USGS NW CASC Final Report: Lummi Natural Resources "Maximizing trap efficiency on Lummi Nation estuarine habitats to reduce

ecosystem impacts from invasive European green crab (Carcinus maenas)."

Lummi Natural Resources Department, Aquatic Invasive Species Division Final report prepared by Bobbie Buzzell November 30, 2023



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SECTION 1. Administrative Information

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Award recipient: Lummi Natural Resources, Lummi Indian Business Council

Project Title: "Maximizing trap efficiency on Lummi nation estuarine habitats to reduce ecosystem impacts from invasive European green crab (*Carcinus maenas*)."

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SECTION 2. Public Summary

This project aimed to study the distribution and abundance of invasive European green crab (Carcinus maenas, hereafter "green crab") on Lummi Nation Reservation Tidelands, and to remove this invasive species from the marshy, estuarine areas on the tidelands. Two estuaries were trapped in the Lummi and Nooksack rivers while simultaneously collecting water quality data (salinity and water temperature) and determined if these parameters will inform the Lummi Natural Resources Department (LNR) green crab trapping team of potential areas of green crab settlement. Invasive green crab are capable of enduring short periods of extreme salinity and temperature conditions but how this adaptability translates to the local environment was unknown, especially during the early invasion status of green crab in the Pacific Northwest. We found there was not clear correlation between the measured water quality parameters and green crab captures at sites trapped along Lummi River. This finding was likely due to low capture numbers of green crab on Lummi tidelands. But the ranges of salinity and water temperature across the gradient of Lummi River suggest green crab settlement upriver is likely. Exploratory trapping in the Nooksack River estuary resulted in no captured green crabs, likely due to the high freshwater output observed in spring and fall seasons, which could prevent green crab movement into the system during the green crab larval dispersal phase. Based on our findings, we plan to prioritize and continue a regular trapping schedule along the Lummi River (e.g., weekly). But trapping in the Nooksack River will be less frequent (e.g., monthly).

SECTION 3. Project Summary

European green crab (*Carcinus* maenas, hereafter "green crab") are an adaptive and resilient invasive species able to inhabit estuarine areas along the Pacific Northwest, USA, and can endure extreme salinity and water temperature conditions. On Lummi Reservation Tidelands, Lummi Bay and Lummi Sea Pond were recently invaded by green crab but less is known about the potential for this deleterious species to settle in the estuarine areas of the Lummi and Nooksack rivers.

To better understand the current distribution and potential of green crab to settle in these estuaries, Lummi Natural Resources Department (LNR) staff collected water quality data alongside trapping and removal of green crab in the Lummi and Nooksack rivers and aimed to answer if water quality conditions (salinity and water temperature) may allow for expanded green crab settlement, especially at more inland locations. Transects of Fukui-style and minnow traps (6-30 traps) were deployed between April and October 2022 at five Lummi River sites and in Lummi Sea Pond (serving as a "control" for green crab presence) every other week with simultaneous collection of salinity (ppt) and water temperature (°C) data. Nooksack River trapping effort and water quality data collection was exploratory, and only two sites were trapped on two separate events in May and June.

Trapping activity in the Nooksack River resulted in no green crab detections which was likely a result of the high freshwater output of the river system. However, trapping activity closest to the mouth of the Lummi River (site 1) confirmed the presence of green crab in the first month of trapping (15 CPUE, CPUE=number of crabs per 100 trap sets) but catch rates were relatively lower in the second half of the study period (4-8 CPUE). Lummi River trapping sites displayed similar salinity and water temperature ranges to Lummi Sea Pond but sites along Lummi River had greater seasonal variability than Lummi Sea Pond.

We were unable to identify a correlation between salinity and water temperature and green crab catch in traps in Lummi River but concluded that the wide range of both water quality parameters in the river will likely allow green crab settlement. Exploratory measurements and trapping in the Nooksack River estuary, however, do not suggest green crab will establish in the short-term. Based on our findings, we plan to prioritize and continue a regular trapping schedule along the Lummi River (e.g., weekly) but trapping in the Nooksack River will be less frequent (e.g., monthly).

