Biological sensitivity of Salish Sea taxa to low oxygen levels: determining observed metabolic demand thresholds of key taxa based on concomitantly measuring abundance, oxygen, and temperature

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Summary:
The primary questions that this analysis proposed to address was: what are the critical oxygen thresholds of key taxa (across life stages), and when and where in Puget Sound do oxygen levels fall below these thresholds? In order to better understand dissolved oxygen (DO) thresholds for Salish sea species, we first processed and collated available Salish sea fish surveys that had concurrent oxygen and temperature information into an initial database repository (Table 1). This data is collated in a Github repository for future research use, and Tim Essington (essing@uw.edu) is the primary contact. Second, we conducted preliminary analysis of all suitable data both qualitatively and quantitatively, using a probabilistic generalized linear model. This was done to identify if critical oxygen and temperature ranges existed among species based on available survey data.

Based on the statistical analysis using all suitable data, we did not find evidence of a strong DO threshold for herring and Chinook salmon (data was collected by Fisheries & Oceans Canada and the University of Washington in the broader Puget Sound). However, exploration of the available presence and absence data provided qualitative information on thresholds for the taxa examined. Interestingly, we found that fish were present at depths with low DO levels even when there was more oxygen available higher in the water column. Specifically, fish are found at lower DO levels, as low as 1.3 mg/L for herring and 2.06 mg/L for Chinook salmon, even when DO levels higher in the water column were >6 mg/L (Figures 3 and 4). Overall, we suggest that the current data does not provide a clear threshold for herring or Chinook salmon. Qualitative analysis of presence and absence data does suggest that any thresholds are likely below 1.3 mg/L and 2.06 mg/L, respectively. Future survey efforts can provide better insight if CTD sampling is conducted immediately preceding or following trawl surveys and key metadata like tow time, distance, and depth are recorded. Additionally, conducting more surveys overall, and specifically targeting these surveys for the fall when lower and wider ranges of DO are typical will likely improve the model inference in future analyses.

Background and research objectives:
Maintaining adequate levels of DO is critical for the survival and well-being of benthic and pelagic marine organisms (Davis, 1975; Vaquer-Sunyer and Duarte, 2008). However, accurately predicting responses and impacts on aquatic species can be difficult (Moriarty et al., 2020; Sato et al., 2016). Currently, our scientific understanding and ability to forecast habitat and species shifts due to changes in oxygen demand and supply are limited by a lack of knowledge on Salish

Sea species' vulnerability to the synergistic impacts of low DO and warming waters. Synergistic impacts are due to the joint effects of oxygen and temperature and emerge from differences in temperature-dependent rates of oxygen intake vs. oxygen expenditure (Deutsch et al. 2015). As a result, the consequences of oxygen changes cannot be considered without also knowing the temperature that an organism will experience (Essington et al., forthcoming). Several topics associated with DO threshold values for Salish Sea species were identified as research needs and critical uncertainties by the Interdisciplinary Team during the Marine Water Quality Implementation Strategy development process. The research undertaken in this project is a first step towards addressing these critical uncertainties. The primary questions that this analysis will answer are: What are the critical oxygen thresholds of key taxa (across life stages), and when and where in Puget Sound do oxygen levels fall below these thresholds?

Methods:

Three steps, and associated methodologies, were applied in this project:

1) Collation and processing of available Salish Sea survey data where there were concurrent oxygen and temperature and fish surveys conducted. Tim Essington will serve as the primary contact for the compiled database for future research.
2) Preliminary data exploration and qualitative analysis of critical oxygen and temperature ranges were conducted for species with sufficient data.
3) Hypothesis testing and model selection to understand if temperature and oxygen levels predicted fish presence.

