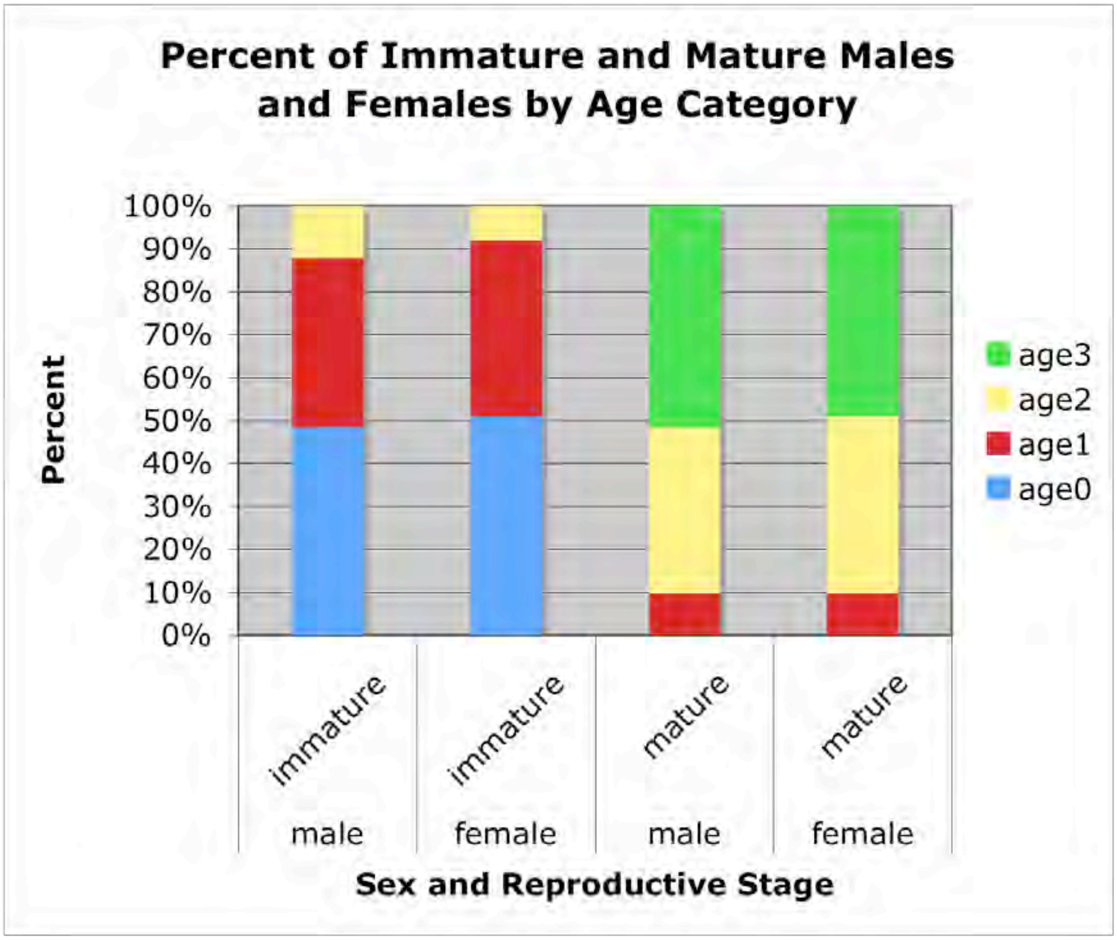


Figure 42. Percent of male and female PSL that are either sexually mature or immature for each age class.