SECTION 4. Report Body

Purpose and Objectives:

The purpose of this project was to determine the current distribution and relative abundance of green crab within the estuarine areas of Lummi Reservation Tidelands and maximize trapping efficiency of green crab during the early invasion status. Lummi Natural Resources Department (LNR) staff deployed traps along a salinity gradient on Lummi River and simultaneously collected water quality measurements (salinity and temperature) to determine the potential of green crab settlement inland. The study extent was scaled back from the original objective due to a delay in project implementation. Additionally, we had originally planned to trap more sites over a regular trapping schedule within the Nooksack River estuary (similar to methods for the Lummi River), but after initial sampling, it was determined the Nooksack River data collection would be exploratory in nature due to limited access. Instead, increased effort was allocated to the Lummi River. We successfully documented the salinity and water temperature gradients along the Lummi River and observed a lack of correlation between water quality parameters and green crab captures. This finding allowed LNR staff to identify and prioritize trap and removal activity in habitats that will likely allow for green crab settlement, and to develop protocols that serve these different priority areas.

Organization and Approach:

All trapping took place between April 4-October 18, 2022, during the regular green crab trapping season in Washington State (Grason et al., 2018; Table 1). LNR staff utilized previously used protocols applied for green crab trapping on the reservation (Mueller and Jefferson, 2019) where baited minnow and Fukui-style traps were placed along transects parallel to the riverbank at distinct sample sites along the freshwater-brackish gradient of the Lummi and Nooksack rivers (Fig. 1; Duncombe and Therriault, 2017; Bergshoeff et al. 2019). Sites along Lummi River (1-5) were chosen based on accessibility and previous water quality sampling work by LNR Water Resources Division (Fig. 1). Lummi River site 4.1 was relocated to site 4.2 in July 2022 after multiple traps were dislodged and lost (Fig. 1); catch and water quality data were summarized together for these sites. Similar trapping was conducted in Lummi Sea Pond (marine water), where there has been confirmed green crab since 2019, and which served as a comparative baseline.

We used HOBO[®] specific conductivity and water temperature loggers that were attached to a PVC station and housing unit at each of the sample sites, collecting measurements at 15-minute intervals (Fig. 3). Water quality measurements for Lummi Sea Pond were collected from a semi-permanent EXO[®] data logger located at the Lummi Sea Pond pumphouse. Salinity and water temperature daily averages were calculated and collated with average daily capture rates of green crab. Correlation analyses (Spearman's Rank Correlation, α =0.05) were conducted to examine relationships between water temperature, salinity, and green crab catch in Lummi River and Lummi Sea Pond. No other statistical analyses were conducted due to the lack of green crab captures in Lummi River, but qualitative summaries for green crab and bycatch captures and monthly/seasonal environmental trends were provided to understand other potential trends and influences on green crab captures.



Figure 1. Lummi Tidelands trapping and water quality sampling sites for April-October 2022; Lummi River site 4.1 was trapped from April-July and site 4.2 was trapped from July-October.

Table 1. Trapping dates for Lummi Sea Pond (LSP), Lummi River, and Nooksack River in2022; includes deploy date and up to 2 check dates.						
Month	lonth LSP Lu		Nooksack River			
April	-	4/4-4/6; 4/18-4/20	-			
May	5/16-5/17	5/16-5/18	5/23-5/24			
June	6/13-6/14; 6/27-6/28	5/31-6/2; 6/13-6/15; 6/27-6/29	6/6-6/7			
July	7/11-7/12; 7/26-7/27	7/11-7/13; 7/25-7/27	-			
August	8/9-8/10;8/11-8/12; 8/23-8/24	8/8-8/10; 8/22-8/24	-			
September	9/8-9/9; 9/19-9/20	9/6-9/8; 9/19-9/21	-			
October	10/5-10/6; 10/17-10/18	10/5-10/7	-			



Figure 2. Fukui-style (left) and minnow (right) traps used for green crab trapping on Lummi Reservation Tidelands in 2022 (photo credit Jonathan Hallenbeck).

