SWINOMISH CRAB ABUNDANCE MONITORING PROGRAM INTERTIDAL METHODS

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INTRODUCTION

Dungeness crab [*Metacarcinus (Cancer) magister*] have a complex multi-phase life history, occupying three spatially distinct marine habitats as they transition from pelagic larvae to juveniles occupying benthic intertidal habitat, and finally to subtidal habitats as adults. After larval crab hatch, they spend up to five months in the water column subjected to vast ranges of conditions as they are transported by tidal currents and circulation (as reviewed in Rasmuson 2013). During this time, larvae transition through five zoeal stages and a megalopal post-larval stage before they begin to settle out of the water column. These late-stage crab larvae are active swimmers that preferentially swim toward favorable habitats for settlement (Eggleston & Armstrong 1995). As *M. magister* transitions from pelagic to benthic environments they settle in mass recruitment events which are temporally pulsed throughout the settlement season from April to September (Dinnel et al. 1993, Eggleston & Armstrong 1995, Shanks 2017, Galloway et al. 2017). After settlement, juvenile Dungeness crab will remain in intertidal benthic habitats for a period of six to 12 months depending on several factors including timing of settlement, habitat quality, and possibly settlement density (reviewed in Rasmuson 2013).

Larval availability and the number of individuals surviving the transition from the pelagic to benthic habitats ultimately influence the number of recruits from a cohort or year-class. This relationship can vary greatly across spatial and temporal scales. Generally, underlying site-specific oceanographic processes (e.g., circulation and tidal currents) and biological responses (e.g., active habitat selection, larval flux) drive variability in settlement density (Eggleston & Armstrong 1995, Roegner et al. 2003, Shanks 2013). Recruitment pulse magnitudes have been shown to vary greatly, with megalopal densities between tens to thousands/m^2 reported (Dinnel et al. 1993, Galloway et al. 2017). These recruitment events represent an important contribution to food web dynamics as nutritional energy is transferred from the pelagic to benthic environment.

Dungeness crab spend the first several months after post-larval settlement occupying highly-structured intertidal habitats (Stevens & Armstrong 1984, Fernandez et al. 1993, McMillan et al. 1995). While occupying these intertidal habitats, Dungeness crab shelter in place buried in sand or under macrophytes and rocks or shell during low-tide periods (McMillan et al. 1995). There are several common sampling tools that have been used to assess the relative density of mobile benthic marine organisms (such as young-of-the-year Dungeness crab) including: excavated quadrats, trawls, trapping, artificial substrate, and visual SCUBA or video surveys (Stevens & Armstrong 1984, Dinnel et al. 1987, Armstrong et al. 1989, McMillan et al. 1995). Each of the sampling tools have their advantages and disadvantages and when choosing a monitoring method, every attempt should be made to recognize their inherent biases. Monitoring for newly settled Dungeness crab (< 30 mm carapace width) in structured intertidal habitats, such as those found in Puget Sound, has been shown to be most efficient using the excavated quadrat method during low tides when the crab are sheltering in place on the beach (Dinnel et al. 1987). Most other monitoring methods either fail to sample smaller size classes (e.g., crab < 10 mm carapace width) of Dungeness crab or are generally less effective in structured habitat types (Stevens et al. 1984, Dinnel et al. 1987).

In addition to tracking settlement and recruitment dynamics across intertidal habitats, tracking the size of Dungeness crab instars can provide insight into settlement cohort dynamics and variability in growth rates between sites and years (Orensanz & Gallucci 1988). Dungeness crab grow through the process of molting, where they shed their exoskeletons and regenerate a new, larger shell. During the first year, a Dungeness crab can molt up to six times as they quickly grow from 5-7 mm to 30-40 mm, at which point they transition out of the intertidal into deeper habitats (Armstrong et al. 1989, Gunderson et al. 1990) and molt an additional six times before reaching sexual maturity (as reviewed in Rasmuson 2013). Because these organisms shed their shells as they grow, aging juveniles is a difficult task for crustacean biologists (as described in Wahle et al. 2004) and most studies have relied upon identifying modes in histograms for interpreting size at instar stage (Hartnoll 1982) (the term instar stage refers to the number of molts an individual has undergone; e.g., stage 2 = second molt after the transition from megalopa to juvenile instar). However, this method of interpreting age can be problematic if, for example, there are multiple recruitment cohorts that start out at significantly different sizes or there is a protracted settlement period during which the environmental conditions driving growth patterns shift (Hartnoll 1982).