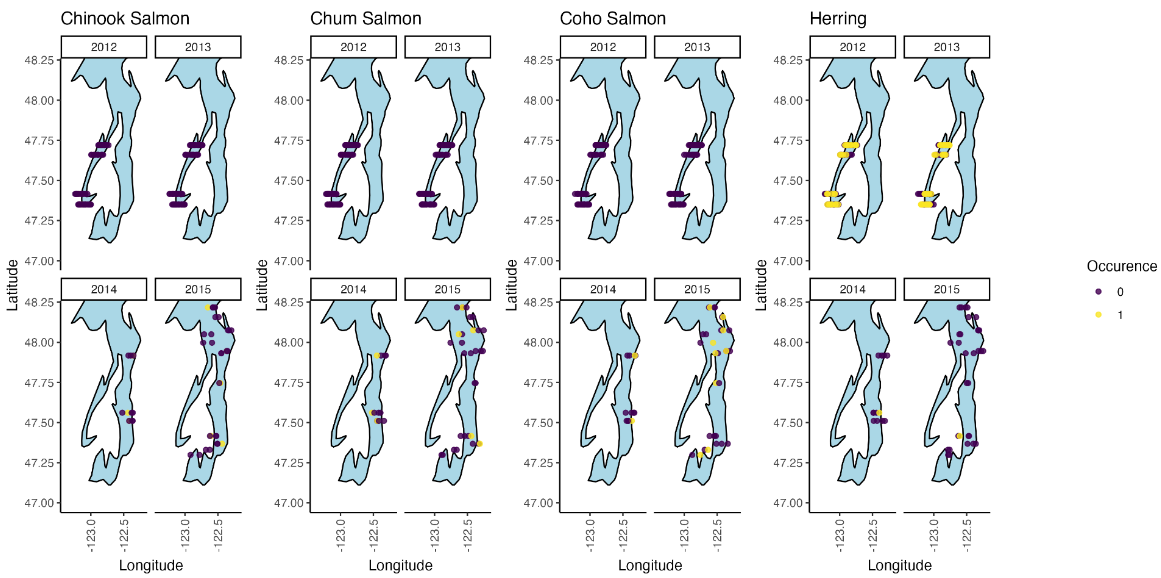
<u>Collation and Processing of Salish Sea Survey Data</u>

Multiple Salish Sea datasets that included fish abundance with concurrent CTD (a conductivity, temperature, and depth instrument) casts were collated and reviewed, including:

- Department of Fisheries and Oceans, Canada pelagic species surveys: RV Ricker mid-water trawl surveys (2014 and 2015 available) (hereafter, DFO).
- Long Live the Kings continuation of RV Ricker sampling sites in the Salish Sea - 2021 and 2022 available, but lacking tow depth and time information needed to calculate CPUE and match with CTD data (hereafter, LLTK).
- Washington Department of Fish and Wildlife bottom trawl surveys – biological data collated (1989-2007), but the availability and extent of associated DO and other physical datasets were unknown (hereafter, WDFW).
- University of Washington Hood Canal dataset, curated by Tim Essington and colleagues combining survey data from Hood Canal with CTD data (hereafter, UW).

After considering all four datasets, only the DFO and UW datasets were found to have the required physical (i.e., DO, temperature) and fish abundance (Catch Per Unit Effort- CPUE) information suitable for this current analysis. Additional information on future survey needs is provided in the discussion.

Figure 1. Map of fish occurrence and survey stations from both UW and DFO surveys in the Southern Salish Sea. Plots are grouped by species and years. Here, purple indicates that fish did not occur in a survey, and yellow indicates at least one fish was caught in that survey. Overlapping points were slightly "nudged' so that multiple surveys were visible in one region.



We received datasets in varied formats and processing levels, thus much of the effort in this project was dedicated to quality control and data processing. For each dataset (DFO and UW), we calculated the Catch Per Unit Effort, based on the net opening for each survey and the length of the tow. CTD data, which surveys the environment along the water column, was matched to the fish survey data to the closest survey depth.

