The non-lethal threat of hypoxia: ecological effects and physiological responses of estuarine species

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Abstract

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Hypoxia [dissolved oxygen (DO) < 2mg L^{-1}] is one of the key threats to some of the most productive regions of the marine environment (e.g., estuaries). Although mortality can occur, mobile organisms have the potential to avoid the most severe low oxygen conditions, but suffer ecologically significant indirect and sublethal impacts as a result. In Washington State, USA, a fjord estuary of the Puget Sound marine ecosystem, known as Hood Canal (110 km), regularly experiences seasonal hypoxia. My dissertation addresses several important gaps in the current knowledge pertaining to the non-lethal biological effects of hypoxia on the mobile benthic and pelagic species of Hood Canal. Using acoustic telemetry, Chapter 1 quantified movement patterns and distributional shifts of Dungeness crab (*Metacarcinus magister*) and English sole (*Parophrys vetulus*), two abundant demersal species. Although highly mobile, both species displayed more localized, rather than large-scale, directional movement relative to hypoxia. Dungeness crab in particular showed significant distributional shifts towards shallower waters. Chapter 2 expanded the investigation of hypoxia's influence in the estuary by assessing spatiotemporal patterns of nearshore macrofaunal community composition. Using underwater video monitoring, the hypoxic region of Hood Canal was found to be primarily composed of hypoxia tolerant invertebrates and fewer fish species compared to the more oxygenated region. Additionally, tolerant and sensitive species displayed distinct DO-tolerance thresholds and responses to reduced oxygen levels. In Chapter 3, we employed experiments and field sampling to measure the hepatic mRNA expression of hypoxia-inducible factor-1α (HIF-1α) as a biomarker for hypoxia exposure of Pacific herring (*Clupea pallasii*). In the lab, HIF-1α mRNA increased in a threshold and a dose-like response under various hypoxia treatments. In the field, the spatial pattern of gene expression was counter to the hypoxia gradient in the estuary, yet the more severe hypoxic time period appeared to correspond with higher mRNA levels. Chapter 4 investigated the generalized relationship between hypoxia and Dungeness crab harvest (3-S) management strategy, an important fishery in Puget Sound. Inferred by the shoaling behavior from the Chapter 1 study, an age-structured population model was constructed to test several hypoxia-scenarios with other stressors, including harvest, illegal crab fishing, and incidental capture mortality. We found the 3-S management strategy most sensitive to the influence of hypoxia when other sources of demographic restrictions were considered, underscoring the uncertainty associated with a data-poor species under multiple anthropogenic and environmental stressors.

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DEDICATION

To curiosity and exploration.

Introduction

Estuaries are among the most productive and biologically rich systems on earth, providing vital nursery habitat for numerous fish and invertebrate species. In addition to the high diversity and abundance of organisms, estuaries provide important commercial and recreational fishing opportunities. However, these ecosystems often occur in close proximity to dense human populated areas and are thus under the threat of numerous human impacts (McGranahan et al. 2007). One of the primary threats is diminished dissolved oxygen (*i.e.,* hypoxia and anoxia), which is increasing in frequency, as well as spatial and temporal extent as a result of anthropogenic eutrophication (Diaz and Rosenberg 2008), emerging as a potential threat to the productivity of the fishing industry by altering the structure and biological interactions of aquatic ecosystems (Breitburg 2002; Diaz 2001). Hypoxia, commonly defined as dissolved oxygen (DO) concentrations less than 2 mg L^{-1}, is now a common feature of estuaries and semi-enclosed seas worldwide (Diaz 2001). Therefore, as oxygen conditions continue to deteriorate, understanding the direct (*e.g.*, mortality) and indirect consequences of hypoxia is pertinent to the conservation of these ecosystems.

Two main types of biological impacts are typically associated with hypoxia. First, low oxygen conditions can directly cause mortality. For example, hypoxia has a particularly high lethal impact on sessile species that lack the capacity to seek out refuges (Breitburg 1992a; Diaz and Rosenberg 1995; Vaquer-Sunyer and Duarte 2008). Second, hypoxia can alter the behavior of mobile organisms (pelagic and benthic); one type being an avoidance response. Avoidance behavior can reduce exposure to hypoxia through distributional shifts, documented as short-term reductions in densities of mobile fauna in hypoxia-impacted areas (Diaz and Rosenberg 1995; Eby et al. 2005; Petersen and Pihl 1995; Pihl et al. 1991). However, hypoxia tolerant species can also exploit hypoxic waters for resources (Long and Seitz 2008) and/or refuge from less tolerant predators (Parker-Stetter and Horne 2009). Although these behaviors reduce mortality, they introduce shifts in species distributions, trophic interactions, hypoxia exposure, and habitat use.

