Padilla Bay National Estuarine Research Reserve
Technical Report No. 28

Monitoring for invasive crabs in seagrass habitat in the Padilla Bay National Estuarine Research Reserve, Skagit County, Washington

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Abstract. Two methods of monitoring for invasive crab were compared: minnow trap collection and shallow trays lined with 3-mm mesh. The traps (3) and trays (7) were set along one transect on the eastern shore of Padilla Bay for one year. The traps were deployed 24-hours prior to tray collection and tray collection occurred once a quarter.

The minnow trap method took two hours per quarter whereas the tray method took 60-90 hours per quarter. The total number of crabs caught in minnow traps was low (63) compared to trays (9,326). In this study, minnow traps caught crabs ranging in carapace width from 15.5–85mm while the trays caught pagurids ranging from 0.9-8.8mm shield length and other crabs (*Cancer magister*, *Hemigrapsus oregonensis*, *Telmessus cheiragonus* and *Pinnixa tubicola*) ranging from 2.3-71.1mm carapace width. No invasive crabs were caught with either method which would indicate there are no invasive crabs at this site in Padilla Bay.

Baseline data for native crabs was obtained from the tray method. The greatest number of new recruits (< 6.0 mm SL) for *Pagurus hirsutiusculus* were present in April, the highest number of females without eggs were present in October and the highest number of females with eggs were present in April. For *Pagurus granosimanus*, the greatest number of new recruits (< 6.0 mm SL) were present in October and the highest numbers of females with eggs were present in January. The greatest number of *Hemigrapsus oregonensis* were present in January and gravid females were only present in July. The highest number of *Cancer magister* were present in January. No females were present during any sampling and no *C. magister* were present in the trays in July.

Pagurids were found exclusively in non-native snail shells (*Batillaria, Nassarius*). Parasites were present on *Pagurus hirsutiusculus* (bopyrid isopods and rhizocephalans).

Acknowledgements

The funding for this pilot project was obtained by Dr. Andrew Lohrer, North Inlet-Winyah Bay NERR, from the NOAA Aquatic Nuisance Species Task Force. Dr. Dave Bushek and Mary Jo Hartman, University of South Carolina, continued the project when Dr. Lohrer accepted a position in New Zealand. The guidance committee for the project included: Dr. Maurice Crawford (NOAA/ERD), Dr. Steve Rumrill (South Slough NERR), and Dr. Kerstin Wasson (Elkhorn Slough NERR).

The project at Padilla Bay would not have been possible without the help of many people. I was aided in sample retrieval by: Carly Althoff, Dave Weinman, Brian Sowell, Christa Strickwerda (PBNERR WCC/Americorps), Doug Bulthuis (PBNERR), Cara Peffley, Keith Anderson (Skagit Conservation District WCC/Americorps). The following people helped with sample sorting: Carly Althoff, Dave Weinmann, Gwen Heisterkamp, Brian Sowell (PBNERR WCC/Americorps), Robin Cottrell, Suzanne Shull (PBNERR), Cara Peffley, Keith Anderson (Skagit Conservation Dist. WCC/Americorps). A few patient women helped with sample identification: Gwen Heisterkamp, Heidi Hendrix, Christa Strickwerda (PBNERR WCC/Americorps).

A special thanks goes out to Pat & Strand Wedul (volunteers) who helped me set minnow traps in the middle of the night on two occasions, and to Heidi Hendrix and Brian Sowell (PBNERR WCC/Americorps) who set minnow traps for me in July.

Our neighbor on Samish Island, Blau Oyster, gave us permission to take shell from their pile, which I greatly appreciated.

I want to thank Pat McLaughlin for her help sexing the pagurids and help with parasite identification and--last but not least--to thank Dr. Douglas Bulthuis for his review and suggestions on the draft.

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Introduction

The National Estuarine Research Reserve System (N.E.R.R.S.) was established in 1972 under the Section 315 of the Coastal Zone Management Act. It includes 25 sites around the coastal United States. The purpose of these sites is research, education, and stewardship in estuaries. The Padilla Bay N.E.R.R. was designated in 1980 and is located north of Seattle, Washington (Figure 1). It consists of approximately 180 upland acres and 10,600 acres of tide flat which includes about 7,500 acres of seagrasses (*Zostera marina* and *Zostera japonica*).

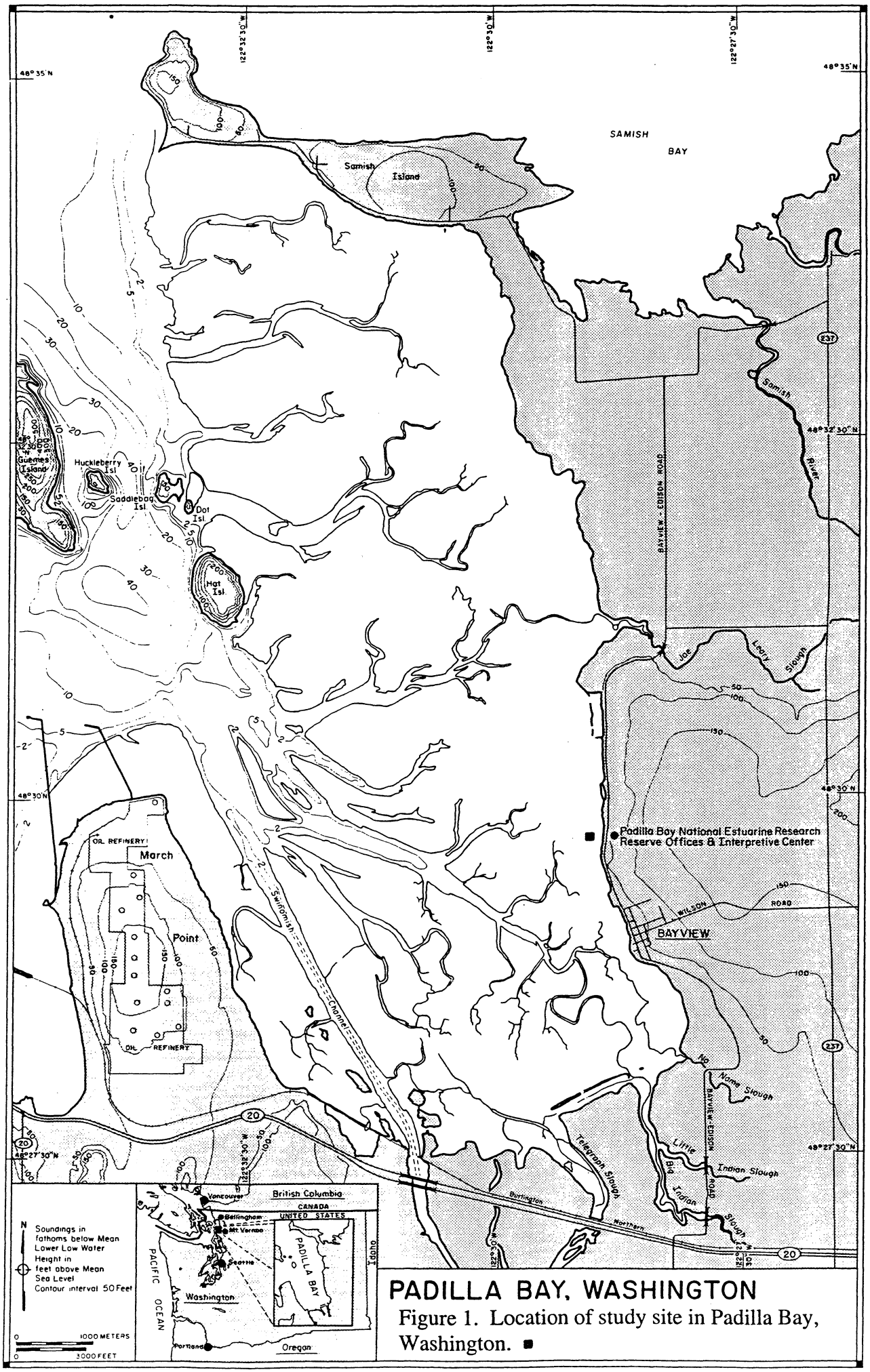
Dr. Andrew Lohrer (North Inlet-Winyah Bay N.E.R.R.,South Carolina) received funding from the NOAA Aquatic Nuisance Species Task Force to coordinate an invasive crab monitoring effort that included eight N.E.R.R. sites (North Inlet-Winyah Bay NERR, SC; ACE Basin NERR, SC; Sapelo Island NERR, GA; Guana Tolomato Matanzas NERR, FL; Rookery Bay NERR, FL; Padilla Bay NERR, WA; South Slough NERR, OR; San Francisco Bay NERR, CA; and Elkhorn Slough NERR, CA). Dr. Dave Bushek (University of South Carolina) was the Principal Investigator for the project. The database from that project will be posted to a website This current report contains only the data collected at Padilla Bay NERR.

The European green crab (*Carcinus maenus*) and the Chinese mitten crab (*Eriocheir sinensis)* are the only two invasive crabs known in Washington State. The European green crab is advancing up the coast of Washington and there have been reports of this crab on the west coast of Vancouver Island. Chinese mitten crab were first discovered in San Francisco Bay in 1992. They are native to the Yellow Sea of China and Korea. One mitten crab was found in the Columbia River in 1997. Mitten crabs spawn in estuaries and juveniles migrate upstream to rear in freshwater (Washington Sea Grant, 1998).