The solubility of oxygen in water is affected by temperature, thus we calculated temperature-adjusted DO values for the analysis. The temperature adjusted DO equation took the following form,

Adjusted DO = DO * exp(KB * (1 / Temperature - Temperature/Temperature Reference))

The key components of the formula are:

- DO: The original dissolved oxygen concentration measurement.
- KB: A constant that represents the temperature coefficient for the solubility of oxygen in water. This value typically ranges from 0.0241 to 0.0272, depending on the specific water conditions.
- Temperature: The water temperature in Kelvin units.
- Temperature Reference: A reference temperature in Kelvin units, often 293.15 K (20°C), used as the baseline for the temperature adjustment.

By using this formula, we can reliably adjust DO measurements to a common temperature, facilitating meaningful comparisons and analysis of the data across different sampling points or time periods. All measurements presented below as DO mg/L, are temperature-adjusted DO values. We included covariates from the CTD in the analysis, with the main focus on DO. We included minimum water column DO, DO at the depth the fish were surveyed, and temperature at the depth the fish were surveyed. Datasets were evaluated for completeness and accuracy, coded based on the data source (i.e., source = “DFO” or “UW”), and assimilated into one dataset.

Exploration and Qualitative Analysis of Oxygen, Temperature and Taxa Data

To understand the range of DO and temperature values across available data we plotted the range of DO and temperature where fish were present and absent for herring and Chinook, chum, and coho salmon (Figure 2). To understand the entire DO profile that might be available to a fish relative to the DO at the depth they were found in surveys, we further analyzed the more detailed UW dataset. This included plotting Chinook salmon and herring CPUE data verses DO depth profiles (Figure 3 and 4).

Statistical Hypothesis Testing

We used generalized linear models to estimate the probability of Chinook salmon and herring occurrence with varying temperature and DO. The model was developed and applied using the Ime4 package in R (Bates et al., 2014). Due to a limitation of statistical power and limited overlap between surveys, we ran these models for just two species: Chinook salmon (*Oncorhynchus tshawytscha*) and Pacific herring (*Clupea pallasii*). We ran separate models for each species and used a binomial distribution to estimate the probability of fish occurrence across temperatures and DO levels. We expected that fish (Chinook salmon or herring) presence may be impacted by DO levels throughout the water column, in addition to the temperature and DO at the depth at which they are surveyed. Specifically, if fish presence was impacted by DO, we expected fish might be present in regions of the water column that had greater DO than other regions.

DO and temperature covariates were obtained from CTD data collected during the fish surveys in similar locations to the trawls. We included CTD temperature and DO at the mean net tow depth as a predictor. Additionally, we hypothesized that minimum DO present throughout the complete water column would have an effect on the presence of fish in the net surveys and thus included minimum DO as a covariate as well.

To control for differences in observed fish occurrence among data sources within the model framework, we included a data source factor (either DFO or UW). We also accounted for survey depth, location, day of year, and time of day (applying a diel factor for day or night survey). Specifically, we incorporated a linear predictor for latitude, to account for changes in fish occurrence based on latitudinal variation in survey locations (there was not enough variation in survey longitudes to necessitate incorporating a full spatial field). Further, we incorporated a linear predictor for depth and day of year to account for changes in fish occurrence based on sample depth and seasonality. We mean-scaled all environmental covariates to allow for meaningful comparison across conditions but present the actual covariate values in the following plots.