In Washington State, USA, Puget Sound, a large marine ecosystem that supports a diverse array of marine species, contains a seasonally hypoxic fjordic estuary known as Hood Canal (110 km) (Newton et al. 2007). Although hypoxia has been reported in Hood Canal since the 1950s, oxygen conditions appear to be deteriorating even further due to a combination of anthropogenic influences, including climate change and nutrient loading (Fagergren et al. 2004; Newton 1995). A sill at the narrow northern opening and the steep bathymetry of Hood Canal reduces tidal currents and hydrological exchange rates from north to south (Newton 1995). As a result, DO levels throughout the southern regions decline throughout summer while the vertical extent of hypoxia expands through the water column, reaching depths of 20–30 m (Newton 1995). Due to the natural environmental (DO) gradient and relatively small spatial scale, Hood Canal was an ideal system to study the effects of hypoxia on marine biota in a fjordic system.

While Hood Canal has experienced fish-kills associated with hypoxic events (Fagergren et al. 2004), recent findings appear to describe large scale hypoxia avoidance by several local species. During the 2007 hypoxic episode, Essington and Paulson (2010) documented reduced densities of demersal fish and macroinvertebrates that occur seasonally, but found less evidence for persistent reductions among the mobile species. The temporary declines in abundance suggested the occurrence of distributional shifts. Studies have also reported spatiotemporal shifts of pelagic species in Hood Canal (Keister et al. 2000; Parker-Stetter and Horne 2009; Parker-Stetter et al. 2009). However, hypoxia’s impact on pelagic versus demersal species can differ greatly due to benthic oriented species being more associated with the bottom, where low oxygen conditions tend to manifest. Consequently, these closely linked systems both require investigation.

The extent of avoidance has important ecological and management implications. Mass movement of mobile organisms into oxygenated refugia can concentrate densities of fish and invertebrates resulting in density-dependent effects, such as reduction in individual growth (Craig and Crowder 2005; Eby et al. 2005). Distributional shifts can also alter the spatial overlap of predators and prey, causing increased predation mortality (Aumann et al. 2006; Long and Sietz 2008) or reduce access to important prey (Bell and Eggleston 2005; Parker-Stetter and Horne 2009). Similarly, overlap of fishing gear and aggregating species may also increase vulnerability to harvest (Selberg et al. 2001). In addition, the magnitude of avoidance can vary due to other biotic (e.g., prey) and abiotic (e.g., temperature) effects, influencing the level of hypoxic exposure that can directly impact growth, reproduction, and development (Pollock et al. 2007; Wu 2002). Consequently, these responses can have important implications for population and community level effects. Such indirect and sublethal effects of hypoxia can thus be as ecologically significant as direct mortality (Diaz and Rosenberg 1995). As a result, evaluation of the single and multi-species (*i.e.*, community) physiological and behavioral responses are essential for management of key commercial species and the ecosystem as a whole.

This dissertation addresses several important gaps in the current knowledge pertaining to the non-lethal effects of hypoxia in Hood Canal, Washington. Proceeding from the findings of Essington and Paulsen (2010), a more detail understanding of individual movement patterns of dermersal species was pertinent for making more robust inferences about the relative impacts of hypoxia in the system. However, the techniques (*i.e.*, acoustic telemetry and underwater video) used for monitoring shifts in individual species and species' densities resulted in some contradictory findings. As a result, an integrative physiological approach (biomarker) is proposed to provide additional information on the level of hypoxia exposure for a specific, sentinel species. Moreover, the biomarker is proposed for a pelagic species for which hypoxic interactions are even less understood. Ultimately, the common thread of the behavioral and physiological studies is to provide valuable information for management purposes. To that end, the last component of this dissertation will directly investigate the generalized relationship between hypoxia and Dungeness crab harvest (3-S) management strategy, a major fishery in Puget Sound. In all, my proposed dissertation work seeks to make an advance by characterizing secondary effects of hypoxia and considering their ecological and management implications.