Data collected in this study also serves as baseline data for native crabs caught in traps or trays. Those crabs include *Cancer magister*, *Hemigrapsus oregonensis* and *Telmessus cheiragonus*.

H. oregonensis are the most abundant and widespread crab in Puget Sound (Jensen, 1995). This crab feeds at night, mainly on diatoms and *Ulva* or related green algae (Garth and Abbott, 1980). In the Puget Sound, ovigerous females are common from December to early August. Eggs hatch beginning in May (Dinnel, 2001).

Cancer magister are common in eelgrass beds. They are carnivores, eating small crustaceans, clams, oysters, and fish (Garth and Abbott, 1980). Mating occurs from March-



PADILLA BAY, WASHINGTON

Figure 1. Location of study site in Padilla Bay, Washington. ■

July in Puget Sound and April – September in British Columbia. The larval period, from hatching through the megalops phase, lasts 3-5 months (Garth and Abbott, 1980). Megalopae settle in May or June on the coast of Washington and as late as August or September in Puget Sound (Dinnel, 2001).

Telmessus cheiragonus are found subtidally in or near eelgrass beds (Jensen, 1995). They mate in the spring, but little is known about mating and egg bearing in Puget Sound (Dinnel, 2001).

Kozloff (1983) reports *Pagurus hirsutiusculus* are common in upper midlittoral zone (+7 to +4) but are also found in the lower midlittoral zone (+4 to 0). It is the most common intertidal hermit crab in Puget Sound (Jensen, 1995; Dinnel, 2001). Females are generally gravid from December through August in California (Dinnel, 2001).

Pagurus granosimanus is common in middle and low intertidal pools and under rocks on protected rocky beaches (Jensen, 1995). They are most common in the infralittoral fringe (0 to –3.5) (Kozloff, 1983). Gravid females are common in April and May in Central California, but little else is known of the reproductive biology (Dinnel, 2001).

Padilla Bay N.E.R.R. currently monitors three sites in Padilla Bay for green crab using Washington State Department of Fish and Wildlife (WDFW) protocols. Three minnow traps are deployed monthly at each site from April - September. Padilla Bay monitored two sites in 2001 and added an additional site in 2002/2003. No *C. maenus* were found with the WDFW method from 2001-2003. This method will be compared to the method used in the current study.

Our objectives with this study were: 1) to document and remove any invasive crab species present in the traps or trays, 2) to document native crabs, size frequencies, reproductive status, parasites, and seasonal differences, and 3) to compare the two methods of sampling (WDFW/NERR).

Materials & Methods

Protocols. Protocols for both approaches to monitoring (NERR and WDFW) are included in Appendix 1. The NERR method used shallow trays lined with mesh (3 mm) and the WDFW method used minnow traps (mesh size 12 mm).

Site Placement. Several sites were scouted (northern Padilla Bay, west of the Sullivan Minor salt marsh, and the Observation Deck site) as possible transect locations.

The observation deck site (Fig. 1) is the muddiest site with sparse *Z. japonica*, and scattered *Z. marina*, *Batallaria attramentaria* (exotic mud snail), and mud shrimp (*Upogebia pugettensis*) holes visible on the surface. Washington State Department of Ecology owns the tide flat at this site. This site was chosen for ease of access and safety during the winter samplings, which occurred in the middle of the night. It is likely crab recruitment is lower here than in northern Padilla Bay due to currents (Dr. Paul Dinnel, pers. comm.), but Dungeness crab (*Cancer magister*) forage on the tide flat at this site and hermit crabs are common, so we assumed we would catch crabs of any species large enough to move around to forage or larval crabs that might settle out of the water column.

Tide Height. Tidal height was determined using a tide chart (Swinomish Channel, entrance Padilla Bay) and the zero foot tide occurred around 10:25 a.m. on 5/11/02. Data collection protocols received from North Inlet-Winyah Bay suggested a zero tide deployment.

Traps.

NERR STUDY. Seven plastic trays were obtained from a local bakery outlet (55 cm x 65 cm x 6 cm). Diamond plastic mesh (Aquatic Ecosystems, Inc.) with 3-mm openings was used to line the trays and extended upwards 15-20 cm from the bottom of the trays. The mesh was attached to the trays using electrical ties. We obtained oyster shell from a local shellfish farm (Blau Oyster, Samish Island) and crushed it by driving over it. The shell was sieved through a 12 mm mesh and pieces larger than 12 mm were used to fill the trays. Five or six whole valves were placed on the surface of the shell hash after the trays were placed in the field. Since European green crab are attracted to shellfish sites, I hoped to increase the chance of attracting and catching this invasive crab by using a substrate to which they are attracted. The east coast study sites were located in oyster reef habitat.

WDFW STUDY. Three minnow traps (6 mm mesh) were deployed north of the plastic trays (75 paces, approximately 60 meters) at the same tide height as the trays approximately 24 hours prior to tray sampling. The minnow traps were baited with cat food (Friskies™ Salmon Dinner).

Location of Transect and Siting of Trays. Seven random numbers were chosen along a 30-meter transect prior to locating the transect in the field (1,6,10,13,18,25,29). The transect was

established on 5/11/02 at the zero tide height west of the Breazeale Interpretive Center observation deck in an area of sparse *Z. japonica*, scattered *Z. marina* and mud shrimp holes. GPS readings were obtained for each tray as it was placed in the field. The trays were buried to the upper edge of the plastic (flush with the surface of the mud), so the netting stuck up about 10 cm beyond the surface of the mud. Each tray was anchored with two wire "staples" at opposite corners and medium-sized rocks in the remaining corners (inside the trays). The mud removed to make room for the trays was placed shoreward of the trays on the mudflat.

Sampling.

NERR METHOD. The trays were placed in the field on 5/11/02. The trays were sampled on 7/11/02, 10/11/02, 1/15/03, 4/17/03 and 7/16/03. Because of the 60-day sampling period and the fact we did not rinse the samples, we did not use the 7/11/02 data in this analysis. The rest of the sampling periods are 90-97 days and the samples were rinsed when sorted (Table 1).

The October 2002, and January, April and July 2003 samples were bagged and carried back to the lab for initial rinsing and sorting (2-4 people, 60-90 minutes per tray) (Table 2). The bagged sample was emptied into two trays (12-mm mesh, 2-mm mesh) stacked inside a wading pool. The larger crabs and shell were retained on the upper tray with smaller crabs on the lower tray. Large forceps were used to pick the crabs from the trays and place them in plastic containers. Shell was transferred from the trays into sieves and rinsed in buckets of saltwater. Water from both the wading pool and buckets were sieved to make sure all crabs were retained. Crabs recovered from the oyster shell were frozen and identification, measurement, and sexing took place within a week. Species identification/sexing took 4-6 hours per tray for each sampling period (Table 2). Crabs were measured with plastic dial calipers (Scienceware, 0.1 mm accuracy).

In October, new oyster shell was deployed in the field immediately after the samples were bagged for transport to the lab. Once sorted, the oyster shell from the October sampling was rinsed, dried and stored for the next sampling event. That shell was deployed in January when the trays were retrieved. The shell collected in January was rinsed and stored and deployed again in April.

Species identification/sexing. Taxonomic keys and photos were used to identify crab to species. Sexing of *C. magister, T. cheiragonus*, and *H. oregonensis* was straightforward (u-shaped flap

Table 1. Sampling periods and sort methods for NERR Invasive Crab Pilot Project in Padilla Bay, Washington, in 2002-2003.

Sample collection months	Jul-02	Oct-02	Jan-03	Apr-03	Jul-03
Sampling period	5/11-7/10	7/11-10/10	10/11-1/14	1/15-4/17	4/18-7/16
Number of days/period	61	91	97	92	90
Sort methods	*sorted in field	*bagged	*bagged	*bagged	*bagged
	*no rinsing	*sorted in lab	*sorted in lab	*sorted in lab	*sorted in lab
	*measured	*rinsed by	*rinsed by	*rinsed	*rinsed
	live & released	pouring	pouring	pouring	pouring
	all but Pagurus	water over	water over	water over	water over
	*Froze Pagurus	*Froze all	and dipping	and dipping	and dipping
		crabs	sieve in	sieve in	sieve in
			bucket of	bucket of	bucket of
			water	water	water
			*Froze all	*Froze all	*Froze all
			but largest	but largest	but largest
			crabs (released)	crabs	crabs

Table 2. Comparison of two methods of monitoring for invasive crabs (Washington Department of Fish & Wildlife (WDFW) protocol, N.E.R.R. Invasive Crab Monitoring protocol). The WDFW method employed minnow traps and the NERR method used shallow trays lined with 3-mm mesh.