The Swinomish Indian Tribal Community (SITC) Crab Abundance Monitoring Program seeks to fill extensive gaps in our knowledge of early life history phases of Dungeness crab in Washington's inland waters. Given these gaps, it is essential to develop a modern baseline of biological and physical metrics in the region so we can determine potential limitations to adult populations and assess the need for adaptive management through time. Thus, our program focused on collecting data on the megalopal and juvenile instar life history stages of this species using light traps (see Cook et al. 2018) and intertidal quadrat sampling, respectively. This document concentrates on our juvenile crab sampling methods by outlining standardized survey procedures utilizing the intertidal quadrat method (adapted from Dinnel et al. 1993) in the greater Puget Sound region.

Data collected will provide SITC with a greater understanding of the spatial and temporal (seasonal and annual) patterns of larval and juvenile young-of-the-year (YOY) Dungeness crab populations in two distinct marine basins, Whidbey and San Juan. The results from this baseline juvenile stage study, the first of its kind in Puget Sound since the 1980s (MacKay & Weymouth 1935, Weymouth & MacKay 1936, Dinnel et al. 1987, 1993, Orensanz & Gallucci 1988), will be used by SITC and project partners to assess YOY nursery habitats, monitor relative changes in population dynamics over time, develop a juvenile Dungeness crab growth model for these areas, and ultimately provide data to improve resource management.

METHODS

Intertidal Dungeness crab survey: Quadrat method

Site selection

We know from research conducted in Washington's coastal estuaries and Puget Sound that YOY Dungeness crab prefer to settle and rear in complex, low-profile, intertidal habitats that offer refuge from predation without high densities of *Hemigrapsus* spp. (Dinnel et al. 1987, Eggleston & Armstrong 1995, McMillan et al. 1995, Banks & Dinnel 2000, Visser et al. 2004). Habitat complexity refers to any three-dimensional structure including *Ulva* spp, eelgrass (both *Zostera marina* and *Z. japonica*), mixed algal composition and/or mixed sediment types (cobble, gravel, and sand), and shell. Using our knowledge of the area and a combination of digital resources (e.g., Google Earth; GIS pictometry and bathymetry), we identified littoral beaches in northern Whidbey and southern San Juan basins exhibiting these habitat criteria (Figure 1 A & B). To further narrow down possible monitoring locations we prioritized sites based on 1) proximity to our larval flux monitoring locations (< 10 km and within the same oceanographic sub-basin), 2) accessibility or proximity to a road or other access point, and 3) granted permission to access tidelands. Sites located in small coves (low current connectivity) were excluded as well as extreme high current areas (e.g., Deception Pass). We also excluded beaches with heavy upland development (e.g., the city of Oak Harbor).

Field visits to the sites were conducted to verify results of the site selection exercise. These site visits, conducted during a low tide, allowed staff to ensure there was adequate area of each desired habitat type (> 0.5 ha). During these site visits we also qualitatively examined the habitat polygons for the presence of crab species (e.g., presence of molts or live Dungeness crab or *Hemigrapsus* spp. instars).