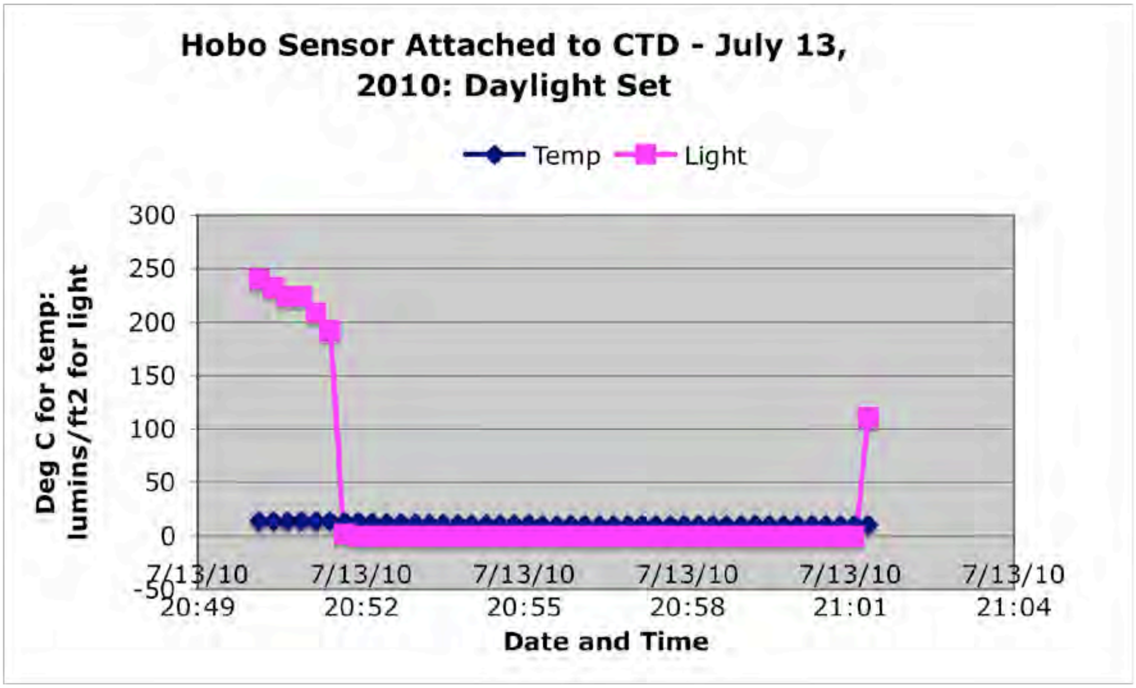


Figure 43. A Hobo sensor was attached to the CTD and gathered temperature and light level data while the CTD was lowered from the surface to the sand wave field. These data were gathered during the July 13, 2010 cruise.

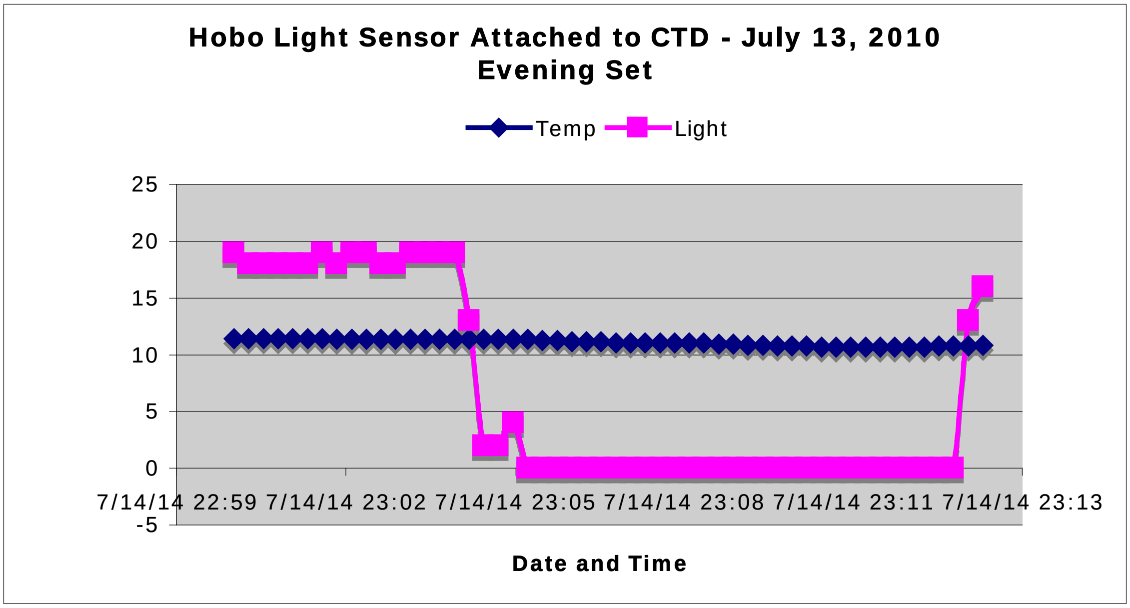


Figure 44. A Hobo sensor was attached to the CTD and gathered temperature and light level data while the CTD was lowered from the surface to the sand wave field during nighttime hours. These data were gathered during the July 13, 2010 cruise.