Chapter 1

Movement patterns and distributional shifts of Dungeness crab (Metacarcinus magister) and English sole (Parophrys vetulus) during seasonal hypoxia[1]

Introduction

Estuarine ecosystems are among the most productive and biologically rich systems on Earth. They serve as nursery grounds and key harvest areas for fish and invertebrates, offer protection to coastal zones by attenuating waves and storms, and provide recreational opportunities. However, because these ecosystems often occur in close proximity to dense human population centers, they are threatened by numerous human impacts (Peterson and Estes 2001). Chief among these threats is diminished dissolved oxygen (*i.e.,* hypoxia and anoxia), which is increasing in spatial and temporal extent and occurring at greater frequency in response to anthropogenic eutrophication (Diaz and Rosenberg 2008). Hypoxia, defined as dissolved oxygen (DO) concentrations less than 2 mg L^{-1} (Eby 2004; Keller et al. 2010; Pihl et al. 1991), is now widespread throughout estuaries and semi-enclosed seas (Diaz 2001). Although hypoxia can be permanent or intermittent, estuaries that stratify during the summer months often experience hypoxia as a seasonal disturbance due to restricted mixing between the deep DO depleted water and oxygen rich surface water (Diaz and Rosenberg 2008; Officer et al. 1984).

Two main types of biological impacts are associated with hypoxia. First, low oxygen conditions can directly cause mortality. For example, hypoxia has a particularly high lethal impact on sessile species that lack the capacity to seek out refuges (Breitburg 1992b; Diaz and Rosenberg 1995; Vaquer-Sunyer and Duarte 2008). Second, hypoxia can alter the behavior of mobile organisms. A common behavioral response is to reduce hypoxia exposure through

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distributional shifts, documented as short-term reductions in densities of demersal fish and macroinvertebrates in hypoxia-impacted areas (Diaz and Rosenberg 1995; Eby et al. 2005; Petersen and Pihl 1995; Pihl et al. 1991). Although these behaviors reduce mortality, they introduce shifts in species distribution and habitat use, which may produce substantial shifts in growth and predation (Eby et al. 2005; Levings and Ong 2004; Matabos et al. 2012). Such indirect effects of hypoxia can thus be as ecologically significant as direct mortality (Diaz and Rosenberg 1995).

Puget Sound is a large marine ecosystem that spans the northwest of Washington State, USA, and supports commercially important species such as Pacific salmon (*Oncorhynchus* spp.) and Dungeness crab (*Metacarcinus magister*), in addition to a diverse community of fish, marine mammals, and seabirds. Hood Canal is a 110 km, fjord-like estuary comprising the western arm of Puget Sound that regularly experiences seasonal hypoxia (Newton et al. 2007). Although hypoxia has been reported in Hood Canal since the 1950s, there is some evidence that suggests oxygen conditions are deteriorating even further due to anthropogenic influences (*e.g.*, nitrogen and carbon loading) (Fagergren et al. 2004; Newton 1995). A sill at the narrow northern opening and the steep bathymetry of Hood Canal reduces tidal currents and hydrological exchange rates from north to south (Newton 1995). As a result, DO levels throughout the southern regions decline throughout summer while the vertical extent of hypoxia expands through the water column, reaching depths of 20–30 m (Newton 1995). While Hood Canal has experienced fish-kills associated with such hypoxic events (Fagergren et al. 2004), recent findings appear to describe large scale hypoxia avoidance by several local species. During the 2007 hypoxic episode, Essington and Paulson (2010) documented reduced densities of demersal fish and macroinvertebrates that occur seasonally, but found less evidence for persistent reductions among the mobile species.

We hypothesize two, non-mutually exclusive types of distributional shifts that might result in localized reductions in density of mobile taxa in hypoxia-impacted regions of Hood Canal. First, individuals may shift into nearby shallow oxygenated areas (Petersen and Pihl 1995; Pihl et al. 1991), which in Hood Canal usually consists of a small band of habitat that is less than 25 m in depth (Essington and Paulsen 2010). However, due to the limited amount of area unaffected by hypoxia this response alone may be insufficient to account for the sharp decline in densities. Alternatively, individuals could follow the regional oxygenated gradient that spans Hood Canal from south to north, thereby reducing hypoxia exposure by moving into more oxygen rich northern habitat.