First, we constructed a null model that estimated fish occurrence while controlling for survey design (Table 1) and sequentially added covariate complexity to address hypotheses regarding temperature and oxygen impacts on fish occurrence (Table 1). The full model took the form:

$$logit(\gamma_i) = \alpha + S_l + V_m + \beta x_i + \beta y_i + \beta z_i + \beta m_i + \beta d_i + \beta t_i$$

where Yi is the expected occurrence, for the *i*-th observation in space and time with a logit-link function, α is the intercept, S_l is the factor for data source which controls for differences in observed fish occurrence among each data source (DFO or UW), V_m is a factor for diel survey time which controls for differences in observed fish occurrence among night and day surveys. βx_i represents the linear effect of latitude, where x_i the latitude for each observation, which is included to control for differences in fish presence that occurred across survey latitudes and β describes the slope of the relationship. βy_i represents the linear effect of depth, where y_i the depth for each observation, which is included to control for differences in fish presence that occurred across survey depth and β describes the slope of the relationship. βz_i represents the linear effect of DOY, where z_i the DOY for each observation, which is included to control for differences in fish presence that occurred across survey DOY and β describes the slope of the relationship. Finally, βm_i, βd_i and βt_i account for linear effects of minimum water column DO, DO at the depth fish were surveyed, and temperature at the depth fish were surveyed, respectively. The complete set of models tested, nested within this full model are presented in Table 1.

Table 1. Datasets considered for this analysis.

Dataset	**Years**	**Further notes and additional data required for analysis**
Department of Fisheries and Oceans Canada (DFO) Main contact: Chrys Neville (Chrys.Neville@dfo-mpo.gc.ca)	2014, 2015; Surveys conducted in July, October and November via mid-water pelagic trawl; sampled day only, 40 tows conducted in total.	NA, used in analysis
University of Washington (UW) Main contact: Tim Essington (essing@uw.edu)	2012 - 2013; approximately 80 tows per year at 4 stations in the Hood Canal, sampled day and night via midwater trawl, June - October.	NA, used in analysis
Long Live the Kings (LLTK) Main contact: Liz Duffy (eduffy@lltk.org)	2021-2023; approximately 47 total tows at stations across the Salish Sea, sampled day only via Purse Seine, July.	Collect gear depth and total tow effort (linear distance or tow time).
Washington Department of Fish and Wildlife (WDFW)	Did not receive data because of lacking CTD	No available CTD data, see accompanying

Main contact: Jennifer Blaine (Jennifer.Blaine@dfw.wa.gov)	information.	recommendations in text for all related CTD recommendations.

To test hypotheses regarding the importance of temperature and DO in predicting fish occurrence, we compared multiple models against a base model (Table 1) and judged the degree of support for each model using corrected Akaike information criterion (AICc) (Akaike 1973, Hurvich and Tsai 1989, Burnham and Anderson 2002). AICc was used to account for small sample sizes (Table 1). We present models in the results ranked by delta AICc (ΔAICc) which represents the difference between each model's AICc value and the lowest AICc value in your set of candidate models (Table 2). A ΔAICc greater than 2 is considered meaningful.

Table 2. Model structure and model selection criteria (ΔAIC_c) applied to the presence and absence of Chinook salmon and herring in the Salish Sea. We evaluated 7 candidate models per species. Overall differences in AIC_c values between the null model and B-D alternative models are small (<= 2) so the null model cannot be dismissed for either species. Covariates not included in the base model are highlighted in bold to demonstrate changes in model complexity.

Model Name	Model	delta AICc
Chinook Mod Null	Latitude + source + diel + depth + DOY	0
Chinook Mod B	Latitude + source + diel + depth + DOY + **min_DO**	0.9
Chinook Mod C	Latitude + source + diel + depth + DOY + **DO**	1.7
Chinook Mod D	Latitude + source + diel + depth + DOY + **temperature**	1.9
Chinook Mod E	Latitude + source + diel + depth + DOY + **DO + temperature**	2.8
Chinook Mod F	Latitude + source + diel + depth + DOY + **min_DO + temperature**	3
Chinook Mod Full	Latitude + source + diel + depth + DOY + **min_DO + DO + temperature**	4.5
Herring Mod Null	Latitude + source + diel + depth + DOY	0
Herring Mod B	Latitude + source + diel + depth + DOY + **min_DO**	1.4
Herring Mod C	Latitude + source + diel + depth + DOY + **DO**	1.9
Herring Mod D	Latitude + source + diel + depth + DOY + **temperature**	1.9
Herring Mod E	Latitude + source + diel + depth + DOY + **DO + temperature**	3.2
Herring Mod F	Latitude + source + diel + depth + DOY + **min_DO + temperature**	3.4