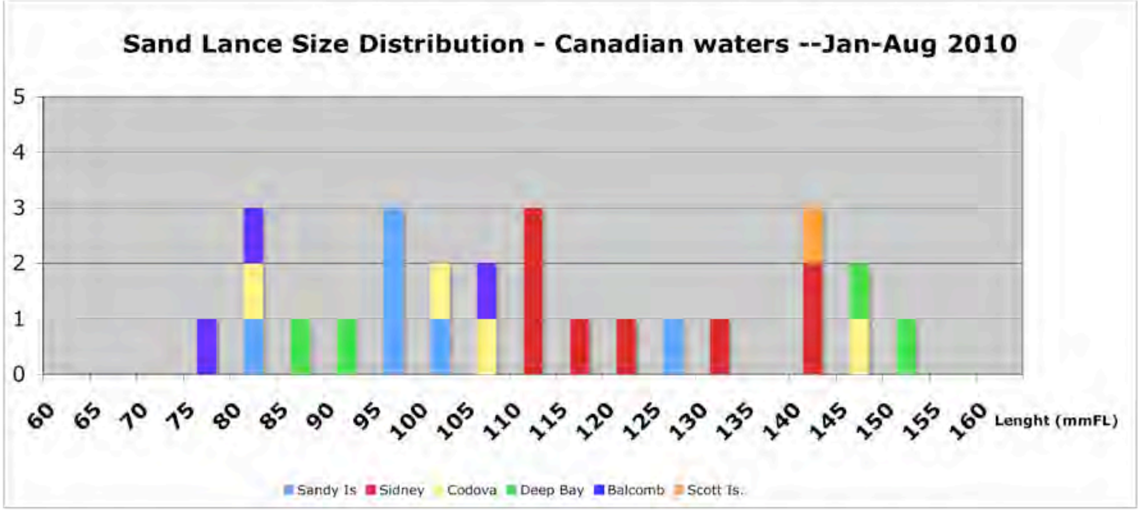


Figure 45. PSL sampled from Canadian waters ranged in size from 78-151 mm FL. Six sites were sampled with no more than eight PSL sampled from any one site (Sidney Channel).

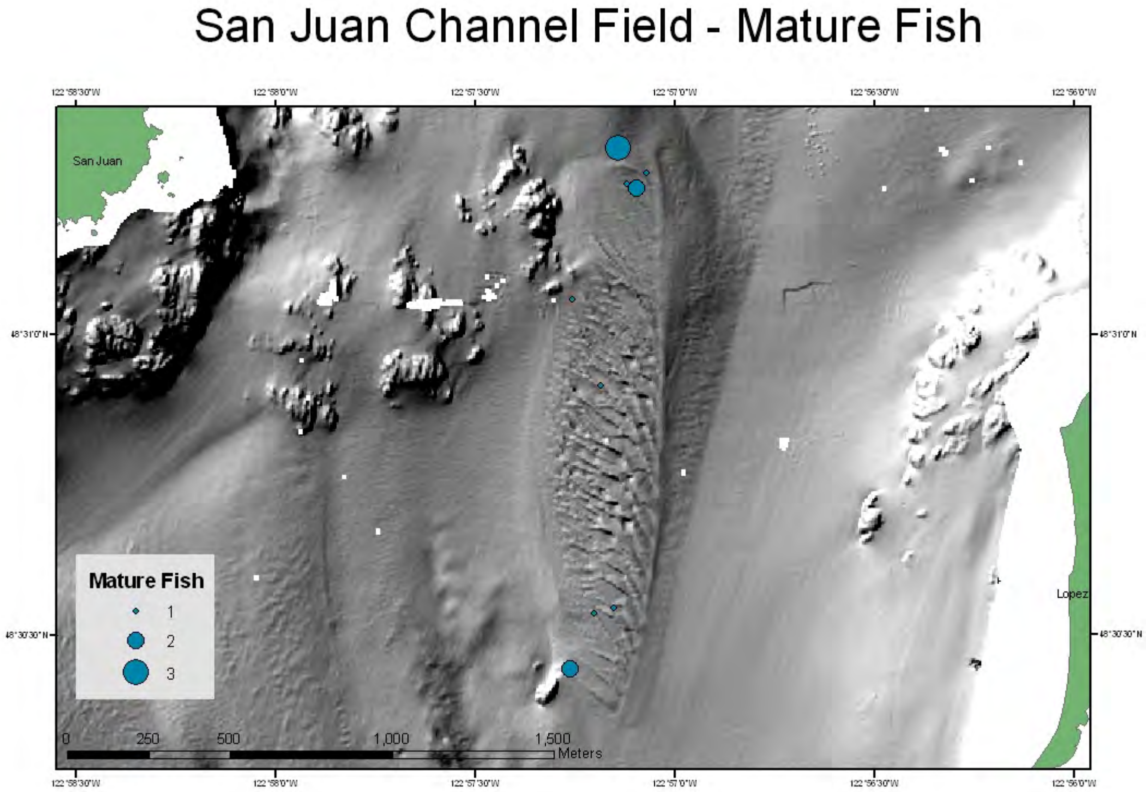


Figure 46. Location of where on the San Juan Channel sand wave field that mature PSL were caught.