Table 2. Trap dimensions and modifications used for green crab trapping in LSP, and Lummi andNooksack rivers in 2022.						
Trap type	Brand	Trap size (in)	Mesh size (in)	Tunnel type	Modification	
Fukui-style	American Maple Inc/Promar	24x18x8	1/2	2 x Mesh	Zip-tied tunnel openings; rebar added	
Minnow	Gee	9x17.5	1/4	2 x Rigid mesh	Expanded opening to ~2"	



Figure 3. PVC housing station for HOBO data loggers deployed at each Lummi River trapping/sampling location (photo credit Bobbie Buzzell).

Project Results, Analysis and Findings:

Trapping

All trapping was conducted between April 4, 2022, and October 18, 2022, with a total of 67 trapping days and 1,341 traps checked and retrieved (Table 1; Table 3). Effort was nearly 1:1 between Fukui-style and minnow traps at each site except for when traps were lost during the study period (Table 3). Two 1" mesh shrimp traps (brand American Maple-Promar[®]) supplemented the Fukui-style and minnow trap effort at the Nooksack River site but were never used at any other site or included in final analyses of this study.

Table 3. Total number of Fukui-style, minnow, and shrimp
traps checked and retrieved regardless of number of soak
nights for each site from April 4-October 18, 2022; "*"
indicates trapping effort at Lummi River 4-2 was later
combined with Lummi River 4-1 due to the proximity of the
two sites.

Site	Fukui Minnow		Shrimp	Total	
Lummi River	459	477	-	936	
1	96	98		194	
2	93	98		191	
3	79	88		167	
4-1	57	59		116	
4-2*	36	36		72	
5	98	98		196	
Lummi Sea Pond	174	174	-	348	
Nooksack River	28	27	2	57	
Total	661	678	2	1,341	

Green crab catch rates were highly skewed towards zero crabs per trap, and varied from 0 to 900 CPUE in traps deployed in Lummi Sea Pond and Lummi River 1 across the study period (Fig. 4). In Lummi Sea Pond, 52 of 318 trap sets captured green crab, and only 11 out of 194 trap sets contained green crab at Lummi River 1. Monthly average catch rates in Lummi Sea Pond peaked the first month of trapping in May (150 CPUE) then decreased through October (0 CPUE; Fig. 4). Monthly average catch rates also peaked the first month of trapping at Lummi River 1 in April (15 CPUE) but afterward captures were only observed in the months of June, July, and September (4-8 CPUE) and no clear trends were discernible.

Across all trapping sites, a total of 27 species and species groups (grouped at Family or a broader taxonomical level) and 19,791 animals were captured (Table 4). The most captured animals were fish and crabs including the hairy shore crab (*Hemigrapsus oregonensis*) with 72.67% of total captures, followed by Pacific staghorn sculpin (*Leptocottus armatus*) with 11.74% of total captures, snails (Gastropoda) with 3.94% of total captures, and Dungeness crab (*Metacarcinus magister*) with 3.73% of total captures.



Figure 4. Box and whisker plot of monthly European green crab CPUE (green crab per 100 trap sets where n=number of trap sets) in Lummi River 1 and Lummi Sea Pond for 2022; CPUE was log-transformed. Points indicate outliers, and crosses indicate monthly average CPUE.



Figure 5. Frequency histogram of green crab carapace widths (mm) by sex captured between April and October 2022 in Lummi River (Top, n=23) and Lummi Sea Pond (Bottom, n=137).

Green crab was the seventh most captured species with 160 captures across all sites, or 0.80% of total captures. No green crab was captured at Lummi River sites 3-5 nor at the Nooksack River. Green crab was the seventh and eighth most captured animal at Lummi River sites 1 and 2, respectively, with 23 total captures. However, all 12 green crab captures recorded for site 2 on August 22 were found in a Fukui-style trap that had been dislodged from site 2 and lost for 70 days prior to its finding near site 1. Nearly all green crab captured in Lummi River were greater than 51 mm in carapace width. One green crab captured in September at Lummi River 1 measured 36 mm (Fig. 5). In contrast, the range of green crab carapace widths measured in Lummi Sea Pond had a broader range and was skewed more toward the 45-50 mm range. Additionally, green crabs from Lummi River had a greater proportion of males than females versus in Lummi Sea Pond where the opposite was observed.