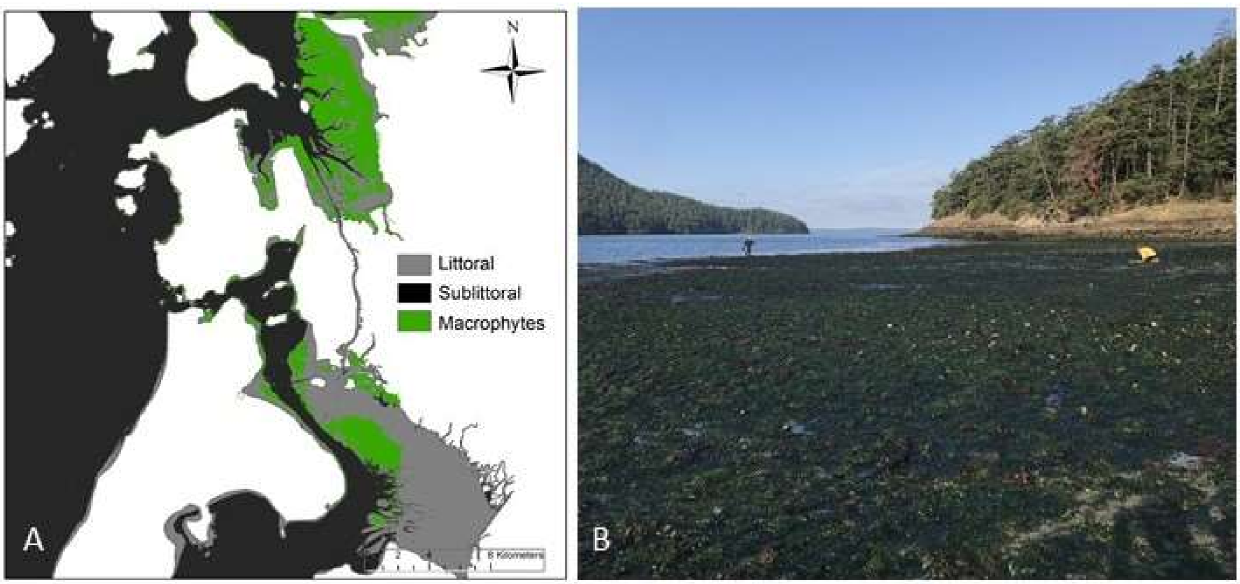

Figure 1. (A) Regional map of the Swinomish monitoring area depicting available littoral zones with mapped macrophytes (Washington Department of Natural Resources, Skagit County Intertidal Habitat Inventory 1996), and B) example target habitat, a low-profile mixed sediment beach with macrophytes.

Sampling design

To capture the annual population dynamics of YOY Dungeness crab, monitoring should take place on all low-tide cycles (roughly bi-weekly) through the settlement season from April to September and periodically (~3 times) during winter month night time low-tides. Over the course of a year, this schedule results in roughly 14 site visits. An alternative monitoring approach is to monitor sites on a monthly schedule, year round (n=12 sampling events per year). Sampling more intensively during the settlement season provides a better picture of the settlement and survival dynamics, whereas consistent monthly monitoring provides more information on recruitment metrics of YOY cohorts and the relative growth patterns of these cohorts. Swinomish initially started following the first method of sampling intensively during settlement season and less intensively in the winter. However, beginning in 2021, Swinomish Fisheries switched to monthly sampling.

Using ArcGIS 10.6, a 0.5 ha polygon is generated for each sampling location, delineating the intertidal zone containing target habitats from roughly -0.3 to +1.8 m relative to mean lower low water (MLLW) (skewed to the lower intertidal or optimal habitat bands for broad expansive beaches). Within the predefined plot, 10 to 12 randomly generated unique points [10 to be sampled and two extras in case one random point is unsuitable for sampling (e.g., point is underwater or on a boulder)] are plotted for each sampling event (Figure 2) and waypoints uploaded to a Garmin GPS unit. Each quadrat waypoint is given a unique identification number (e.g., 21COR101; 21 = year, COR = site code, 101 = waypoint number) which is carried through to all data types collected at that location and date.