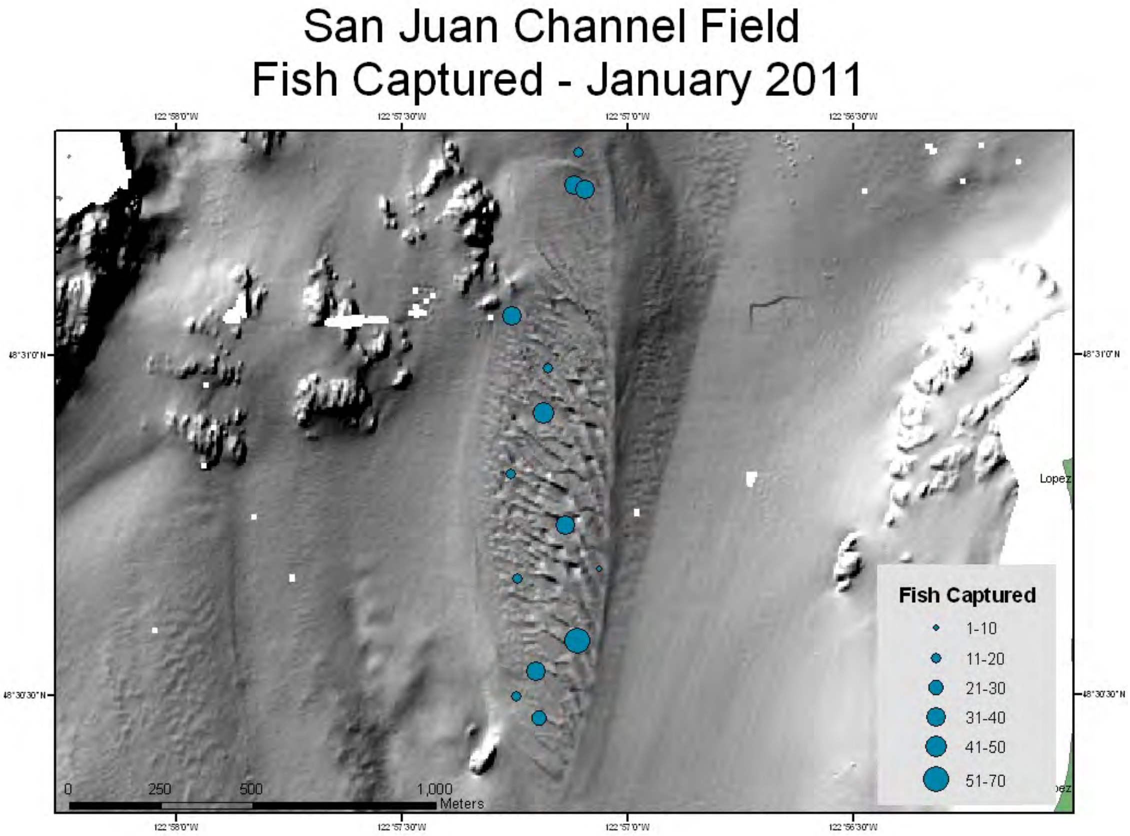


Figure 47. Location and numbers of PSL captured per Van Veen grab during the month of January 2011 and indicating a large number of fish reside in the bedform during winter months.