Herring Mod Full	Latitude + source + diel + depth + DOY + **min_DO + DO + temperature**	5.3

Results and Discussion:

Collation and Processing of Salish Sea Survey Data
We found that two of the available data sources could be applied in this analysis, DFO and UW. Unfortunately, WDFW was not able to access CTD data files that coincided with these fish surveys. The LLTK data will be viable for this type of analysis in future years, however, in previous years there was no record of trawl depth or trawl time (i.e. minutes), which is needed to calculate CPUE and to match the CPUE data with the DO data. For future integration of LLTK survey data into subsequent analyses we have two recommendations. First, we recommend that the linear distance traveled for each tow be recorded, or as a minimum, the tow start and end time (as was available with the DFO data). This allows standardization of catch data by sampling effort and across datasets. Second, we recommend that the depth(s) of the survey net is recorded (i.e. start and end net depth). Depth information allows the matching of depth specific CTD data and provides context to understand the conditions where fish were caught versus conditions throughout the water column.

The following is recommended for any future survey efforts aiming to collect data that can improve understanding of fish DO thresholds in the Salish Sea:

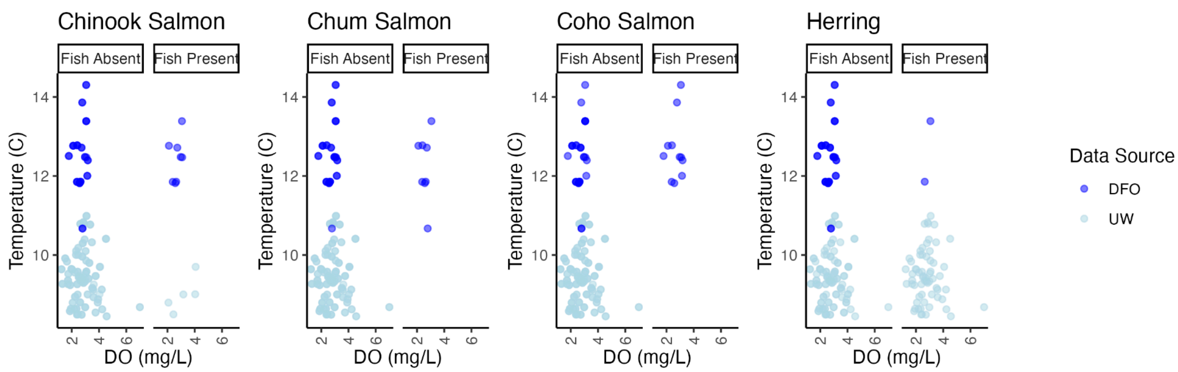
- Conduct CTD sampling (DO and temperature) immediately preceding or following trawl surveys for fish abundances, recording the tow effort (i.e. tow time or distance traveled), gear type, gear depth, location of trawl start and end (latitude and longitude). This is likely more accurate with two boats; however we acknowledge the added survey costs associated with a multi-boat approach likely make it not feasible.
- Ensure the instruments, for example a CTD, have been calibrated and tested, and data processed on a regular cadence.
- Focus surveys seasonally in the Fall to cover the widest range of water column DO concentrations. We suggest the Fall because this is when lower DO values are generally most likely to occur widely. Increased spatial effort across a range of DO values, and low DO values, will allow for increased inference related to DO and temperature thresholds.
- Provide consistent metadata for data-users to provide the necessary context to ensure that data is applied correctly.