	WDFW minnow traps	**NERR trays**
Size range/presence		
Cancer magister	67-85 mm CW	11.3-71.1 CW
Hemigrapsus oregonensis	15.5-26 mm CW	2.3-24.5 mm CW
Pagurus granosimanus	not measured, present	1.2-4.8 mm SL
Pagurus hirsutiusculus	not measured, present	0.9-8.8 mm SL
Pinnixa tubicola	not present in traps	3.1-7.1 mm CW
Telmessus cheiragonus	not present in traps	28.1-52.3 mm CW
Total numbers caught	total counts low	total counts high
Cancer magister	7	203
Hemigrapsus oregonensis	38	3016
Pagurus spp.	18	n/a
Pagurus granosimanus	n/a	295
Pagurus hirsutiusculus	n/a	5807
Pinnixa tubicola	0	5
Telmessus cheiragonus	0	30
Calipers	General, 1 mm accuracy	Scienceware, 0.1 mm accuracy
Mesh size	12 mm	3 mm
Length of deployment	24 hours	3 months
Months traps/trays collected	10/02,1/03,4/03/7/03	10/02,1/03,4/03,7/03
Time estimates		
Equipment deploy or collect	1 hour	2 hours (2 people)
Sample sort per tray	n/a	60-90 minutes (4 people)
Sample sort, all traps or trays	minutes	7-10 hours (4 people)
Sample I.d./sexing per tray	n/a	4-6 hours/tray (1 person)
Sample I.d./sexing, all traps or trays	minutes	28-42 hours (1 person)
Total person-hrs per sampling	2 hours	60-90 hours
Parasites	not noted	noted, found only on P.
		hirsutiusculus
Gravid females	noted for H. oregonensis and	noted for all crabs
	C. magister, not for pagurids	
Tide height	0.0	0.0
Vegetation	Zostera japonica, Z. marina	Zostera japonica, Z. marina
Substrate	muddy	muddy

on abdomen on females, v-shaped flap on abdomen on males) for specimens greater than 6.0-mm carapace width. In order to measure the pagurids and sex them, we had to freeze them. Sex was determined under microscopic examination. Females had gonopores on the coxae of the second walking legs and males had gonopores on the coxae of the fifth walking legs.

WDFW METHOD. The minnow traps were deployed 10/10/02, 1/14/03, 4/16/03, 7/15/03. They were retrieved approximately 24 hours later, just before or just after the plastic trays were sampled. Carapace width of *Hemigrapsus* spp. and *Cancer magister* were measured with stainless steel slide calipers (Brand: General, 1 mm accuracy), and the crabs were sexed and released in the field. Total counts of *Pagurus* spp. were recorded and the crabs were released. We could not identify to species, measure or sex them without removing them from their shells which involves preserving them in some way and this was not in the WDFW protocol.

Results

NERR METHOD. The most abundant crabs in the trays were *Pagurus hirsutiusculus* (5,807), followed by *Hemigrapsus oregonensis* (3,016), *Pagurus granosimanus* (295), *Cancer magister* (203), *Telmessus cheiragonus* (30) and *Pinnixa tubicola* (5) (Table 2). *H. oregonensis* ranged in size from 2.3-24.5 mm carapace width (CW) while *C. magister* ranged in size from 11.3-71.1 mm CW.

Pagurus hirsutiusculus. In October, all crabs were sexed, regardless of size. There were no gravid females and a ratio of 5 females to every male. There were more females (13) with one parasitic isopod (bopyrid) than males (3) and two additional females had 2 bopyrids each. In January, April, and July, the crabs < 6.0 mm shield length (SL) were not sexed but were present in high numbers (1,034; 1,891; 320). Of the crabs ≥ 6.0 mm SL, all females were gravid with 46 females/male (Jan), 473 females/male (Apr), and 85 females present with no males (July). The greatest number of new recruits (< 6.0 mm SL) was in April. The highest number of females without eggs (< 6.0 mm SL) were present in October and the highest number of females with eggs (< 6.0 mm SL) were present in April (Fig. 2).

Pagurus granosimanus. In October, 3 of the 148 females were gravid. There were 2.4 females for every male ≥ 6.0 mm SL. Crabs under 6.0 mm SL were present in January, April and July

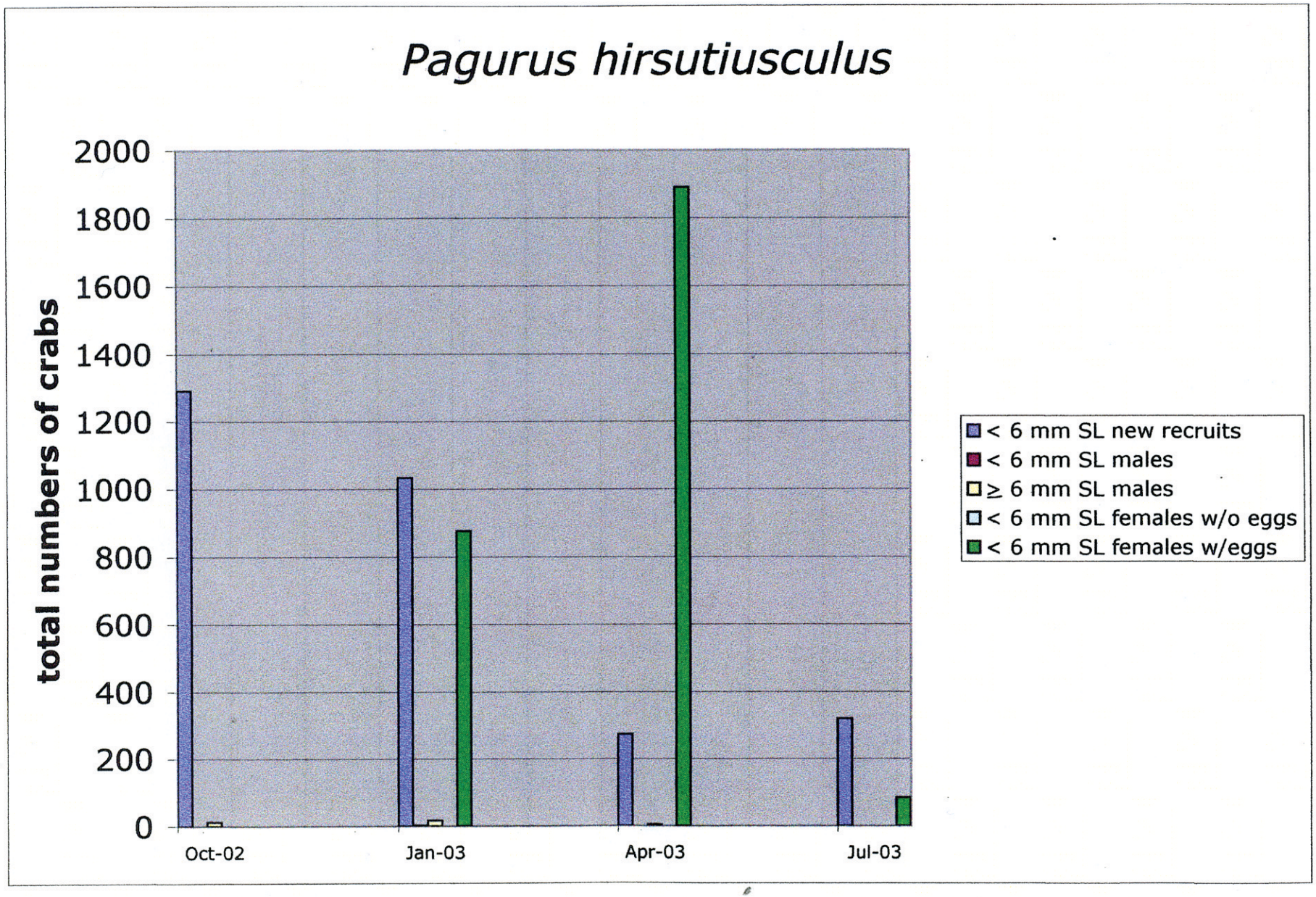


Figure 2. Seasonal comparison of total numbers of *Pagurus hirsutiusculus* caught in shallow trays (n=7) along one transect in Padilla Bay, Washington, in 2002-2003. SL=shield length.

(7,6,50) with gravid females present (15,1,1) as well. No males were present in January or April and only one male was present in July. The greatest number of crabs (< 6.0 mm SL) were present in October as well as the greatest numbers of females (< 6.0 mm SL) without eggs and males (< 6.0 mm SL) (Fig. 3). The highest numbers of females (< 6.0 mm SL) with eggs were present in January (Fig. 3).

Hemigrapsus oregonensis. The greatest number of *H. oregonensis* were present in January (1,666). More males than females (≥ 6.0 mm CW) were present in all months except January. Gravid females were only present in July and only 1 in 4 females were gravid that month. Crabs with < 6.0 mm CW were present in October, January and April (105;1,092; 255) but not in July. Females were present in greatest numbers in January, April, and July (348,139, 55) and males were present in greatest numbers in October, January, and April (312,218,340). The greatest number of new recruits (< 6.0 mm CW) and females without eggs (≥ 6.0 mm CW) were present in January (Fig. 4).

Cancer magister. No females were present in the trays during this study. The highest number of males were present in January (169) followed by April and October (30, 7). No *C. magister* were present in July. All *C. magister* were ≥ 6.0 mm CW.

Size Frequencies. Quarterly size frequency data was compiled for the most numerous crabs. *Hemigrapsus oregonensis* had the highest percent frequencies in the following size classes: 4-8 mm (Oct '02, Jan. '03, Apr '03) and 9-13 mm (Jul. '03) (Fig. 5). For *Cancer magister*, single crabs were scattered through size classes ranging from 19-53 mm CW in October and no *C. magister* were present in the trays in July '03. Highest percent size frequencies for *C. magister* in January and April '03 occurred in the 19-20 mm and 37-38 mm size classes, respectively (Fig. 6). The highest percent frequencies for *Pagurus hirsutiusculus* occurred in the 3-4 mm SL size class, irrespective of season (Fig. 7). This was also true for *P. granosimanus* with the exception of July '03 when the 1-2 mm and 2-3 mm size classes had the highest percent frequencies (Fig. 8).