The relative importance of these two hypotheses concerning the alternative types of distributional shifts (local and regional) has important ecological and management implications. Localized shifts would imply a high potential for local indirect ecological effects in shallow areas. More specifically, widespread movement of mobile organisms into non-hypoxic shallow depths can lead to concentrated densities of fish and invertebrates resulting in density-dependent effects, such as reduction in individual growth (Craig and Crowder 2005; Eby et al. 2005). These distributional shifts can also alter the spatial overlap of predators and prey, causing increased predation mortality (Aumann et al. 2006; Levings and Ong 2004; Long and Seitz 2008). Similarly, overlap of fishing gear (*e.g.*, crabs pots) and aggregating species may also increase vulnerability to harvesting (Selberg et al. 2001). Alternatively, a predominately regional shift would suggest that even though hypoxia is more severe in the southern regions, the effects of hypoxia are felt throughout Hood Canal. Indeed, the degree of ecosystem connectedness (*i.e.*, transfer between regions) can influence the magnitude of impact from a perturbation and is thus an important component for management and conservation efforts (Bonsdorff 1992; Estes et al. 1998; Lenihan et al. 2001). Extensive movements of organisms from the southern region of Hood Canal in response to hypoxia would support current recreational fishing restrictions that prohibit angling throughout the estuary.

To better understand the distributional shifts of benthic mobile species in response to hypoxia we used acoustic telemetry to monitor the local and regional movements of two common species in Puget Sound: Dungeness crab (*Metacarcinus magister*) and English sole (*Parophrys vetulus*). Although the primary focus of this research revolves around Dungeness crab, English sole movements provide valuable insight into the habitat and behavioral responses of demersal fish in Hood Canal. Dungeness crab is an abundant target of commercial and recreational fisheries in the Pacific Northwest (Butler 1961a; Schmitt 1921; Stevens and Armstrong 1984). English sole is also an abundant species in the region (Lassuy and Morgan 1989) and has proven an effective sentinel marine organism for numerous environmental water monitoring programs, including the Puget Sound Assessment and Monitoring Program (PSAMP). In addition, both adult Dungeness crab (Collier 1983; Smith and Jamieson 1991) and English sole (Jow 1969; Pattie 1969) generally exhibit relatively restricted movement patterns, but are capable of traversing large distances. There is also evidence that other crab and flatfish species exhibit hypoxia-avoidance and habitat selective responses (Bell and Eggleston 2005; Bell et al. 2003; Switzer et al. 2009). As a result, Dungeness crab and English sole can potentially act as indicators of the types of hypoxia-induced distributional shifts expressed by benthic fish and macroinvertebrate species in Hood Canal.

We hypothesized that our study species would potentially exhibit one or both of the following distributional responses: (1) vertical (local) movement into shallower, more oxygenated water; (2) horizontal (regional) movement from the hypoxic southern region to the normoxic northern region. Thus, we predicted the net direction of local (vertical compression) and/or regional (south to north) movement of mobile fauna would be away from hypoxic conditions. Although other factors can influence behavior, our aim was to detect potential changes in species distributional patterns that were coincident with the onset of hypoxia.

Methods

Study Design

We tracked individual movements in two years, 2009 and 2010. The 2009 sampling was a pilot study that tracked individuals from July – November in the hypoxia-impacted region (Figure 1.1). The 2010 primary sampling spanned June – September, and featured a paired study design consisting of two study regions, one in the southern portion of Hood Canal and a second site located approximately 10 km northward (Figure 1.1). By treating the northern site as a reference location we could potentially identify unique changes in behavior due to hypoxia from seasonal patterns that were phenological shifts not related to low oxygen conditions. Assessing the effect of hypoxia was achieved by evaluating the difference between movements at the north control site and the south impact site before and during hypoxia's extent into shallower waters. Inferences were primarily drawn from the 2010 data, but results from 2009 were included to evaluate the consistency of distributional responses.