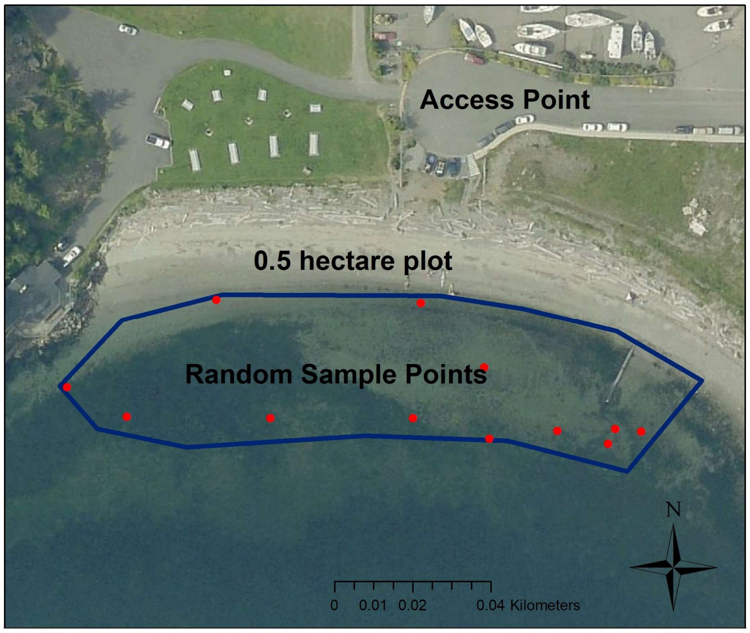

Figure 2. Example 0.5 hectare polygon plot on a mixed sediment beach with macrophytes and 12 randomly generated waypoints.

At the beach, monitoring should begin either (1) roughly when the tide reaches the 0 m relative to MLLW tide level on the ebb before the low or (2) one hour before the low, whichever is earlier. Sampling should not occur on days when the tide fails to reach the 0 m relative to MLLW tide level. A field team of two to four people can conduct a 10-sample survey and process samples in the field in 1.5 to 2 hours, depending on weather conditions and crab densities. Roughly 45 to 60 minutes are spent setting up the survey and collecting samples and 30 to 60 minutes are spent processing samples and measuring Dungeness crab on site.

Materials list - based on a two to four-person field team

- 2 -19-liter (5 gallon) buckets
- 10 - 4 mm mesh laundry bags (laundrybags.com; 18” x 24” mesh)
- 2 - small garden trowels
- 2 - clip boards and field forms (Appendix I)
- 10 - survey flags
- Sample ID tags (waterproof paper)
- 2 to 4 - enamel 13x17 inch butcher trays, preferably white (or equivalent)
- 10 - sample jars (preferably plastic) with lids
- 2 plastic metric dial calipers (precision, 0.1 mm)
- GPS unit and spare batteries
- 2 - 0.25 m^2 quadrats
- Crab field identification guides
- Gloves and other protective field gear – recommended

Sample collection

In the field, a surveyor navigates to the randomly generated waypoints and places sample ID tags pre-labeled with unique identification numbers into the substrate using survey flags. At each sampling location, a 0.25 m^2 quadrat is placed at the base of the flag in the upper left corner of the quadrat (Figure 3, upper left with surveyor facing the uplands). Samples should not be collected if the flag is still in the water or has become inundated during the course of the sampling. If necessary, a flag can be placed at one of the two extra random waypoints or haphazardly moved to a more optimal tidal elevation above the water line and a new waypoint taken for the site. The surveyor(s) then fill out the header information on the field data sheet (Appendix I). Using methods described below, at each flag, surveyors will first note the waypoint ID, record the percent cover of macrophytes, and then record substrate percent cover. Surface sediment material is then excavated from the quadrat and all crab are identified to species, enumerated, and measured (Dungeness crab only).