The number of species and species groups also varied across sites, especially for the Nooksack River where three-spined stickleback (*Gasterosteus aculeatus*) and prickly sculpin (*Cottus asper*) were the most captured species, with 78.91% and 20.36% of total captures, respectively (Table 4). Lummi River sites 1-2 were most similar in incidental catches, while sites 3-4 had more similar bycatch. Across the river sites, site 5 was unique in incidental catch composition, where hairy shore crab and Pacific staghorn sculpin were nearly equal in captures (44.15% and 43.85%, respectively), and where the most three-spined sticklebacks were captured among Lummi River sites (11.11%). Dungeness crab was the second most captured bycatch species at sites 1 and 2, which was in contrast with sites 3 and 4 where Pacific staghorn sculpin was the second most occurring bycatch species. Lummi Sea Pond had the greatest number of green crab captures (137 green crabs, 3.69% of all animals captured) across all the sites and was most similar to Lummi River site 5 in terms of bycatch composition, where hairy shore crab comprised 43.45% of total captures and Pacific staghorn sculpin comprised 34.25% of total captures and Pacific staghorn sculpin comprised 34.25% of total captures. However, Lummi Sea Pond had greater catch diversity than site 5.

For brevity, the least observed bycatch species and species groups (<0.01% overall) are omitted from Table 4. Omitted bycatch included shiner perch (*Cymatogaster aggregata*), frogs (Ranidae), Japanese oyster drill (*Ocinebrellus inornatus*), tidepool sculpin (*Oligocottus maculosus*), plainfin midshipman (*Porichthys notatus*), unidentified flatfishes (Pleuronectidae), unidentified surfperch (Embiotocidae), unidentified beetle (Coleoptera), and an unidentified fish (Teleost) that was lost from the trap before it could be identified confidently.

Table 4. Percent captured species and species groups in European green crab (*Carcinus maenas*) trapping between April and October 2022 (percent
captured=number of animals for a given species divided by total number of animals for the site multiplied by 100); n=total number of traps
deployed and retrieved for each sample site regardless of soak nights.

	Lummi River				Lummi	Nooksack	Total	
Species	1 (n=194)	2 (n=191)	3 (n=164)	4 (n=188)	5 3) (n=196)	- Sea Pond (n=348)	River (n=57)	(n=1,341)
Hairy shore crab (Hemigrapsus oregonensis)	78.19	72.26	92.24	92.29	44.15	43.45	0.00	72.67
Pacific staghorn sculpin (Leptocottus armatus)	3.60	3.92	3.60	5.41	43.85	34.25	0.73	11.74
Snails (Gastropoda)	4.48	9.46	0.03	0.00	0.40	5.88	0.00	3.94
Dungeness crab (Metacarcinus magister)	9.88	9.05	0.37	0.12	0.10	0.08	0.00	3.73
Three-spined stickleback (Gasterosteus aculeatus)	0.15	0.18	0.35	1.25	11.11	2.05	78.91	2.40
Sand shrimps (Cragonidae spp.)	1.80	4.29	3.28	0.73	0.10	0.00	0.00	1.99
Hermit crabs (Paguridae)	1.28	0.55	0.00	0.00	0.00	5.91	0.00	1.44
European green crab (Carcinus maenas)	0.34	0.25	0.00	0.00	0.00	3.69	0.00	0.80
Non-native Asian shrimp (<i>Palaemon macrodactylus</i>)	0.00	0.00	0.00	0.00	0.00	3.02	0.00	0.57
Prickly sculpin (<i>Cottus asper</i>)	0.00	0.00	0.00	0.03	0.10	0.00	20.36	0.29
Broken back shrimps (Pandalidae & Hippolytidae)	0.00	0.00	0.00	0.00	0.00	0.73	0.00	0.14
Hairy helmet crab (<i>Telmessus cheiragonus</i>)	0.00	0.00	0.00	0.00	0.00	0.49	0.00	0.09
Gunnels (Pholidae)	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.03
Saddleback gunnel (Pholis ornata)	0.06	0.00	0.08	0.00	0.00	0.00	0.00	0.03
Purple shore crab (Hemigrapsus nudus)	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.03
Bay pipefish (Syngnathus leptorhynchus)	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Starry flounder (<i>Platichthys stellatus</i>)	0.06	0.02	0.00	0.00	0.00	0.00	0.00	0.02
Coho salmon (Oncorhynchus kisutch)	0.00	0.00	0.00	0.06	0.10	0.00	0.00	0.02
Total Captures	3,278	4,333	3,752	3,437	1,008	3,708	275	19,791