Acoustic Tagging

We used acoustic telemetry to describe the movements and distributional shifts of Dungeness crab and English sole. This technique has been used on a much smaller spatial (Bamfield Inlet, British Columbia) and temporal (48 hr) scale to monitor Dungeness crab (n=6) movement in relation to brief foraging behavior and low oxygen conditions (Bernatis et al. 2007). However, our larger sample size, expansive study area, and longer monitoring period(s) were employed to answer inquiries regarding large, distributional patterns relative to hypoxic extent and progression. To do so we began by collecting Dungeness crabs from the north and south sites in 2010 and the south site in 2009 using baited crab pots (Figure 1.1). All collection was conducted after seasonal molting in June (males) and early July (females). Acoustic transmitters (VEMCO V9-2 and V9P-2; transmission rate = 45-60 sec) were glued to the shell carapace using cyanoacrylate adhesive (super glue), following the procedures described by others (Bernatis et al. 2007; Holsman et al. 2006). All individuals were released at their capture location within 24 hours of tagging. It was important that we tagged crabs that were not vulnerable to commercial or recreational harvest. In Hood Canal, the fishery is allowed to retain males exceeding a minimum carapace limit (158.8 mm). Thus, female crabs or sub-legal male crabs were selected for tagging. Due to differences in the timing of collection between 2009 and 2010 and the distinct molting cycle of females and males, females were more available in the 2009 collection efforts and males were more available in the 2010 collection. For this reason, the 2009 and 2010 data are not directly comparable because there may be sex-specific distributional patterns that would confound interannual comparisons. In 2009, we tagged (all VEMCO V9P-2 pressure-sensing tags; transmission rate = 45-60 sec) ten females (mean carapace width (CW) ± SD = 155.9 ± 13.9 mm), however four pressure-tags appeared to have detached or were never detected and were thus excluded from the dataset. In 2010, we tagged (19 pressure-sensing tags; transmission rate = 45-60 sec) 34 males (mean CW ± SD = 146 ± 7.2 mm). Three pressure-sensing tags malfunctioned and one individual was never detected.

Due to the size of fish captured, timing and field constraints, English sole were only collected and tagged from the southern sites in 2010 and 2009. All acoustic transmitters were < 2% of the fish's weight to ensure minimal behavioral effects from tagging (Winter 1996). Fish were collected with an otter trawl. The trawl catch was immediately sorted and individuals > 270 mm were retained for tag implantation. Anesthetization and surgical implantation of acoustic transmitters (VEMCO V9-2; transmission rate: 45-60 sec) were performed based on Moser et al. (2005). English sole were released at their point of capture after recovery from the procedure. We tagged ten individuals (mean TL ± SD = 295.5 ± 24.4 mm) in 2009 and 14 individuals (mean TL ± SD = 304.2 ± 20.3 mm) in 2010. Pressure sensing tags were not used in English sole because of the small size of their peritoneal cavity.

Acoustic Receivers

Our primary method for monitoring the individual movements of acoustically-tagged fish and crabs were arrays of stationary (passive) acoustic receivers. These receiver arrays provided two distinct types of spatio-temporal data: (1) fine scale depth (m) coverage of crabs from the pressure-sensing tags and (2) coarse scale flatfish and crab locations (Latitude-Longitude) relative to the receiver detection range (Heupel et al. 2006). Pressure-sensing tags have a high degree of vertical accuracy (2.5 - 5 m) and resolution (0.22 - 0.44 m) (VEMCO: http://www.vemco.com/education/faqs). In contrast, data on the horizontal location of tagged species is less detailed due to the wide detection radius of each receiver. Thus, determining the detection range of the passive receivers was critical for calculating the areal extent of coverage and interpretation of the 2-D acoustic data (Klimley et al. 1998). Range-testing was conducted in 2010 on six passive receivers (two shallow, one deep each in the two regions), following the protocol described by Andrews et al. (2007). We fit a logistic regression model describing the probability of tag detection as a function of receiver-to-transmitter distance and used the fitted model to estimate the detection distance associated with a 10% probability of detection (termed maximum detection diameter = 700 m). If a tagged individual was within the detection range, the receivers recorded tag transmissions continuously, documenting the tag identification, date, time (hh:mm:ss), and transmitter depth (m).