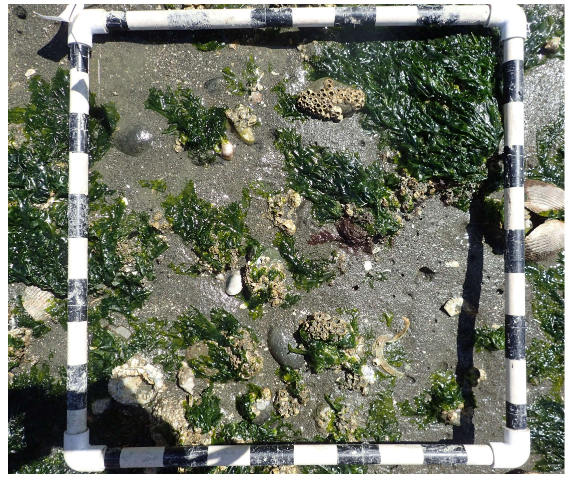

Figure 3. A 0.25 m^2 quadrat placed at the base of the flag marking the sample waypoint.

Percent cover - macrophytes

First, the surveyor(s) categorizes the percent cover of macrophyte groups present. For this survey we have classified the macrophyte types as eelgrass (*Zostera marina, Zostera japonica*), algae (green blade, green filamentous, brown blade, red filamentous), other (define), or bare. When quantifying the percent cover of macrophytes, record the 'true' cover percentage in 5% increments, not to exceed 100% for any category, allowing for the documentation of overlapping vegetation. Also, document the percent cover bare substrate to calculate the percent of the plot that is covered with overlapping vegetation species. For example, if a quadrat has 55% cover of green blade, 20% brown blade, and 30% bare, we will know that 5% of the green blade and brown blade are occupying the same space. If a trace amount of a particular macrophyte type is present but less than 5% cover, record 1%.

Percent cover - substrate

After the macrophyte cover has been recorded, if needed, carefully move aside vegetation in order to visualize the surface sediments. When performing this step be sure to thoroughly examine the removed material for crab megalopae and/or instars that may be sheltering in the vegetation. Sediment composition should be recorded by percent cover of the following size classes: cobble (64 to 256 mm), coarse gravel (16 to 64 mm), fine gravel (4 to 16 mm), sand (0.06 to 4 mm), mud (< 0.06 mm), shell (whole, half, or hash), and other (e.g., wood waste, hard pan, boulder) (Wentworth 1922). Percent cover data is collected in 5% increments, ensuring that the sum of the percent cover of all sediment classes does not exceed 100%. If a trace amount of a particular substrate size class is present but less than 5% cover, record 1%.

Excavate quadrat

Position a bucket with a 4 mm mesh bag draped over the opening adjacent to the quadrat. Using a small trowel, excavate the interior of the quadrat to a depth of approximately 3 to 5 cm. With the sample material in the bag, remove the sample identification tag from the flag and place it in the bag, secure the top, and remove from the bucket. Wash the bags of sediment in the water on site, removing fine sediment and debris less than 4 mm from the bag. The sample is then set aside and surveyor(s) proceed to the next flag.

Sample processing

Empty the contents of the bag onto an enamel (or equivalent, preferably white) sorting tray. Batch processing may be necessary for samples containing large volumes of gravel. Carefully sort through the sediment and vegetation and retain all crab species (megalopae and instars). All crabs found are identified to species and enumerated. For each Dungeness crab in the sample, carapace width (CW), carapace height (CH), and total height (megalopae only) are recorded to the nearest 0.1 mm using manual dial calipers (Figure 4). Measurements can be taken in the field once researchers are familiar with species identification and measurement protocol. Instar CW is measured anterior of the 10th antero-lateral spines, while CH is measured from the back of the carapace to the tip of the rostrum (Figure 4). Megalopa CW is measured at the widest point of the carapace while CH is measured from the back of the carapace to the tip of the rostral spine with calipers (Figure 4). Total height of the megalopa is measured from the rostral spine to the posterior spine (Figure 4).