Water Quality

A salinity gradient was observed in Lummi River, where the overall average salinity decreased as sites increased in distance from the river mouth (Fig. 6). Overall salinity averaged from 26.16 ppt at Lummi River 1 to 16.66 ppt at Lummi River 5. Lummi Sea Pond had an average salinity of 27.08 ppt which was most like site 1 at Lummi River. Monthly average salinity increased at each river site over the course of the study period while salinity in Lummi Sea Pond remained relatively similar (daily average 24.05-29.00 ppt). The largest observed range of salinity measurements across the river sites occurred in April through June (daily average 0.48-27.48 ppt) but after the max tide height and total precipitation peaked in June, the range of salinity values decreased and was skewed towards higher values (daily average 22.55-41.54 ppt). Lummi River site 5 not only had the lowest monthly average salinity (April, 0.52 ppt) but also the highest (October, 41.29 ppt).

Overall averages of water temperature for each trapping site ranged between 17.60 °C to 20.34 °C (Fig. 7). The minimum water temperature measured was 7.2 °C at site 5 on Lummi River in April, and maximum water temperature recorded was 30.14 °C at site 4 in July. Monthly average water temperatures for all river sites and Lummi Sea Pond mirrored the variation of air temperature over the course of the study period, where water temperature increased until July and then decreased through October.

The Nooksack River site measurements for water temperature and salinity were taken every 10 minutes starting June 5 at 14:40 to June 7 at 10:30 for a total of 120 measurements. Average water temperature was 16.51 °C and average salinity was 0.59 ppt, which was the lowest average salinity measured across all sample sites. (Fig. 6).



븆 Lummi Sea Pond 📫 Lummi River 1 📫 Lummi River 2 📫 Lummi River 3 🖨 Lummi River 4 🖨 Lummi River 5

Figure 6. Salinity (ppt) measurement boxplots for Lummi Sea Pond and Lummi River trapping/sample sites by month (top), boxplots of the highest high tide height (m) by month in Lummi Bay (middle), and total precipitation by month from April 1 to October 7, 2022 (bottom); boxplot charts include 95% confidence intervals, medians, and outliers (points); "*" indicates limited sampling for each river sample site (n=2) and "**" indicates measurements missing from Lummi River 1 and Lummi River 4.



Figure 7. Monthly box and whisker plots for water temperature at each European green crab and water quality sample site (top), and monthly boxplots for air temperature on Lummi Reservation from April 1-October 7, 2022 (bottom); boxplot charts include 95% confidence intervals, medians, and outliers (points); *"*" indicates limited sampling for each river sample site (n=2) and "**" indicates measurements missing from Lummi River 1 and Lummi River 4*.

Correlation

Spearman's rank correlation was used to determine the monotonic relationship between salinity and number of green crabs, and water temperature and number of green crabs. For both water quality variables the correlation was overall weak when river and sea pond sites were examined together (Fig. 8). We first used green crab catch data and water quality data from all river sites and Lummi Sea Pond (Fig. 8 A & C). Then we examined Lummi Sea Pond data separately to determine which traps had the most green crab captures (Table 4; Fig. B & D). Salinity and number of green crabs had a weak positive correlation but with slight confidence (R=0.14, p=0.14). However, Lummi Sea Pond data alone had a strong positive correlation with high confidence (R=0.73, 0.03). Water temperature and number of green crabs had a weak negative correlation but with little confidence across Lummi River and Lummi Sea Pond sample sites (R=-0.07, p=0.45). Lummi Sea Pond data alone had a moderate negative correlation with high confidence (R=-0.045).