The acoustic arrays between years differed in extent and coverage. The 2009 array consisted of 13 acoustic receivers (VEMCO VR2W-069K-100) anchored underwater, running along the shoreline and center of Hood Canal (depth placement (DP): 15.2-111.9 m) spanning an approximately 7 km reach. To reduce uncertainty pertaining to horizontal location, the 2010 array was composed of 45 acoustic receivers and focused on two regions of Hood Canal, with one row of receivers between them to detect northward or southward movements (Figure 1.1; DP: 12.5-170 m). This paired design provided a fair degree of coverage (9%; 15.7 km^2) over the study areas (176 km^2) (Figure 1.1). To compensate for gaps in the 2010 receiver coverage, we ran transects every week using a mobile acoustic receiver (VR100) to capture additional transmissions (Figure 1.1). The maximum detection distance for the mobile receiver was estimated to be 419 m at a gain setting of 48 dB.

All 2010 field observations were also accompanied by weekly monitoring of water quality at ten fixed stations throughout Hood Canal (five north, five south). Temperature, salinity, and dissolved oxygen profiles through the water column (casts) were measured using a Sea-Bird 19 plus SEACAT profiler, fitted with a dissolved oxygen sensor. As there was a minimal difference between the center-channel and nearshore, only the deep casts were averaged for each week's DO profile (Figure 1.2a). The difference between weekly mid-channel and nearshore casts in the north averaged 0.24 mg L^{-1} (± 0.24 SD); the largest discrepancy (0.74 mg L^{-1}) occurred when shallow oxygen levels increased during the week of August 22nd (week 12).

For the southern region, DO-at-depth measured from the deep and shallow casts each week differed approximately 0.18 mg L^{-1} (± 0.11). The 2009 weekly water quality data was provided by a moored buoy near Hoodsport, Washington, USA, (southern region) that automatically conducted hourly CTD casts (ORCA: http://orca.ocean.washington.edu/) (Figure 1.2a).

Analysis

The local and regional movements were analyzed separately using the vertical and horizontal telemetry data, respectively. To detect local movements into shallower water, vertical changes in depth were assessed using the fine scale pressure-sensing telemetry data. Alternatively, horizontal movements were considered changes in position along a 2-D plane and were used to identify large-scale regional distributional shifts. To reduce inclusion of erroneous detections, we removed data if total detection frequency was less than three per day at a single receiver or less than 20 detections per week total. Removal of false detections from telemetry data is common and important for proper interpretation of the data (e.g., Andrews et al. 2010) . The 2009 and 2010 data were also analyzed separately.

We evaluated local-scale movement of Dungeness crab by modeling changes in depth over time. Depths were first assessed at a weekly time scale to reduce temporal autocorrelation and correspond to the weekly water quality and mobile tracking efforts. We tested for changes in depth distribution and position in the water column by using linear mixed-effects models (LMM) on maximum depth and mean depth data, respectively. LMMs allowed the inclusion of crab identity as a categorical random effect into our candidate models to account for the unique depth preferences of each crab (Gelman and Hill 2007). The weekly 2010 and 2009 LMMs evaluated the relationship between weekly crab depth and time using week, region, and a week x region interaction term as fixed effects, and crab identity as a random effect. We selected models using Akaike Information Criterion corrected (AICc) for our finite sample sizes to quantify the strength of evidence among a set of candidate models composed of all combinations of the fixed and random effects (Burnham and Anderson 2002). Models with AICc values within two units of the smallest AICc model were considered equivalent (Burnham and Anderson 2002). The AICc evaluation of the crab depth models allowed us to detect significant differences between southern and northern shifts. By limiting the south datasets to an equivalent time frame (July 7th – September 16th), we also measured the magnitude of the week effect between the southern 2010 and 2009 crabs, predicting weekly movement to be more rapid during the severe hypoxic event of 2010. All mean depth data were natural-log transformed to stabilize the variance. Residuals of all best fit models were assessed to ensure they were approximately normally distributed with constant variance.