Pagurid shells. Pagurids were found almost exclusively in empty *Batillaria attramentaria* shells, a non-native species of mud snail. The largest males were often found in shells with most of the spire missing. *P. granosimanus* were also found mostly in *Batillaria* shells but also in *Nassarius fraterculus* shells, another non-native snail. *Crepidula convexa* were attached to many of the *Batillaria* shells pagurids were using.

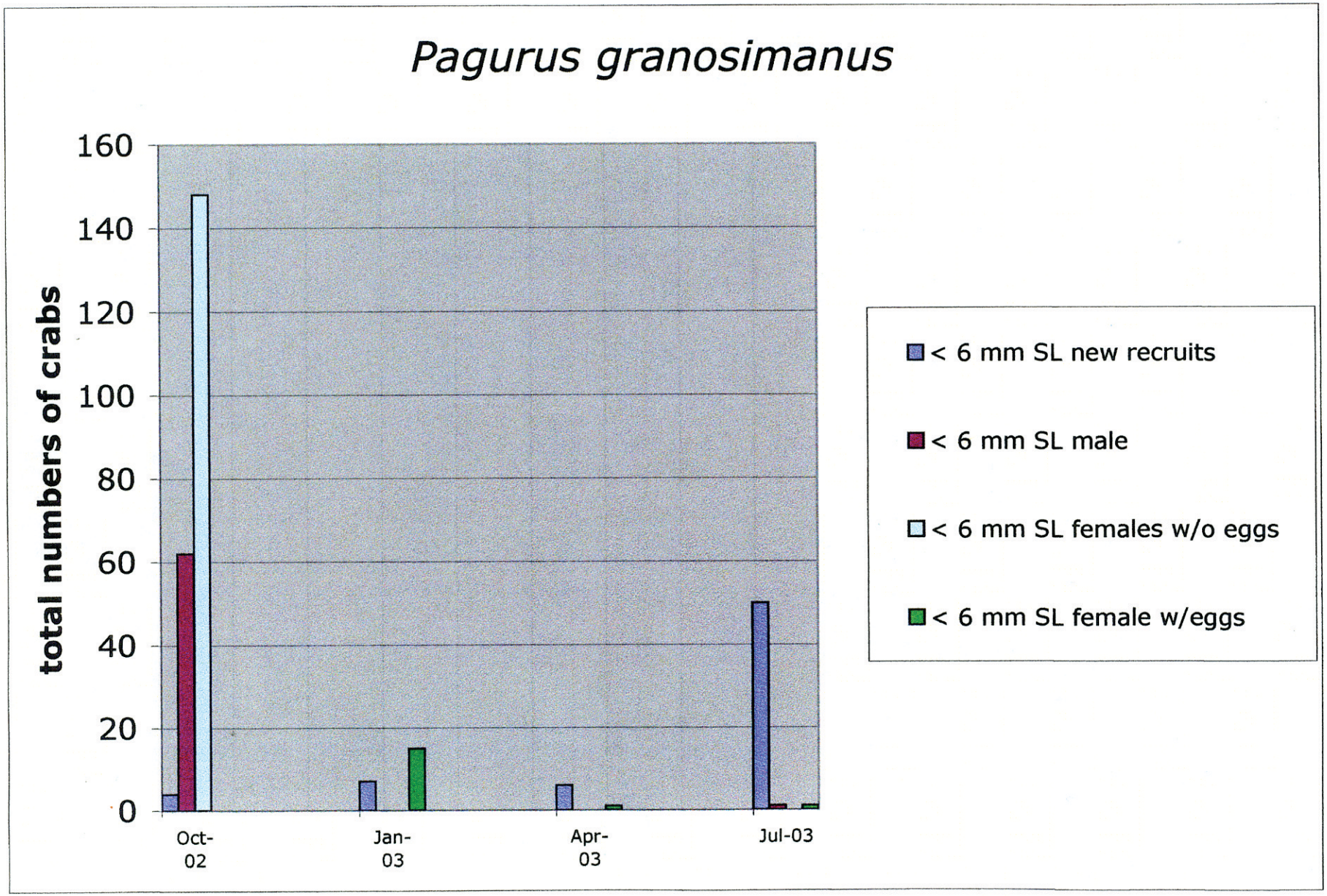


Figure 3. Seasonal comparison of total numbers of *Pagurus granosimanus* caught in shallow trays (n=7) along one transect in Padilla bay, Washington, in 2002-2003. SL=shield length.

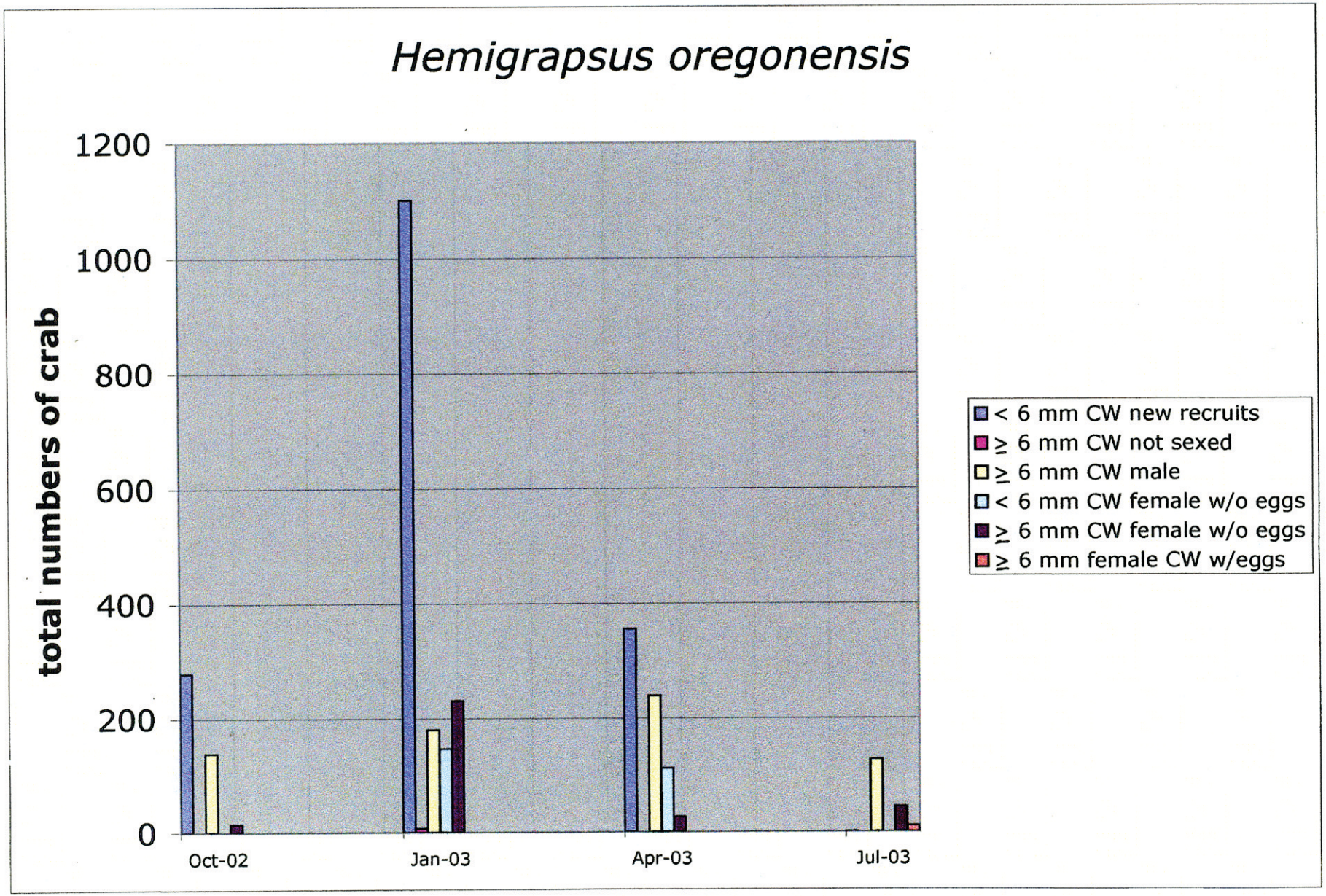


Figure 4. Seasonal comparison of total numbers of *Hemigrapsus oregonensis* caught in shallow trays (n=7) along one transect in Padilla Bay, Washington, in 2002-2003. CW=carapace width.

