APPENDIX I. STABLE ISOTOPE EVALUATION FOR CRAB

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Acronyms

LDW	Lower Duwamish Waterway
QAPP	quality assurance project plan
TTL	target tissue level
WDFW	Washington Department of Fish and Wildlife

I1 Stable Isotope Evaluation for Crab

An important factor in interpreting the crab tissue data for the Lower Duwamish Waterway (LDW) is the inclusion of two species of crab to assess site conditions: graceful crab (also called slender crab) or Dungeness crab.[1] Dungeness crab were the primary crab target species in the 2017 baseline sampling event, because they are the species of the most interest from a human health perspective. However, the abundance of Dungeness crab can vary considerably from year to year; in 2017, graceful crab was needed as a surrogate despite considerable effort expended to collect Dungeness crab.[2]

The relatively low numbers of Dungeness crab encountered in the LDW during sampling is consistent with the Washington Department of Fish and Wildlife's (WDWF's) characterization of 2017 as a "downturn year" for Dungeness crab in Puget Sound (Rothaus 2017). WDFW further noted that the catch rates for Dungeness in south Puget Sound were unusually low in 2017. This information, coupled with the fact that the siltier substrate of estuarine systems such as the LDW is not preferred habitat for Dungeness crab, resulted in the decision to collect graceful crab (which are more tolerant of short-term salinity fluctuations and siltier substrates) as the alternate species.

To evaluate the use of graceful crab as a surrogate, carbon and nitrogen stable isotope analyses were conducted to assess the similarity of trophic position and general habitat use of the two crab species. This appendix discusses the results of the stable isotope analyses conducted.

Carbon and nitrogen stable isotopes are commonly employed in toxicity and food web studies to investigate trophic dynamics. Specifically, $\delta^{15}N$ provides information about the trophic position and diet of consumers (Peterson and Fry 1987; Fry 1988; Peterson et al. 1985). $\delta^{13}C$ is useful for distinguishing among different food web types (e.g., different types of primary producers; terrestrial vs. marine sources) or the locations in which consumers feed along a salinity gradient (e.g., Stewart et al. 2004; France 1995). Stable isotope results for Dungeness and graceful crab in the LDW are shown in Table I1 and Figure I2.

[1] Several species of red rock crab were also collected in 1998, but will not be monitored in future events.

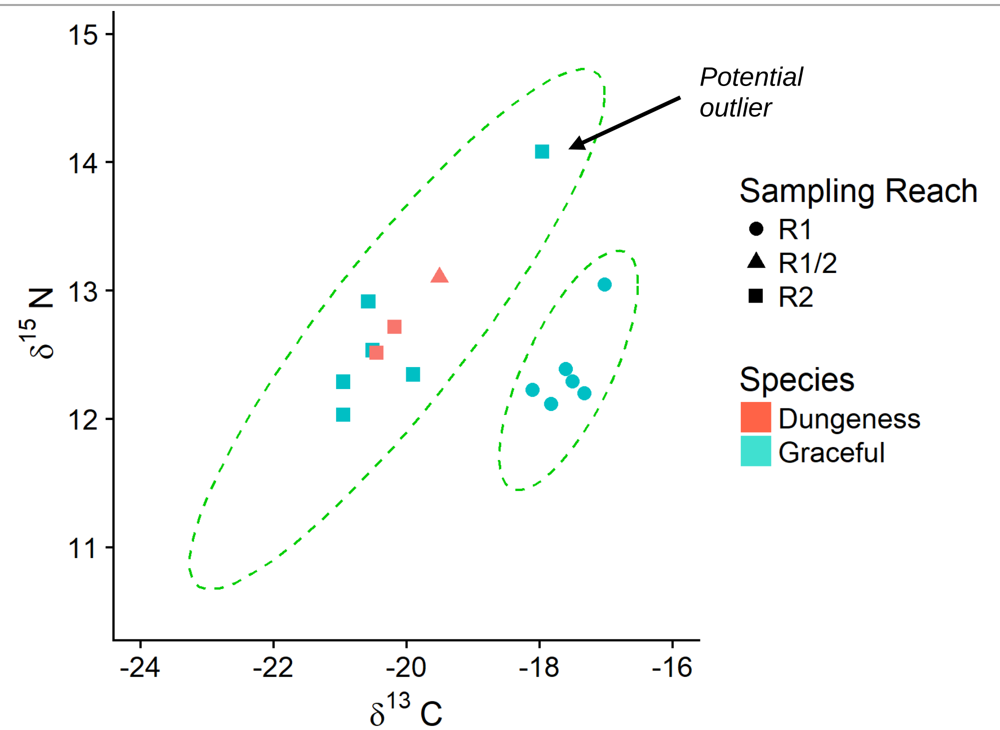
[2] Crab traps were deployed in the LDW over the course of five days, with soak times ranging from two to four hours or up to overnight. This increased level of effort from what was specified in the fish and crab quality assurance project plan (QAPP) was an effort to catch more Dungeness crab (Windward 2017). Despite this, a total of only 9 Dungeness crab (7 of which were larger than the legal size limit of 6.25 in.) were collected during the 2017 sampling (as compared with the target of 60).