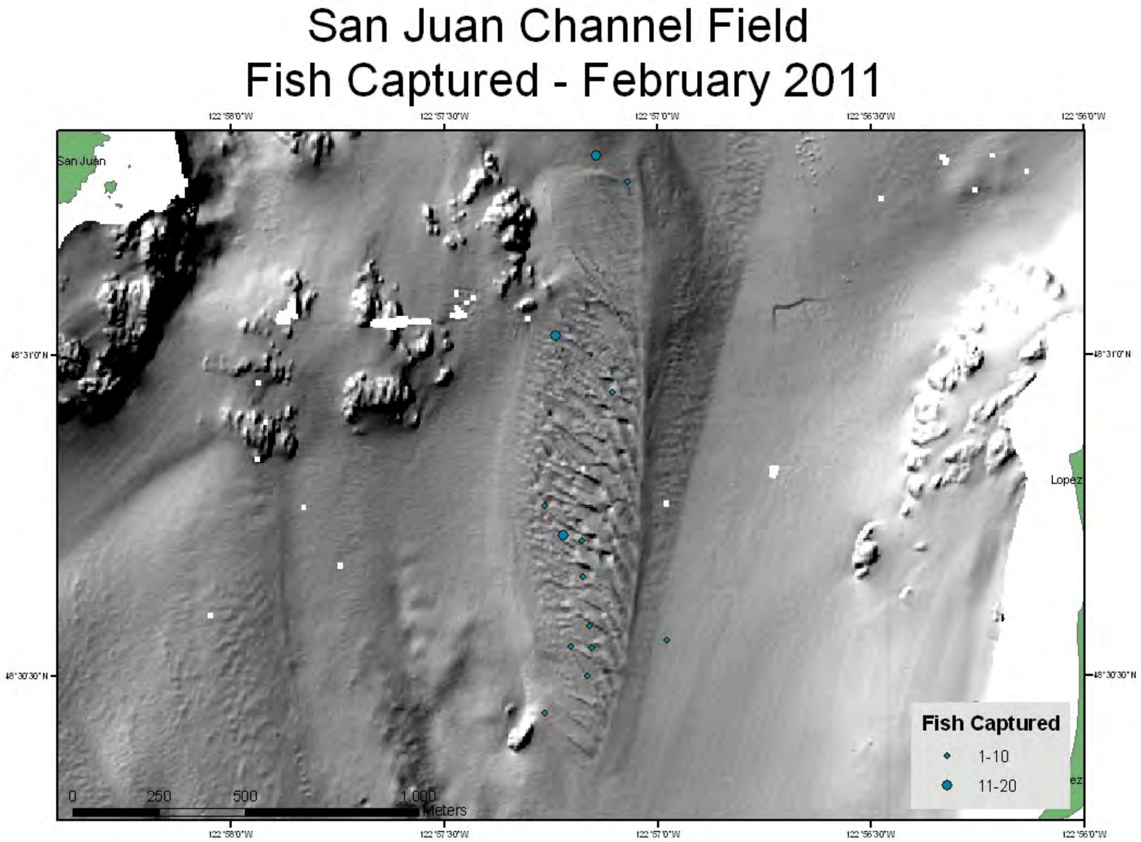


Figure 48. Locations and number of PSL captured in Van Veen grab samples taken in February 2011 from the San Juan Channel sand wave field indicating a large number but a decline from that captured January 2011.