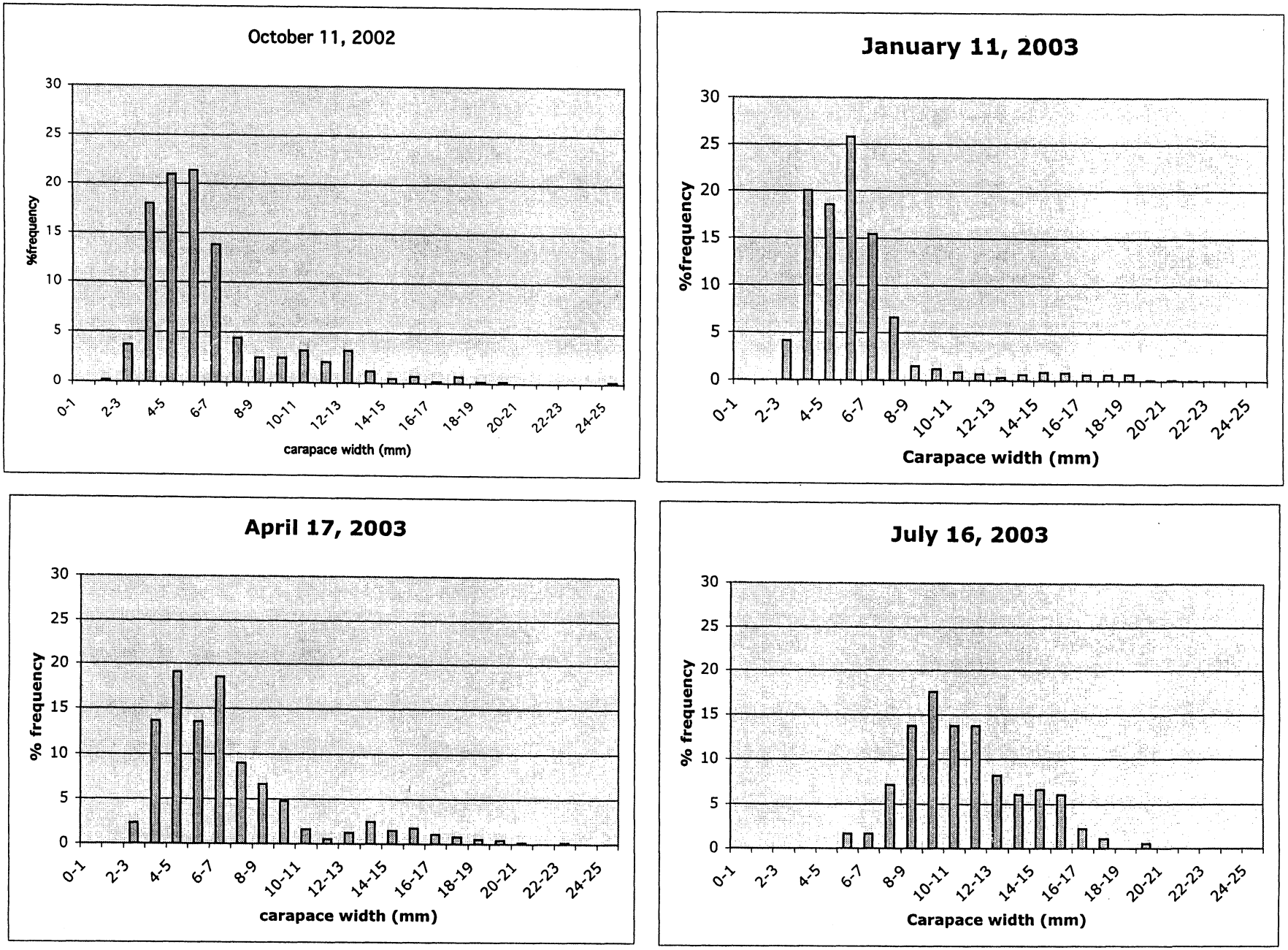
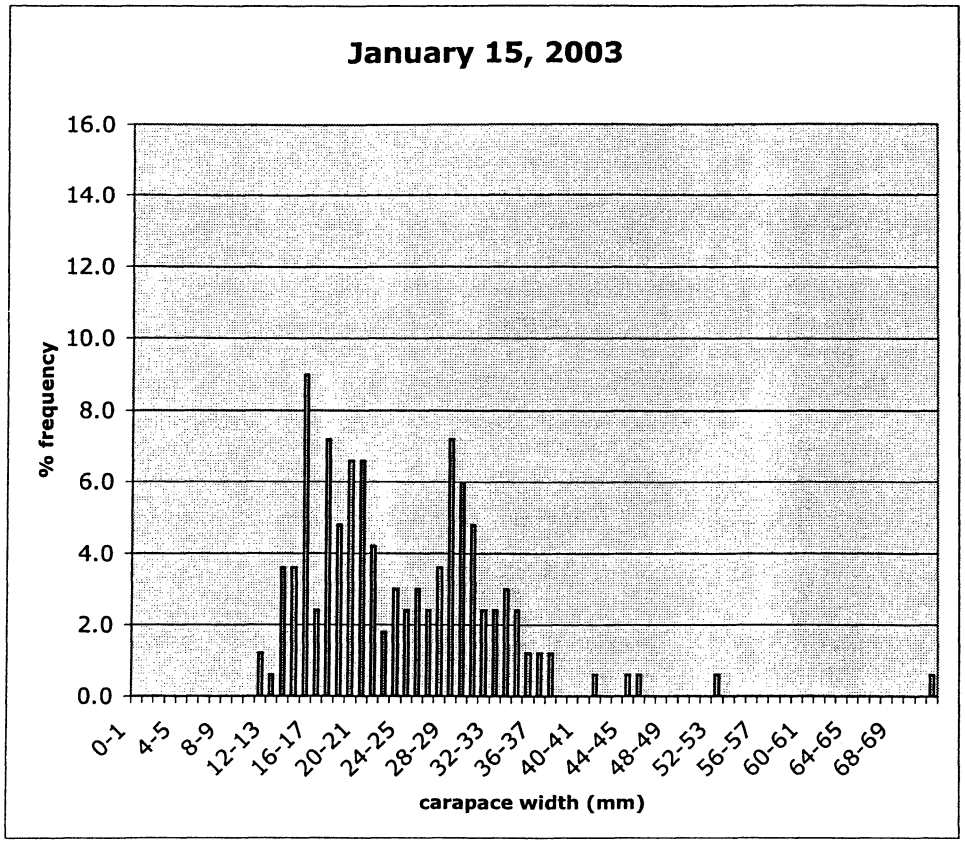


Figure 5. Percent frequency of size classes of *Hemigrapsus oregonensis* caught in shallow trays (n=7) along one transect in Padilla Bay, Washington, in 2002-2003.



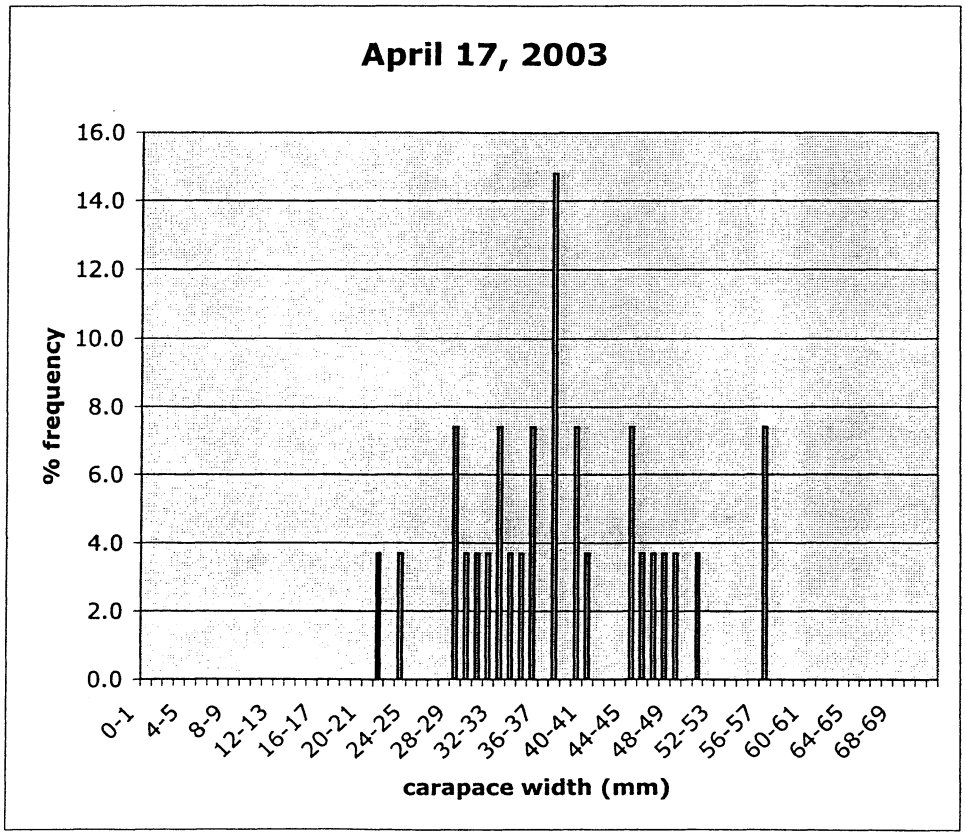


Figure 6. Percent frequency of size classes of *Cancer magister* caught in shallow trays (n=7) along one transect in Padilla Bay, Washington, in 2002-2003. In October 2002, single crabs were scattered through size classes (from 19-53 mm CW) and no *C. magister* were present in July 2003.