Figure 8. Scatterplot and Spearman correlation (with linear regression, correlation coefficient "R" and pvalue) of total daily captures of Carcinus maenas (CAMA) from April-October 2022 and daily mean salinity (ppt) of A) Lummi River sites 1-5 and Lummi Sea Pond, B) Lummi Sea Pond only; and daily mean water temperature (°C) of C) Lummi River sites 1-5 and Lummi Sea Pond, D) Lummi Sea Pond only.

Supplementary Results

The catch rates of the three most common bycatch (snails excluded) were plotted to examine the difference in CPUE across the study period and sites (Table 4). Hairy shore crab was the most captured bycatch species averaging between 0 to 120 crabs per trap during the study period (Supp. Fig. 1). Lummi River sites 3 and 4 captured the greatest number of hairy shore crabs, 3,461 and 3,172 total crabs, respectively. Catch rates of hairy shore crab generally increased at all sites over the study period.

Although Lummi River 5 had the highest monthly average catch rates of hairy shore crab in June and October, traps in Lummi Sea Pond more consistently caught hairy shore crabs over the course of the study duration (Supp. Fig. 1).

Pacific staghorn sculpin was the second most captured incidental species overall and was captured consistently at all trapping sites (Table 4; Supp. Fig. 1). Pacific staghorn sculpin was captured most frequently at Lummi River site 5 and Lummi Sea Pond (226 and 360 fish per 100 traps, respectively). Lummi River sites 1 and 3 on average captured the least amount of Pacific staghorn sculpins (60 and 82 fish per 100 traps, respectively). Average catch rates were overall highest in June and October across all trapping sites (253 and 367 fish per 100 traps, respectively) and lowest in April and May (10 and 30 fish per 100 traps, respectively).

Dungeness crab was the second most captured crab species overall but was more consistently captured at Lummi River sites 1 and 2 with an average of 167-203 crabs per 100 traps (Table 4; Supp. Fig. 1). Lummi Sea Pond and Lummi River site 5 captured on average the least amount of Dungeness crabs (0.31 and 0.51 crabs per 100 traps, respectively). May was the peak month of average capture rates at Lummi River sites 1 and 2 (475 and 785 green crab per 100 traps, respectively) but no traps at any of the sites captured Dungeness crab in the month of August.

Spearman Rank correlation analyses suggested moderate positive relationships between captures of green crab and hairy shore crab (R=0.21, p=0.23), Pacific staghorn sculpin (R=0.27, p<0.05), and hairy helmet crabs (R=0.44, p<0.05; Supp. Fig. 2). Dungeness crab had a weak positive correlation with green crab, but this result was insignificant (R=-0.06, p=0.52).



🚔 Lummi Sea Pond 📫 Lummi River 1 🗰 Lummi River 2 🚔 Lummi River 3 🛱 Lummi River 4 🛱 Lummi River 5

Supplementary Figure 1. Box and whisker plots of monthly CPUE (log-transformed) of hairy shore crab (*Hemigrapsus oregonensis* HEOR), Dungeness crab (*Metacarcinus magister* MEMA), and Pacific staghorn sculpin (*Leptocottus armatus* LEAR) at Lummi Sea Pond and 5 sites on the Lummi River from April 1-October 7, 2022. Boxplot charts include 95% confidence intervals, medians, and outliers (points).CPUE is equal to number of animals per 100 traps.



Supplementary Figure 2. Correlations between *Carcinus maenas* (CAMA) and A) *Hemigrapsus* oregonensis (HEOR), B) *Leptocottus armatus* (LEAR), C) *Metacarcinus magister* (MEMA), and D) *Telmessus cheiragonus* (TECH). Test statistics (R) represent the Spearman correlation coefficient. Replicates represent all individuals captured at a site within a day of the 2022 trapping season, April-October.

Conclusion and Recommendations:

At the current population status of green crab on Lummi Reservation tidelands, we observed no clear correlation between water quality parameters (salinity and water temperature) and trapping densities of green crab in Lummi River (Fig. 8). River sites captured few too crab to distinguish a relationship with water quality parameters. But in Lummi Sea Pond, higher salinity and lower water temperature conditions had a moderate, positive correlation with higher catches of green crab. Early invasion conditions at the mouth of Lummi River and documented salinities and water temperatures across all study sites suggested green crab will likely persist seasonally in Lummi River, especially in spring (April-June; Fig. 6 and Fig. 7).