Exploration and Qualitative Analysis of Oxygen, Temperature and Taxa Data

We qualitatively explored the oxygen threshold limits of herring and multiple salmon species by plotting fish presence and absence across temperature and DO values to demonstrate the range of conditions that these fish occurred in (Figure 2). Together, these datasets provide insight into the range of temperatures and DO conditions in which Chinook salmon and herring occur. That is that any threshold values must be beyond the range of the environmental conditions represented within the currently available data. We found that the DFO data captured a smaller range of DO values and overall warmer temperatures than the UW surveys (Figure 2). The UW CTD captured DO levels from 1.22 to 6.9 mg DO/L, while the DFO CTD dataset surveyed had a lower and narrower DO range, 1.78 to 3.17 mg/L (Figure 2). The UW CTD captured temperatures from 8.4 -10.9 ℃, while the DFO CTD dataset captured temperatures from 10.6 - 14.3 ℃ (Figure 2).

While chum and coho were not caught frequently enough to incorporate in a statistical model, plots of presents and absence (Figure 2) offer insight into the oxygen conditions that these fish experienced. Qualitatively, there did not appear to be a threshold where fish no longer occurred, fish were caught at very low DO levels (herring: 1.2 - 6.99 mg /L, Chinook salmon: 2.06 - 4.06 mg/L, chum: 2.1 - 3.1 mg/L, coho: 1.79 - 3.17 mg/L).

Figure 2. Fish occurrence by the range of temperature (C) and dissolved oxygen (DO mg/L, adjusted for temperature) values at the same depth where fish were caught. Plots are grouped by species, and colors indicate the data source.



Further examination of the more detailed UW data indicates that herring and Chinook salmon do not appear to "prefer" higher DO regions in the water column (Figures 3 and 4). We found that fish were present at depths with low DO levels even when there was more oxygen available higher in the water column. Specifically, fish were found at lower DO levels (as low as 1.3 mg/L for herring and 2.06 mg/L for Chinook), even when DO levels at other places in the water column were >6 mg/L (Figures 3 and 4). Overall, this qualitative review of the UW data do not indicate a specific threshold for herring or Chinook salmon, but the data do indicate that thresholds are likely below 1.3 mg/L and 2.06 mg/L, respectively, at least for the temperatures experienced in these sampling events.

Figure 3. Depth (ft) and water column DO for UW surveys that caught adult Chinook salmon. The catch per unit effort (CPUE) is represented by the size of the red dot, and the horizontal dashed line indicates the depth where the fish was caught. Plots are grouped by survey month and year (month.year) and the survey location. These surveys took place in Hood Canal, and Da = Dabob Bay, Hp = Hoodsport, and Un = Union.