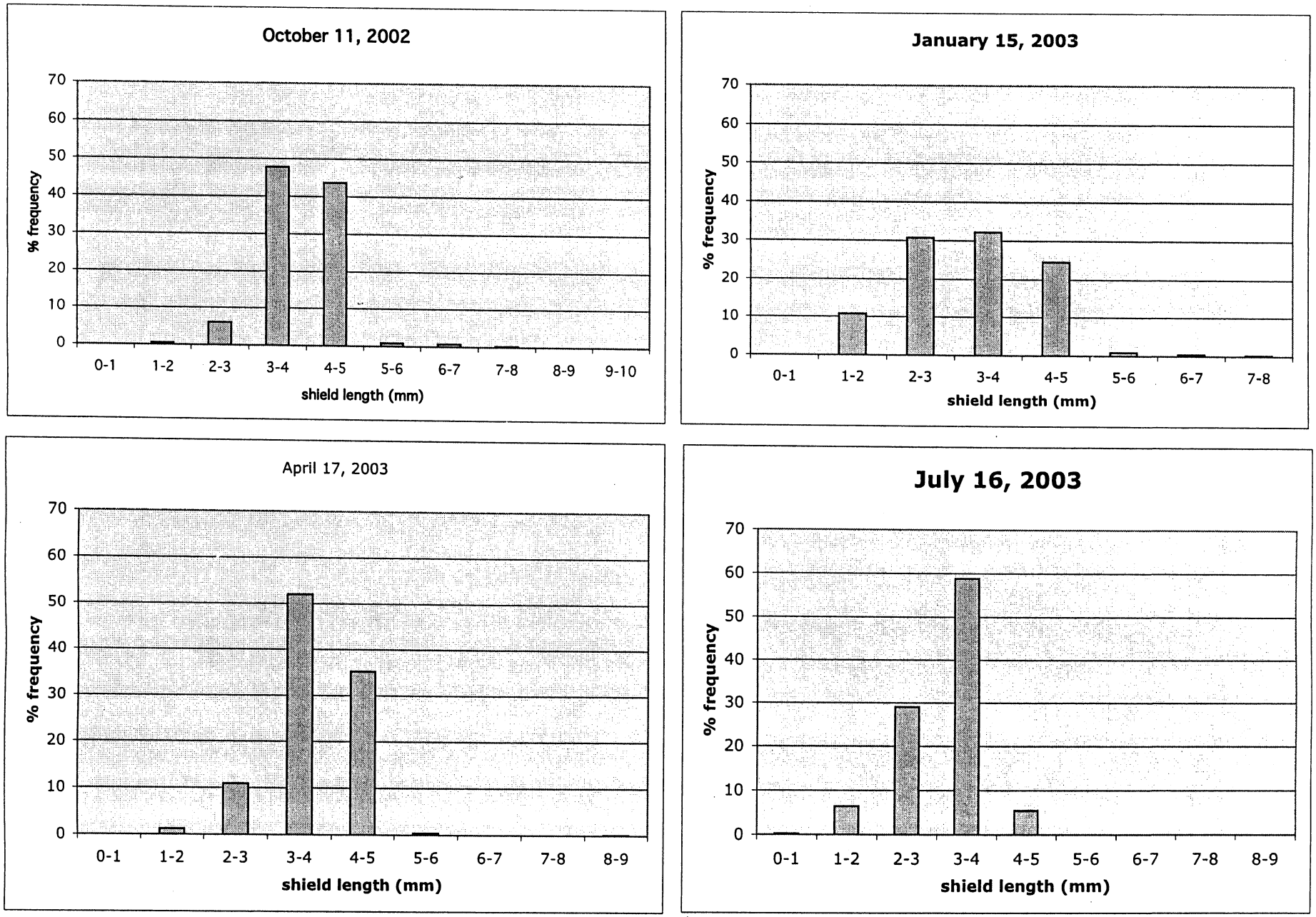


Figure 7. Percent frequency of size classes of *Pagurus hirsutiusculus* caught in shallow trays (n=7) along one transect in Padilla Bay, Washington, in 2002-2003.

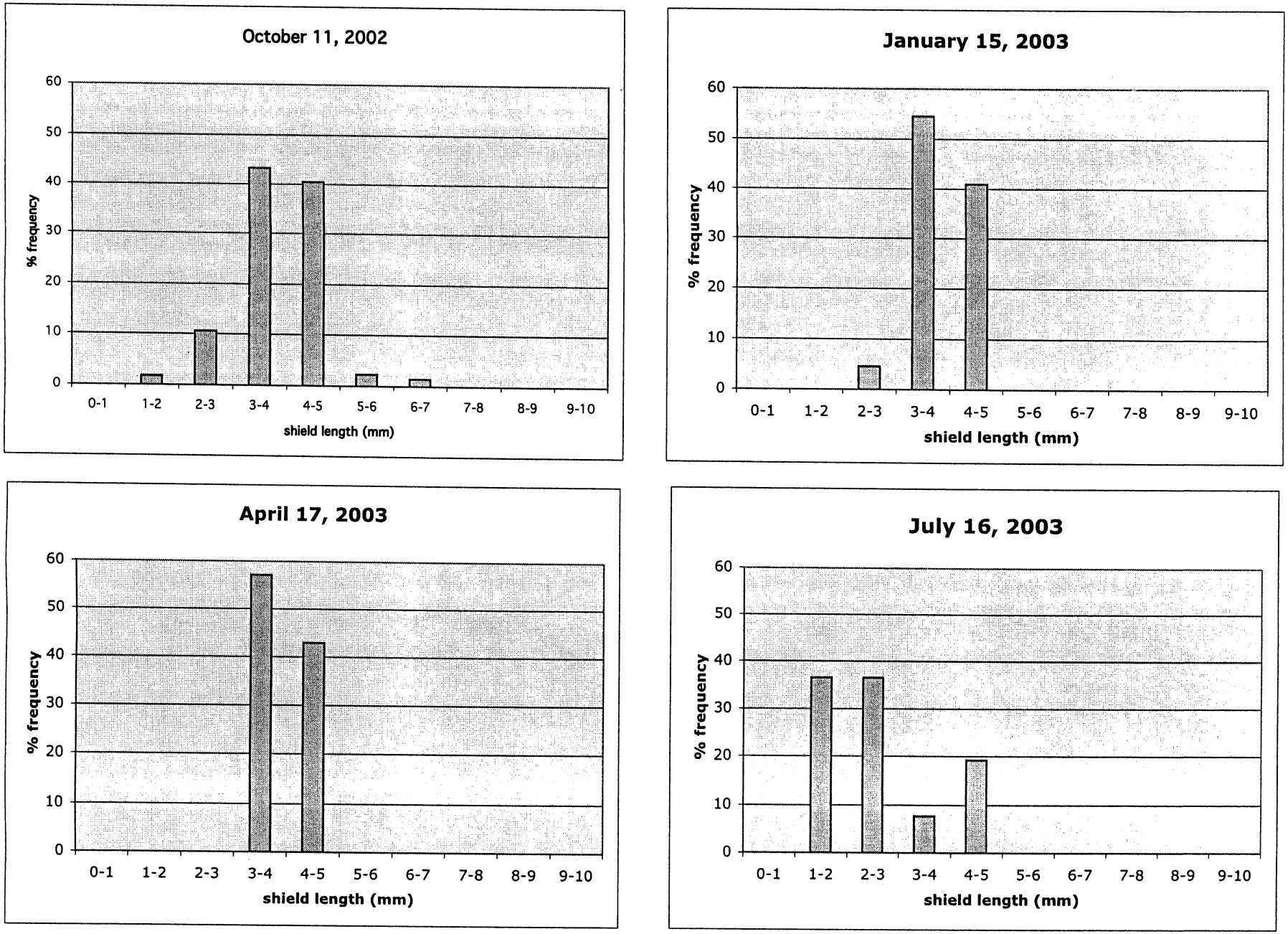


Figure 8. Percent frequency of size classes of *Pagurus granosimanus* caught in shallow trays (n=7) along one transect in Padilla Bay, Washington, in 2002-2003.

Parasites. *Hemigrapsus oregonensis*, *C. magister*, and *T. cheiragonus* were not examined for parasites. Parasitic isopods (bopyrids) were only present on *P. hirsutiusculus* (< 6.0 mm SL) and in low numbers January, April, and July (2,2,7). No parasitic isopods were present on *P. granosimanus*. Some rhizocephalans were present (banana-shaped, attached to abdomens) on *P. hirsutiusculus*.

Commensal crabs. *Pinnixa tubicola* was the only species of commensal crab found during the study. It is a commensal in tubes of polychaete worms (Garth and Abbott, 1980).

WDFW METHOD. The traps were set out on tidal mudflat in mixed *Zostera marina* and *Zostera japonica* on muddy substrate. The traps soaked for: 25.25 hours (Oct. '02); 23 hours (Jan. '03); 22.5 hours (Apr. '03); 23.75 hours (Jul. '03).

The most abundant crabs in the minnow traps were *Hemigrapsus oregonensis* (38), followed by *Pagurus spp.* (18) then *Cancer magister* (7) (Table 2). More *H. oregonensis* males were present than females and only male *C. magister* were caught in the traps. *Hemigrapsus oregonensis* ranged in size from 15.5-26 mm CW and *C. magister* from 67-85 mm CW. *Hemigrapsus oregonensis* were present in the traps in spring and summer. *Cancer magister* and pagurids were present fall, winter, and spring (none in summer).

Discussion

We did not detect any invasive crab during the study (Table 2). The NERR method is capable of catching smaller crabs, more species of crab, and provides a more detailed seasonal comparison of crabs caught in trays than the WDFW method (Table 2). The NERR method would provide for earlier detection of invasive crab if set at the right tidal elevation in a suitable habitat. However, the NERR method is much more time-consuming than the WDFW method (Table 2).