Salinity and water temperature conditions of Lummi River were highly influenced by seasonal conditions that followed tide, precipitation, and air temperature trends (Fig. 6 and Fig. 7). The salinity gradient in Lummi River was better observed in spring and early summer (April-July) when precipitation was relatively high and high tides were at the highest, but the gradient became nearly nonexistent in the absence of precipitation, especially in August (Fig. 6). There was also an observable water temperature gradient across river sites where upriver sites were on average colder than downriver sites. But all sites reflected the seasonal increase and decrease in average daily air temperature (Fig. 7). However, as with salinity, the range of water temperatures across sites was less variable in late summer.

Given the known physiological tolerances of green crab and the water quality parameters in Lummi Sea Pond, Lummi River sites 1-5 provided survivable conditions during the entire study period, ranging in average daily salinities of 5-38 ppt and water temperatures of 9-29 °C (Cuculescu et al. 1998; Young and Elliott 2018; Fig. 6-7). Even during periods of high and low extreme temperatures and salinities, green crabs could outlast these conditions in the short-term. However, green crab captures were highest in spring months for both Lummi River 1 and Lummi Sea Pond, which overlaps with higher salinity and colder temperatures. Thus, this may be the most likely time of year to detect green crabs, especially using trapping as a form of detection (Fig. 4, Fig. 6, Fig. 7).

Another scenario that may have resulted in decreased catch rates during the progression of this study is that trap and removal activity in 2022 was successful in both Lummi River and Lummi Sea Pond (Fig. 4). This has been shown to be true from 2021-2023 in Lummi Sea Pond where trapping effort was extended to a full calendar year and increased more than two-fold (unpublished data; Duncombe and Therriault, 2017). Trap and removal effort outside of this study also gradually increased between April and September 2022 in Lummi Bay and Lummi River mouth, and thus could have contributed toward reduced catch rates in late summer as well as a lack of an observable correlation with water parameters.

Green crab demographics in Lummi Sea Pond also suggested effective trap and removal activities diminished catch rates (Fig. 5). The female to male ratio was roughly 3 to 1 in Lummi Sea Pond; which typically occurs when larger, more aggressive, adult male crabs are overfished and allows for more opportunity of smaller female crabs to enter traps (Duncombe and Therriault, 2017; Flynn et al. 2023). This trend was not evident in Lummi River, however, where males nearly dominated female green crab catch (roughly 1 female for every 5 males, Fig. 5). Increased trapping effort in Lummi River and Lummi Bay was not applied until April 2022; whereas in Lummi Sea Pond heightened trapping effort began in August 2021.

In addition to water quality conditions, distribution of native species could also influence distribution of green crabs, as has been observed with Cancrid crab species. But correlations with estuarine species have been less studied (Hunt et al. 2003; Howard et al. 2019). While not originally proposed in this study, we also explored the correlation of green crab captures and the four most captured fish and crab bycatch species to understand overlap in trapping occurrences of native species and green crab (*see supplementary results*). Hairy shore crab, Pacific staghorn sculpin, and hairy helmet crabs showed a moderate positive correlation with green crab captures, and Dungeness crab showed an insignificant weak positive correlation (Supp. Fig. 2). Therefore, we suggest future exploratory and early green crab detection trapping should target tideland areas with populations of hairy shore crabs, Pacific staghorn sculpins, and hairy helmet crabs. Outside the scope of this study, shrimp traps deployed at the mouth of Lummi River captured more juvenile Dungeness crabs and green crabs overall than Fukui-style traps (*unpublished data*). Therefore, it is likely trap type influenced the lack of correlation between Dungeness crab and green crab catches (Bergshoeff et al. 2019; Buzzell, 2023).