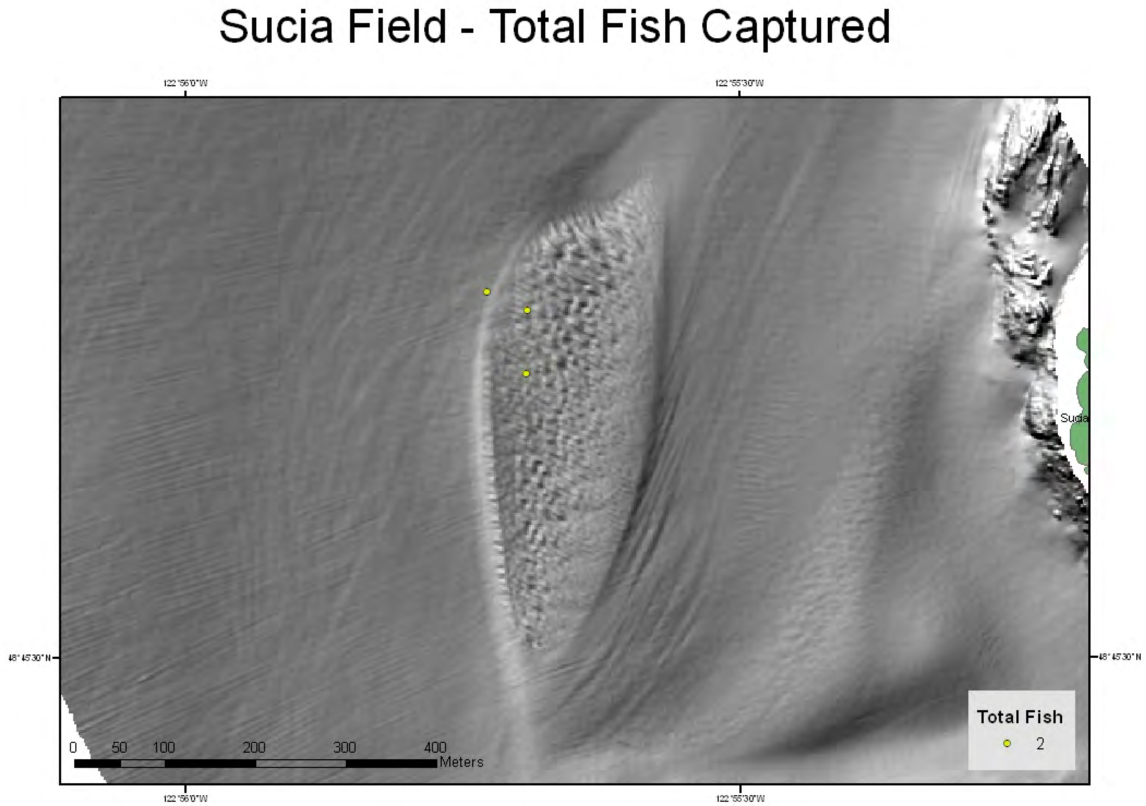

Sucia Field - Total Fish Captured

Figure 49. Locations and number of fish collected from the sediment wave field off Sucia Island indicating that PSL reside here but not in the numbers found in the San Juan Channel sand wave field where the grain size is finer and less biogenic material is found.

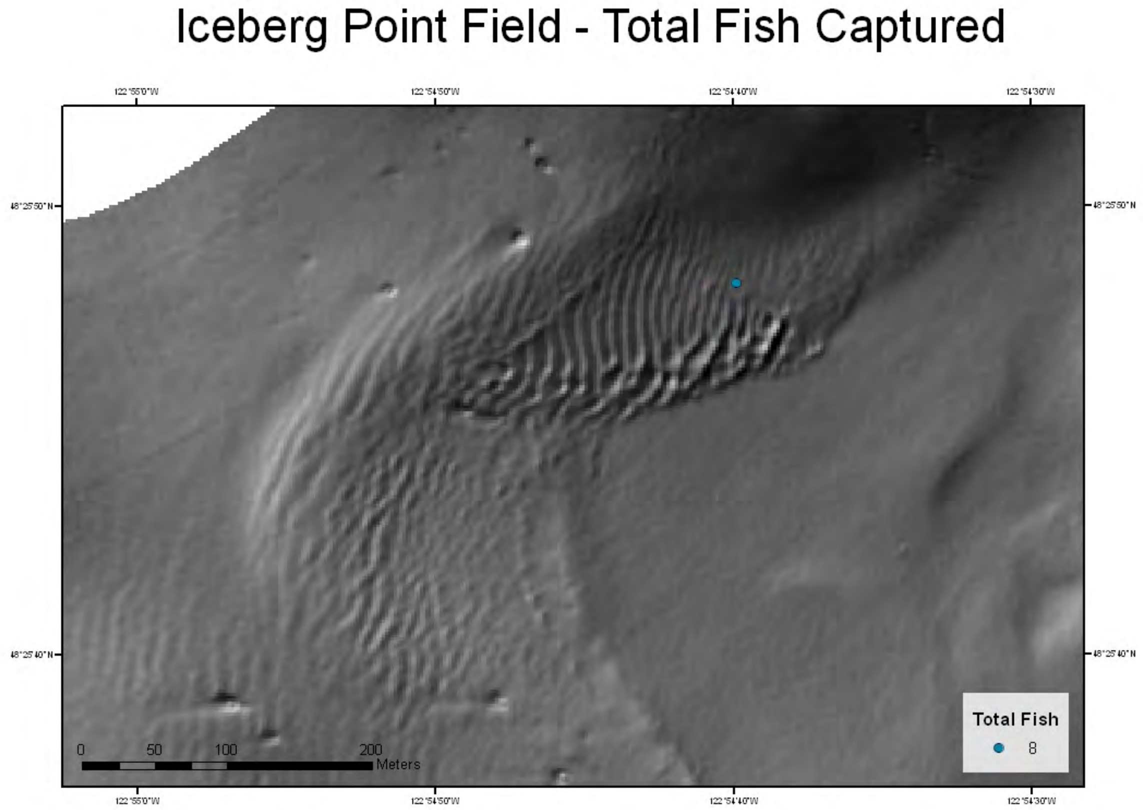


Figure 50. Location and number of PSl captured in a Van Veen sediment grab sampler from the sediment wave field off Iceberg Point, southern Lopez Island.