European green crab (*Carcinus maenas*) has about 17 crab stages (Crothers, 1968). *Carcinus maenas* are the characteristic adult shape by stage 5 (5-6 mm CW) (Klein Breteler 1975). This size would be "caught" in the NERR method trays but would likely go undetected in the minnow traps (based on the crab sizes caught with the WDFW method in this study). Either method should catch green crab after their first winter as they are 32-60 mm carapace width at about a year old (Behrens Yamada *et al.*, 2000).

We did find parasites on *Pagurus hirsutiusculus* but did not key them to species. The parasitic isopod *Pseudione giardi* has been noted with *P. hirsutiusculus* in the San Juan archipelago (Haig and Abbott, 1980). We also found rhizocephalans but did not key them, either. The rhizocephalan *Peltogasterella gracilis* occurs gregariously on hermit crabs along the central California coast (Newman, 1975). There is a parasitic isopod that occurs in *H. oregonensis* and can be detected through dissection (Garth and Abbott, 1980). We did not have the resources to accomplish that level of detail.

We sexed pagurids in October 2002, but the NERR protocol did not call for sexing anything < 6.0 mm CW. When the counts increased dramatically in January 2003, we stopped trying to sex anything < 6.0 mm CW for the sake of expediency.

Because most of the pagurids use non-native snail shells, interesting questions to explore might be: What native snails were present before *Batillaria* invaded and in what numbers? What would pagurid populations have been at that point in time (fewer shells/fewer pagurids)? This might be a case where the presence of empty non-native snail shells has led to greater populations of native hermit crabs in the bay.

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Appendix 1

Washington Department of Fish & Wildlife Sampling Protocol

N.E.R.R. Sampling Protocol

CRAYFISH/MINNOW TRAP SURVEY METHOD

EUROPEAN GREEN CRAB MONITORING

Washington Department of Fish and Wildlife
2001

This document provides detailed instructions on monitoring with crayfish/minnow traps (hereinafter referred to as "crayfish traps") not provided in the Washington Department of Fish and Wildlife's **"Survey Methods Overview - European Green Crab Monitoring"**. Please read this in full before setting any traps.

SAFETY

Please be aware that coastlines can be hazardous areas to work in. If you think an area is potentially hazardous and are uneasy about accessing it, DON'T DO IT! Be mindful of the tides and if possible, conduct your surveys during low tides. Be sure to let someone know where you are going and when you plan to return. Be careful when traversing uneven ground, especially slippery surfaces such as wet rocks or seaweed. If you are carrying out work on the southwest coast (e.g., Willapa Bay) or other areas with similar conditions, be aware that mud flats can be like quicksand. Do not go out too far on a mud flat.

In Washington State, citizens can privately own tidelands down to various tide levels. Much of Puget Sound's shorelines are privately owned. When choosing a site to monitor, please respect private property boundaries, and only access a privately-owned tideland if you have permission from the owner. Most likely, if you approach a tideland owner and explain your project and how it will help protect our marine resources, including those on their own property, they will probably be quite cooperative and may even want to volunteer their help!

SCIENTIFIC COLLECTION PERMITS

A scientific collection permit (or "sub-permittee status") is required to collect and/or transport any live green crab in Washington State. Sub-permittee status may be obtained from the Washington Department of Fish and Wildlife (WDFW). If you are interested in receiving a sub-permit, please contact your monitoring coordinator to apply for sub-permittee status. If you are not working with a monitoring group, please contact the following WDFW offices: for Puget Sound, the San Juan Islands, and the Strait of Juan de Fuca, call the Pt. Whitney Laboratory in Brinnon, Washington at (360) 796-4601; for the southwest Washington coast, call the Willapa Bay Field Station at (360) 665-4166. Additional information on the sub-permitting process and guidelines are contained in WDFW's **"Monitoring and Transport Permission Protocol"**.

HOW TO CONDUCT THE SURVEY
Crayfish Trap

1. TRAPS - **PREPARATION**: 1) Obtain a crayfish/minnow trap(s) and modify the tunnel openings to 2 1/4 inches if necessary (crayfish trap openings are typically 2 1/4 inches wide), 2) Attach a bait container to the edge of one half of the trap, so that it will hang in the center of the trap when both sides are joined. Make sure that the bait container doesn't block either one of the tunnel openings, 3) Fill out a red plastic WDFW research trap identification tag with a permanent ink pen (see Appendix A). If you are monitoring independently, please place your phone number and address on the tag. If you are associated with an organization, put the organization's phone number on the tag, not the surveyor's. If the trap becomes lost, the finder of the trap will be more likely to reach someone at an organization's number. Attach the tag to the trap.

2. TRAPS - **SECURING**. These traps are light and need to be anchored to the ground. You can secure your trap(s) to the beach by staking it with either a 2-3 foot (approx. 1 meter) piece of rebar, pencil rod, or narrow PVC pipe when deploying them in the intertidal zone. Some type of anchor (chain, metal ball, rocks, etc.) with a float as a marker will also work, especially if they are deployed and retrieved from a boat at high water.

3. TRAPS - **BAIT**. WDFW has not yet scientifically tested which bait works best, but most types tried have worked. In Puget Sound (including the San Juan Islands and the Strait of Juan de Fuca) the standard bait is cat food which consists of fish products (WDFW uses Friskies Salmon Dinner). On the outer Washington coast, WDFW's standard bait is mackerel (not canned).

4. **LOCATION/SITE SELECTION**.

A. PUBLIC BEACHES. Public beaches can be good choices for monitoring because there is usually safe and easy access to them, and the boundaries are often well marked. The downside to monitoring at public beaches is that they are just that, public. If there is a lot of foot traffic on the beach, then it is more likely that your trap(s) will be tampered with or even stolen. Before you place traps on public beaches, try to contact the beach ranger or any other overseer.

B. YOUR OWN PRIVATE TIDELANDS. If you are a tideland owner, then you probably don't need to search for a site any further than your own backyard. It will be easy for you to access the site, you know where the boundaries of your property are, you may be able to keep an eye on your trap(s) from your home, and there is most likely less foot traffic there than at a public beach.

C. OTHER PRIVATE TIDELANDS. In the event that you would like to monitor private tidelands other than your own for whatever reasons (e.g., optimum conditions, *Spartina* present), **DO NOT TRESPASS.** Always obtain permission from the property owner prior to monitoring.

D. HABITAT. Apart from having legal, safe and convenient access, the most important things to consider when choosing a monitoring site are the environmental conditions of the tidelands and the body of water they are on. Habitat varies greatly from site to site, and also within a site, however, look for structures that may provide potential cover for green crab if they were to move into the area. Green crab have been found in a variety of different conditions within their native range and also in sites they have colonized. On the U.S. west coast, the European green crab has only been observed in protected embayments and estuaries. On the southwest Washington coast, the European green crab has been found primarily in the lower salt marsh zones, **particularly in *Spartina,*** a non-native invasive marine grass. However, on the east coast of North America, they are common in moderately exposed rocky areas (Grosholz and Ruiz, 1996). We recommend that traps be placed in areas with any of the following characteristics:

- Macro algae (algae visible to the eye)
- Eel grass spp.
- *Spartina* and other lower salt marsh vegetation
- Large structures (e.g., boulders, tires, etc.)
- Low wave action
- Freshwater influence (e.g., creeks, streams or river mouths)
- Oyster or mussel beds

E. TIDE HEIGHT. The tide heights where most green crab on the North American West Coast have been found have been in the mid to upper-intertidal range, around 0 to plus 5 foot tide heights. Sampling should be focused within those tides heights, but you may also set traps at lower tidal elevations, especially if the habitat looks favorable.

5. **TRAPS - DISTANCE BETWEEN.** If you are setting multiple traps at a given "site", set them far enough apart so they won't interfere with each others effective fishing range. Unfortunately, the approximate effective radius of a trap is not yet known. As a rule of thumb for Puget Sound trapping, set them at least 30 feet apart (10 meters [See Appendix B for trap-spacing example]). Generally, the longer the shoreline you are sampling, the farther apart the traps should be situated. If the habitat you are sampling is fairly uniform, you can place the traps a set distance apart. If the habitat on the beach you're sampling varies widely, it's more important to look for suitable habitat instead of just placing the traps a uniform distance apart. For example, if *Spartina* is on the beach, it is advisable to place at least one trap in the *Spartina* as it has proved to be a preferred habitat on the Washington coast. Since we aren't absolutely sure where we might find the green crab in our waters, you can also sample areas of unknown suitability, especially if it gives you broad geographic coverage of a beach. However, your first few traps should definitely be placed in known green crab habitat.

6. **TIME PERIOD**. Deploy the gear for a 24-hour soak period which encapsulates both a full tidal and day/night cycle. It is crucial to check and empty your trap(s) after the designated soak period, particularly during the months of April through September, when the probability of catching large quantities of crab and other animals is higher. The longer the trapped animals are unprotected from the effects of sun and air, the less likely they will survive until they can be released. Leaving large quantities of crab in this confined condition for an extended period of time also increases the number of animals that will be damaged/killed due to their interactions, including by-catch (Liz Carr, pers. comm., 1999). **If you suspect the trap(s) can not be checked within a 24-hour period, reschedule your survey for a more convenient time.**

7. TRAPS - **RECOVERY**. After your gear has soaked for 24 hours, pull the gear and record your catch information onto a survey data form. See WDFW's "**Data Collection Procedures - How to Fill Out the Data Sheet**" for data collection instructions. **Please record all observations, even when no crabs are caught. "Zeros" are important information.** Please be sure to clean all debris, plants and animals out/off of your traps between surveys, especially if you will be setting your traps at a different site. It is very important not to inadvertently transfer other "pest" species (e.g., oyster drills) from an infested site to a pest-free site through your activities.