Lummi River's current conditions will likely allow the establishment of green crab in the future; however, exploratory conditions of the Nooksack River (low salinity) indicate less habitable conditions in the short-term. Nooksack River freshwater discharge ranges from 600-1800 ft³/second during spring and summer months but can exceed 19,000 ft³/second in fall and winter months¹. High flows likely displace organisms and may prevent the settlement of green crab (Kimmerer 2002). In the long-term however, sea level rise, reduced snowpack melt, and other climate changes could increase brackish habitat within the Nooksack River estuary and thus increase opportunity for green crab establishment (Huppert et al. 2009; Yamada et al. 2021).

Another confined population of green crab exists in Bellingham Bay within Squalicum Harbor, Bellingham, roughly 5 km from the mouth of the Nooksack River, although the current population status is unknown (Yamada et al. 2021). Due to its proximity, Squalicum Harbor poses an increased risk of future green crab establishment in the Nooksack River estuary. In the meantime, regular trapping at monthly or at minimum, quarter-year intervals, will suffice for early detection of green crab in the Nooksack River. Shifts in water parameters will be important for predicting potential establishment of green crab in the Nooksack River estuary; however, as previously mentioned, the settlement of other species (e.g., hairy shore crab) may be an important indicator of suitable green crab conditions, and so, green crab detection efforts should be in conjunction with monitoring other brackish crab and fish species. Ultimately, since the Nooksack River is the least accessible of trapping sites on the reservation tidelands, the frequency of trapping the Nooksack River will be dependent on staff and equipment availability.

Consistent trapping at Lummi River sites 1-3 should be continued with the use of the more efficient shrimp trap style on a biweekly schedule, or as the tide schedule allows (Buzzell, 2023). Shrimp traps are not only more efficient but are less likely to become dislodged and can withstand more biofouling compared to minnow and Fukui-style traps (*personal observation*). For management purposes, capturing so few green crabs in Lummi River was informative and provides evidence of an early invasion status

¹ USGS Nooksack River at Ferndale, WA – 12213100 Accessed November 6, 2023, <u>https://waterdata.usgs.gov/monitoring-</u> location/12213100/#parameterCode=00060&period=P365D&showMedian=true

outside of Lummi Sea Pond. But if the population is left unmanaged, the green crab population will likely increase within Lummi River and the surrounding tidelands.

Outreach and Products:

Results and the final report from this study are readily accessible to Lummi Natural Resources Department directors, managers, and staff through request to the AIS Division Manager. Part of the outreach for this project included coordination with the Northwest Indian College Intern Program which resulted in the participation of four NWIC interns and collaboration with the Salish Sea Research Center post-doc Dr. Brandi Kamermans. Dr. Kamermans and the interns collected water samples for eDNA sampling at multiple trapping sites at Lummi Sea Pond, Lummi Bay, and Lummi River to investigate the sensitivity of the technique in detecting green crab on Lummi Reservation in conjunction with trapping methods. This project was presented at the Indigidata Workshop (Indigenous Data Science Education) held at the Silver Reef Casino on the Lummi Reservation. More information regarding this workshop can be found at: <u>https://indigidata.org/</u>. A copy of the final presentation and results is also provided with this report.

Articles in prep: Buzzell, 2023 (*In prep*). Potential peer-reviewed journals include Knowledge of management of Aquatic Ecosystems and Fishery Bulletin.

Presentations:

"Lummi Reservation Tidelands European Green Crab" PowerPoint presentation for Washington Conservation Corp (virtual), April 7 2022

"Contextualizing the Research Question: Using eDNA to Detect Invasive Species" Indigidata Workshop (in-person) July 31 2022

Website containing project information: The public summary will be available on the Aquatic Invasive Species Division website, which can be found here <u>https://www.lummi-nsn.gov/Website.php?PageID=845</u>

Photos: See below.



First young-of-the-year European green crab (EGC) captured at Lummi River site 1 in September 2022. Photo credit: Bobbie Buzzell



Example of habitat and terrain variation in the salinity gradient study. Lummi River site 2 (left) and site 5 (right); LNR technician, Daniel Washington, pictured. During trapping periods, each site has 6 total traps deployed over 2 consecutive nights. Photo credit: Bobbie Buzzell



NWIC intern, Scott Boyd, and LIBC youth worker, Elias Plaster, assist with green crab trapping at Lummi River site 1. Photo credit: Bobbie Buzzell

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