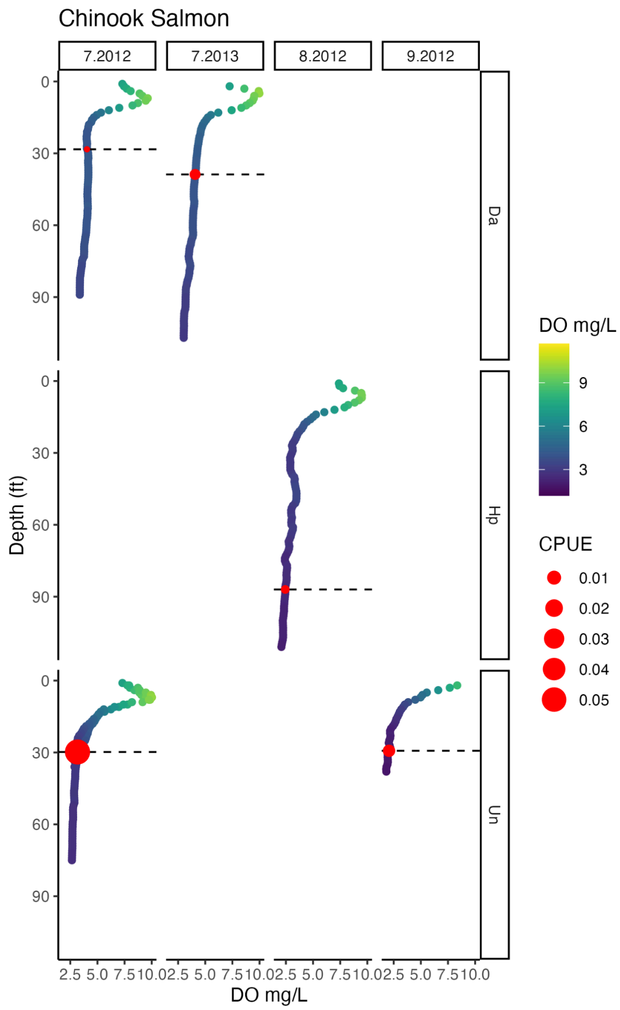
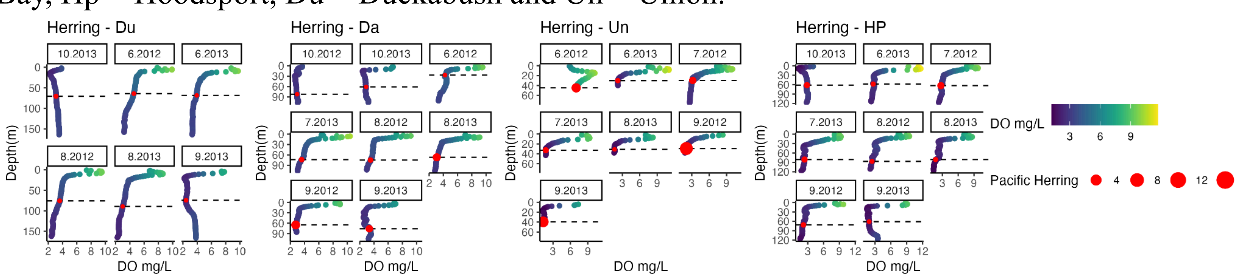


Figure 4. Depth (ft) and water column DO for UW surveys that caught adult Herring. The catch per unit effort (CPUE) is represented by the size of the red dot, and the horizontal dashed line indicates the depth where the fish was caught. Plots are grouped by survey month and year (month.year) and the survey location. These surveys took place in Hood Canal, and Da = Dabob Bay, Hp = Hoodsport, Du = Duckabush and Un = Union.



Hypothesis Testing and Modeling of Environmental Drivers

We used a generalized linear model to estimate the effects of DO on the probability of capturing a herring or a Chinook salmon. For both species, there was no support for models that contained any combination of DO or temperature covariates over a simpler (null) model that only considered location, depth, and day of year without environmental covariates (Table 2). We used AIC_c to identify the most appropriate model among the seven candidate models (Table 2). AIC_c balances model complexity against how well the model fits the data, with a specific correction for small sample sizes. We calculated ΔAIC_c by subtracting the lowest AIC_c from the remaining models. A Δ AIC_c greater than 2 is considered meaningful because it represents a substantial difference in model support based on statistical theory. We found that overall differences in AIC_c values between the null model and B-D alternative models were small ($<= 2$) so the null model cannot be dismissed for either species. This means that there was no support for the proposed hypotheses using the data collated for this project. We found that DO does not statistically predict the probability of observing a Chinook salmon or herring.

Given the lack of statistical support for including DO or temperature relationships in models estimating fish presence, we suggest that the best way to gain insight into DO and temperature limits from the current dataset is to evaluate the plots of the data qualitatively, as presented prior. This also provides insight into why the available data presents limitations in drawing inferences about DO thresholds. In particular, there was minimal overlap in survey location and timing between both data sets which resulted in fish species being caught in variable environmental conditions from each other (Figure 1 and Figure 2). Conducting more surveys overall, and specifically targeting these surveys for the fall when lower and wider ranges of DO are typical will likely improve the model inference in future analyses.

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