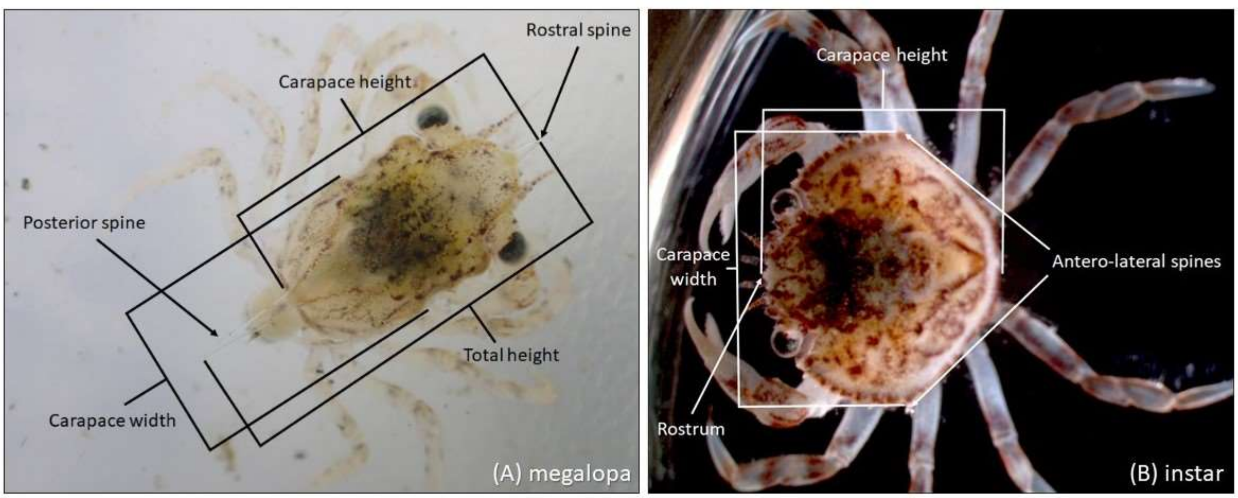

Figure 4. Measurement locations for carapace width, carapace height, and total height (megalopae only) on Dungeness crab megalopa (A) and instar (B).

If species cannot be identified or measured at the field site, they are transferred to plastic jars and kept on ice or frozen until laboratory processing. The rest of the organisms can be returned to the beach once they have been counted and measured (Dungeness crab only).

Crab species common to Puget Sound can be identified using *Crabs and Shrimp of the Pacific Coast* (Jensen 2014) and *Marine Invertebrates of the Pacific Northwest* (Kozloff 1996).

Dungeness crab growth and instar stage assignment

Carapace widths of Dungeness crab by instar stage vary throughout their range from California to Alaska and can exhibit significant temporal variability within regions (Appendix II).

Currently, this project is limited to differentiating between recent settlers (J1 instars) and recruits (defined here as J2+ instars). The presence of multiple settlement cohorts with significantly different CWs of J1 instars precludes us from assigning instar stages past J2 based on CW alone. Measurements of CW and settlement timing, and ratios of CH to CW will ultimately be used to develop cohort specific growth models that will allow us to differentiate between instar stages [Juvenile (J) 1 through J13]. Identifying J1 instars is a relatively straightforward process as they have a CH to CW ratio of 1:1 (e.g., CH = 6.0 mm, CW = 6.0 mm) despite the relatively wide range of initial sizes (Dinnel et al. 1993, SITC unpublished data). After the molt to J2, the CH is roughly 88% of the CW (Dinnel et al. 1993) and the CH:CW ratio progressively decreases as the growth of the CW outpaces the growth of the CH with each successive molt (MacKay & Weymouth 1935, Dinnel et al. 1993). SITC Fisheries is currently conducting a laboratory and field analysis of Dungeness crab CW to CH ratios to determine if the CH:CW ratio is consistent across all instar stages by cohort.