8. TRAPPING FREQUENCY. The preferred and recommended minimum sampling frequency during April through September is once a month per site. During these months, the green crab is more active in the intertidal zone, and you are more likely to catch one in your trap(s). If you would like to survey more frequently, please do so. By sampling more often, you increase your chances of finding a European green crab if it is in the area. If a green crab is found, sampling frequency and trapping density would need to increase.

NOTE: While walking to and from sites where you choose to set your traps, it is very important to do a visual scan of the beach for European green crab molts (exoskeletons) and carapaces (only the back shell). The inside of the carapace tends to be very white. Frequently, the presence of European green crab will be initially revealed through the discovery of a molt, not through a live animal sighting. This was the case in Willapa Bay, Washington.

REPORTING AND STORING GREEN CRAB

In the event you catch a European green crab, after you've recorded the standard data, retain the animal, place it in a zip lock bag or other suitable container, and place it in your freezer. This container should be labeled with the date the trap was set, trap location (be specific), the name of the surveyor and phone number, and the organization name (if applicable). If you set more than one trap in that location, identify which data sheet each sample(s) is linked with. IMMEDIATELY contact your monitoring coordinator (if applicable) to confirm the crab's identity, or contact WDFW at (360) 796-4601, ext. 0 for Puget Sound, or (360) 665-4166 for the Washington coast. As stated in the green crab Scientific Collection permit, **"ALL CAPTURED GREEN CRAB NEED TO BE TURNED OVER TO WDFW SHELLFISH STAFF IN ORDER TO CONFIRM IDENTIFICATION".** It is very important to get identification confirmation as soon as possible, and not talk with the press until the sighting is verified by WDFW. If you are with a monitoring group, your monitoring coordinator will contact WDFW after the coordinator has confirmed your sighting. WDFW staff will provide their home phone numbers and/or e-mail addresses to monitoring coordinators for easier contact outside of working hours in case of a green crab sighting in Puget Sound.

NERR Invasive Crab Monitoring Pilot Project

Data Collection Protocols for Quarterly Crab Sampling

Preparation for cage deployment

All sites should get 7 plastic bread trays, a roll of vexar mesh, a bag of cable ties, and some bricks. MJ can show you all how to create a "habitat tray" using these materials. You need to finish construction of the trays and deploy them early in the summer 2002. Hopefully, they will have been deployed at least 1 month prior to the first census (Fall 2002).

Seven habitat trays should be deployed at least 1 meter apart from one another, and ideally, spanning a lateral distance of 20-30 m. Deployments should be intertidal, at a standardized tidal height. Every site has a different tidal range, but deployments at a height of 0.0 ft would be a decent lower intertidal elevation. More details on this later.

Each habitat tray should be filled with materials that represent the ambient habitat well. For example, southeastern sites should deploy to intertidal oyster reef habitats and, therefore, should dump 1 – 1½ buckets of oyster clusters (living and dead material) into each tray. Empty cages WILL float away. Thus, a few bricks can be placed in each cage as ballast.

Preparation for decapod sampling

Start gathering materials approx. 1 wk prior to scheduled sampling date:

- Obtain a 17-gallon plastic box with a lid ($4 at Walmart, Lowes, or Home Depot). This will serve as a dry box where you can keep all your important stuff clean and dry. Most of the things listed below need to placed inside and brought to field
- Print 10 data sheets (one for each habitat tray, plus a few extras). Punch holes and put the sheets into a sturdy 3-ring binder. Pencil can be attached to binder with a string.
- Work gloves. Essential for people working in oyster reef habitats. Cotton gloves coated with a rubber/plastic material work best, since they maintain their shape and integrity for multiple uses even when working in wet, muddy environments.
- Metric dial calipers, plastic kind. Did I mention that you must get the metric ones? Can be obtained from Ben Meadows catalogue for like $30 bucks.
- Long tweezers/forceps…the kind that are about ¾ of a foot long.
- A thick Sharpee pen (permanent marker) and some flagging tape or labels.
- Hat, sunscreen, water, bug spray, cell phone, GPS unit, digital camera

Other things.

- 10 jars. These could be large Ricotta cheese containers…or they could be official sampling jars. Doesn't matter. Something that won't break would be best. Crabs need air holes of some sort—unless you plan to freeze or preserve all crabs anyway. One jar is needed for each tray sampled. Sometimes more than one jar per tray is needed…especially useful if you get numerous or large-sized crabs.
- 3-4 large buckets.
- 1 wood-framed metal sieve (with 1 mm mesh metal screening, reinforced by 1 cm screening). Size of frame should be approx. same as habitat tray size.
- 2 additional trays with no vexar mesh
- 1 hard plastic kiddie pool with some drain holes punched in it.

Field sampling protocol: Be in field at least 2 hr prior to the time of a spring low tide

1. Using the Sharpee pen and flagging tape, prepare a label for "habitat tray no. *x*".
2. Put label into a jar.
3. Place several items into the hard plastic pool, stacking them in this order (from bottom to top): 1 tray with no vexar mesh [upside-down], the wood-framed screen [upside right], the 2nd tray with no mesh [upside right]. See figure.
4. Fill out top of data sheet so that some important information gets documented...Specifically, when/where/by whom this particular tray was sampled.
5. Put on gloves, pick up tray from its position in the field (you may want to take out bricks), and begin dumping contents of habitat tray onto the stack of trays/screens. The top layer in the stack will capture the largest bits of shell etc. Most crabs will fit through, however, and will try to escape downward. They will get only as far as the second level—the screen, framed by the wood pieces. The bottom layer of the stack just keeps the framed screen up above the dirty muddy water. Buckets of water can be dumped over the shell hash to get rid of additional mud and to encourage the crabs to move downward (to separate themselves from the shells).
6. Pick through the oyster shells/clusters and make sure no crabs are clinging to them. The long forceps come in handy here. After satisfied that all crabs have gone downward onto the finer screen, the top layer of the stack (the tray with the shell hash) can be set aside.
7. Collect all crabs from the screen and place into sample jar. (A label should be inside the sample jar already, but double check). Use forceps if necessary, but try to avoid damaging crabs.
8. When done (satisfied that you've gotten all specimens greater than about 5 mm CW), cover the sample jar to prevent escapes. Set jar aside for later examination (at the laboratory)*.
9. Return the shellhash to the habitat tray, and re-deploy to the field in the same location as before.
10. Move on to next tray. Repeat steps 1-9 until all trays have been sampled. Sampling of each tray should take about 30-40 min.**

* If there is an unusually large specimen, or if you don't want to keep something even temporarily, use the calipers to measure it. Record all relevant data, and release. Sometimes, large specimens will prey upon other crabs inside the jar, or they may simply take up too much room in the jar. Thus, there are valid reasons to measure and release right away. This should NOT be done for exotic specimens, however.

** If sampling starts 2 hr before low tide, the entire set of trays can be done in a single day (during a single low tide). If an additional day of sampling is required, it is best to sample cages on successive days.

Laboratory sampling protocol. You have some flexibility here.

(1) You can choose to sort and measure the crabs alive.

Positives: -You are not a killer.
-No impacts on native populations or rare individuals
-Samples get processed immediately, so there's no data backlog

Negatives: -Must be done the same day as field sampling (otherwise, large crabs will eat the smaller ones, affecting density and size frequency estimates).
-Crabs move around and are harder to identify and measure
-When crabs pinch your sore field fingers, it ain't much fun.

(2) You can choose to freeze the crabs, and then thaw and sort dead crabs.

Postitives: -Ease of identification
-no pinching
-species id's and data quality (densities/ sizes) perhaps better

Negatives: -You have to kill crabs, even native ones
-often leads to procrastination...samples linger in your freezer for months. (that is particularly bad for us, since rapid data dissemination and info transfer is explicit in our proposal.)

Option 2 may be more feasible, especially until everyone is familiar with the procedures and identities of the crabs. Negative aspects will be minimized, as long as samples are processed within 7 days of sample collection.

For each jar,

1. Empty the contents into a large dish (e.g., the size of a brownie pan)
2. Identify the largest individual to species*
3. Write down the numeral 1 on the data sheet, and write the name of the species next to it.
4. Note the sex by examining the abdominal plate.
5. Using the dial calipers, measure the width of the carapace across its widest point (usually from point to point on crabs that have serrated "teeth" along the outsides of the carapace). Accuracy should be ±1 mm (though data should be entered with 1 decimal place, e.g., 26.1 mm)
6. If the crab is gravid (bearing eggs), or has a soft-shell (post-molt), or is parasitized by something funky (e.g., a rhizocephalan), or is missing appendages, write this down in the "Qualitative" portion of the data sheet.
7. Write down the number 2, and pick up a second specimen from the same species. Repeat steps 4-6 until all crabs in that species have been measured. **
8. Repeat steps 2-7 for the next species, and the next, until all crabs in the jar have been examined.

* Though crabs are generally a well-known taxonomic group, some species identifications can be difficult. Use published keys and consult with local experts to identify the crabs accurately. We may also send each other preserved specimens as a QA/QC procedure.

**Crabs <6 mm CW are usually exceedingly hard to sex (because they are often not yet sexually mature). They are also hard to collect, identify, and even measure accurately. Thus, these can be counted as "recruits < 6 mm" and left at that.