We excluded several potential predictor variables based on statistical and ecological significance of the covariates. Although there is high autocorrelation at shorter time scales, we assessed vertical movements at an hourly time interval to determine whether there were important effects of tide and/or time-of-day on depth. Although there was marginal evidence for a time-of-day effect, a less parameterized model (day + region + day:region) was less than two units (ΔAICc = 1) from the time-of-day model. As a result, we concluded that tide and time-of-day were weak predictors of adult crab depth, consistent with other studies on adult crab (Armstrong et al. 2010). Temperature and salinity were also excluded from all models as they were relatively constant through space and time (mean temp ± SD = 10.3 ± 1.6°C; mean salinity ± SD = 29.6 ± 1.0 PSU) and never exceeded Dungeness crab temperature (> 25°C) (Curtis and McGaw 2012; Des Voigne 1973) or salinity tolerance (< 12 PSU) (Cleaver 1949; Curtis and McGaw 2012). In addition, only four crabs (2010 $n_{north} = 1$ and $n_{south} = 2$; 2009 n = 1) experienced extremely shallow average weekly depths (5-10 m).

We assessed three measures of horizontal movement for Dungeness crab and English sole to evaluate regional distributional shifts: (1) transfer rate between sites; (2) movement magnitude and direction; and (3) total distance traversed. Transfer rate, only applicable to the 2010 data, was calculated as the number of individuals from one region that were detected in the other region over the study period. Due to the small sample size, a Fisher's exact test was used to compare the proportion of transfer from both sites. Next, we calculated approximate weekly locations for each individual as the geographic centroid of passive and mobile receiver positions where the animal was detected, weighted by the total number of detections at each receiver in a given week (ArcGIS v.10). The precision of horizontal locations was low because of the large detection range of individual receivers. Indeed, based on our receiver range testing, displacement less than 700 m (*i.e.*, maximum detection diameter) may not have been actual movement, but rather an individual positioned between adjacent receivers. As a result, we made conservative estimates of regional patterns by assigning movements less than the maximum detection diameter (*i.e.*, < 700 m) equal to zero. We therefore only used these data to address larger regional movements into and out of study sites. Using this conservative dataset, directionality and magnitude of movement was determined by assigning negative values to southerly movements from the initial weekly geographic centroid and positive values to northward movements. Based on the signed centroid values and initial detection sites, we calculated the total directional displacement for each individual. Similarly, estimates were made for total distance by calculating the sum of all weekly movements that were greater than 700 m.

Although not an initial goal of the study, we also evaluated the relative vertical activity level of each crab in relation to mean depth DO level. Due to the unexpected pattern, we report our findings without drawing strong inferences concerning the possible effects. We describe activity from the vertical-depth dataset as the number of depth changes per total number of detections of each crab within a specified period, in this case one week (Beaudreau and Essington 2011). In addition, mean DO associated with the weekly average depth of each crab was used as the predictor variable in the analysis. A Spearman rank correlation coefficient was calculated to establish any monotonic association between activity and mean DO among and between groups. We used Spearman rank correlation to evaluate this relationship because assumptions of generalized linear models were not upheld. All vertical movement data were analyzed with R 2.15.2 and regression was performed using the lme4 package (Faraway 2006).

Results

Weekly dissolved oxygen profiles indicated substantial differences in the vertical extent and intensity of hypoxia between the southern and northern sites. In early June 2010, the deep waters had uniformly low DO throughout the southern channel of Hood Canal; the shallowest hypoxic depth was initially near 90 m (Figure 4. 2a). As the summer progressed, the extent of hypoxia rapidly expanded vertically into the shallows, reaching approximately 20 m by week 12 (August 22nd) (Figure 4. 2a). In contrast, the northern DO conditions stayed relatively constant. Only by the tenth week did oxygen in shallow waters of the north begin to decline, but conditions never became hypoxic in shallow habitats (Figure 4. 2a). The 2009 hypoxia was less severe throughout Hood Canal, and did not expand into the southern shallows until late September (Fig 2a).