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APPENDIX I: Swinomish Fisheries Department Dungeness crab intertidal monitoring data sheets

Juvenile Crab Intertidal Data Sheet

Site Information		
Site ID:	Date(yyyymmdd):	Quadrat Size: 1/4 m^2
Surveyors:	Low Tide: @	

Weather: Clear Partly Cloudy Mostly Cloudy Heavy Rain Light Rain Fog/Mist

Quadrat	Q1	Q2	Q3	Q4	Q5
Waypoint #					
Time					

Algae / Seagrass % Cover					
Quadrat	Q1	Q2	Q3	Q4	Q5
Zostera marina					
Zostera japonica					
Green blade					
Green filamentous					
Brown blade					
Red blade					
Red filamentous					
Other:					
Bare					

Substrate % Cover					
Quadrat	Q1	Q2	Q3	Q4	Q5
Cobble 256-64mm					
Coarse Gravel 64-16 mm					
Fine Gravel 16-4 mm					
Sand 4-.06 mm					
Mud <.06mm					
Shell					
Other:					

Crab Counts (Record Other Species on Reverse)					
Quadrat	Q1	Q2	Q3	Q4	Q5
No crab	☐	☐	☐	☐	☐
M. magister instars					
Hemigrapsus oregonensis					
Pagurus hirsutiusculus					
Pagurus granosimanus					

SITE NOTES:

SampleID = SiteID+YYMMDD+Wpt# Page ______ of ________ Data Entry Complete: ________________

Juvenile Crab Measurement Sheet

Measurer(s): Date measured

Recorder: Fresh / Frozen

Site ID+Date	Quad ID	Crab Species	n	CW mm	CH mm	TH mm	Sex	QA

CW = Carapace Width; CH = Carapace Height; TH = Total Height; Sex = M: Male or F: Female for individuals >30mm

*Preserved Sample Page ______ of ______ Data Entry Complete: ______________

APPENDIX II: Literature review of juvenile Dungeness crab size at instar stage across their range. All carapace widths (mm) reported exclude the 10th anterolateral spines.

	WASHINGTON							
	Dinnel et al. 1993			Orensanz & Gallucci 1988		Armstrong et al. 1987		Cleaver 1949
Instar stage	Coastal	Hood Canal	Puget Sound	San Juan Island 1982 early settlers	San Juan Island 1984 late settlers	Grays Harbor (Nearshore)	Grays Harbor (Estuary)	Grays Harbor
J1	6-8	4-6	4-6	7.4	6.7	7.3	7.2	6.0
J2	10-12	6-8	6-8	11.7	9.8	10.9	10.1	9.0
J3	13-15	9-11		16.6	14.2	15.3	14	12.0
J4	15-19			24.4	19.4	20	19.8	16.0
J5	20-28			31.7	26.9	25.9	25.1	22.4
J6				42.1	39.9	32.3	30.9	28.8
J7				53.8		40	37.1	35.2
J8				67.2		52.5	46	44.5
J9				84.6		55.8	58.6	56.4
J10						84.2	73.7	68.3
J11							87.8	84.9
J12								
J13								

	CALIFORNIA		OREGON		BRITISH COLUMBIA	
	Collier	Poole	Waldron 1958		Butler	Mackay & Weymouth
	1983	1967	Spring 1948	Summer 1954	1961	1935
Instar stage	San Francisco Bay	Bodega Bay	lab-reared from OR coast		Queen Charlotte Islands	Boundary Bay
J1	7.3	7.1	7.4	8.1	6.5	4.9
J2	10.7	11.4	10.4	11.7	9.4	6.9
J3	14.2	15.8	12.8	16.3	12.9	9.1
J4	18.4	21.0	16.3	21.9	17.3	12.5
J5	23.5	28.3	18.9		22.6	17.0
J6	29.9	37.0	22.0		29.1	22.4
J7	37.5	47.5			37.0	29.4
J8	47.0	63.4			46.6	38.3
J9	58.6	85.0			58.4	49.0
J10	72.6				72.7	61.3
J11	90.0				90.2	75.2
J12						89.4
J13						