Table I1. Summary of stable isotope results for crab tissue samples

Species/Area	Count of Edible Meat Composite Samples	Range of Stable Carbon Isotope Values (i.e., $\delta^{13}C$ Values) (per mil)[a]	Range of Stable Nitrogen Isotope Values (i.e., $\delta^{15}N$ values) (per mil)[a]
Dungeness Crab			
Reaches 1 and 2[b]	1	-19.5	13.1
Reach 2	2	-20.5 to -20.2	12.5 to 12.7
Graceful Crab			
Reach 1	6	-18.1 to -17.0	12.1 to 13.0
Reach 2	6	-21.0 to -18.0	12.0 to 14.1

a Stable isotope values are reported as “delta” values in parts per thousand, which are also commonly referred to as “per mil.” These values indicate the enrichment or depletion of the stable isotope relative to the standard (Stable Isotope Ecology Laboratory 1997).

b Because relatively few Dungeness crab were collected during the 2017 sampling, this Dungeness crab composite was made up of three crabs (one crab from Reach 1 and two crabs from Reach 2).



Note: The 90% probability ellipses shown in this figure are the 90th quantiles from a bivariate normal distribution with the mean, variance, and correlation exhibited by the graceful crab samples in each reach. There were insufficient data to calculate ellipses for Dungeness crab.

Figure I1. Scatterplot of stable isotope results for crab

The stable isotope results indicate the following:

- **Dungeness crab and graceful crab have similar trophic positions** – Excluding one potential outlier,[3] $\delta^{15}N$ values for both crab species are similar and range from 12 to 13.1. In general, a difference in trophic level corresponds to a 3 to 5 per mil[4] difference in $\delta^{15}N$ (Peterson and Fry 1987). In Figure I1, the probability bounds are drawn around the graceful crab data only, because insufficient data were available to estimate probability bounds for Dungeness crab. The Dungeness crab samples fall within the 90% probability ellipses for graceful crab from the same reach, an indication that the results are similar. The similarity of $\delta^{15}N$ in Dungeness and graceful crab in the LDW indicates that the two species occupy similar trophic positions and are ecologically comparable.
- **No difference in trophic level by reach** – The overlap in stable nitrogen isotope values between Reaches 1 and 2 indicates that the trophic positions of Dungeness and graceful crabs and their prey are similar by reach.
- **Stable carbon isotopes results correlate with salinity** – $\delta^{13}C$ values are similar for the two crab species and differ only by reach, with higher $\delta^{13}C$ for crabs in Reach 1 (as compared with those from Reach 2). Increased salinity (and more marine food sources) generally correlates with higher carbon stable isotope values (e.g., Claudino et al. 2013; Garcia et al. 2007). Thus, the higher $\delta^{13}C$ values for the Reach 1 samples match the expected result.

Based on the results of this evaluation, the use of graceful crab as a surrogate for Dungeness crab to evaluate progress towards the target tissue level (TTL) is reasonable, given the species' similar trophic position and general habitat use. Future sampling events will target both types of crab. Graceful crab, which are commonly present in the LDW (and less subject to "downturn years") can reliably be collected to evaluate trends. Dungeness crab will be targeted and collected when present because they are the species of greatest interest from a human health perspective.

I2 References

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[3] One sample was identified as a potential bivariate outlier using an adaptive outlier detection procedure based on squared robust Mahalanobis distances within each reach (in R: *mvoutlier::aq.plot(alpha=0.1)*, (Filzmoser and Gschwandtner 2018).

[4] Stable isotope values are reported as "delta" values in parts per thousand, which are also commonly referred to as "per mil." These values indicate the enrichment or depletion of the stable isotope relative to the standard (Stable Isotope Ecology Laboratory 1997).

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