We found evidence consistent with hypoxia inducing local compression of Dungeness crab vertical range in the southern site (Figure 4. 2b). The maximum weekly depth of individual crabs was best described by a model including week, region, and a week x region interaction term (Table 1.1). Based on the best fitting model coefficients, crabs in the southern site displayed deeper initial average maximum depths (mean depth ± SE = 90.9 ± 0.51 m, N = 8) than the north

(59.1 ± 0.21 m, N = 8) and the rate at which maximum depth changed was greater in the southern site (5.02 ± 0.05 m week^{-1}) than in the northern site (0.55 ± 0.02 m week^{-1}). Thus, maximum weekly depth decreased 10 fold faster in the hypoxia-impacted site compared to the reference site. Although region could not be included in the 2009 model due to lack of a northern tagged group, the most parsimonious 2009 model incorporated an effect of sampling week, where the initial depth (88.2 ± 0.25 m, N = 6) and weekly rate of change (2.88 ± 0.01 m week^{-1}) were comparable to 2010 (Table 1.1). When we compared crabs in the southern site between years, we found that the individuals displayed a faster rate of change in maximum depth (6.77 ± 0.05 m week^{-1}) in 2010 than those in 2009 (4.82 ± 0.02 m week^{-1}). Similar results were found for the analysis of weekly mean observed depth (Table 1.1); the coefficients from this model indicated a general decrease in mean depth over the study period, a greater initial depth in the southern site, and a greater change in depth through time in the southern site. However, the crabs in the southern site appeared to remain near the oxycline despite these shifts in maximum and mean depth (Figure 1.2b).

Although we found some evidence indicative of a localized response to hypoxia, the 2010 crabs and sole exhibited little transfer regionally between the northern and southern sites. Only one of the 13 crabs tagged in the south site was detected in the north, whereas none of the 17 crabs from the northern site appeared in the southern site. Although the direction of the one crab (south to north) and the northern crabs' lack of transfer south were consistent with our hypothesis, the low frequency of inter-site movement provided insufficient evidence to reject the null-hypothesis that the north and south transfer rates are equivalent (Fisher's exact test, P > 0.05). Similarly, only one of the 14 English sole tagged in the southern site transferred between regions. Again, the direction of flatfish movement was consistent with our hypothesis, but the rate of between site movements was very low.

Overall, tagged crabs also exhibited limited large-scale displacement in either study site, while English sole demonstrated more movement. All but four crabs in 2010 (87%) remained within 5 km of their initial mean geographic centroids. The four exceptions (maximum displacement distance = 19.8 km) were from both the northern (n = 1) and southern sites (n = 3). In 2009, all crabs (N = 6) from the southern site remained within 4 km of their initial centroids. Although 10 of the 14 English sole in the southern site exhibited displacement exceeding 5 km, the majority of sole (79%) moved less than 15 km from their initial mean centroids in 2010. Two of the 14 flatfish moved approximately 20 km. Although not directly comparable, the movements of English sole during the 2009 pilot study were also generally short, with tagged individuals detected 5 km from their initial centroids.

We also found individuals in the southern sites no more likely to exhibit northward movement than individuals in the northern site. The frequency of northward movement among crabs in the north site was more than two-fold greater than crabs in the southern site (Table 1.2). These differences were counter to our hypothesis, which predicted greater northward movement in the hypoxia-impacted southern site. Moreover, the majority of the crabs in the southern site moved southward or remained near their initial detection southern location. It was not possible to compare English sole movement across sites, yet tagged individuals in the southern site more commonly moved southward than northward (Table 1.2). These results were consistent with those from 2009, when the majority of crab and sole in the southern site moved southward (Table 1.2).

Although individuals in the southern site did not exhibit directional movements northward, the cumulative total distances of crab and sole were notable. In 2010, crabs in the southern site moved an average total distance of 5.50 km (± 7.43 SD, N = 13). Similarly, crabs from the northern site exhibited an average total distance of 4.36 km (± 4.25, N = 17). These cumulative distances would only span approximately half the distance between the southern and northern sites. In contrast, English sole in the southern site accumulated distances to move beyond the southern reaches of Hood Canal, with an average total distance almost eight times that of the crabs (32.8 ± 47.1 km, N = 14). Although the detection of crab and sole movements was restricted by the extent of the smaller array in 2009, crabs (4.47 ± 4.33 km, N = 6) and sole (5.31 ± 4.48 km, N = 10) showed similar relative movement patterns.

We also found an association between individual crab activity (no. of depth changes week^{-1}) and mean weekly dissolved oxygen (Figure 1.3). The correlation coefficient of the 2010 dataset indicated a significant inverse relationship between activity and DO (Spearman rank correlation, $r_s = -0.30$, $N = 30$, $p < 0.01$). Although the crabs in 2009 also exhibited a similar pattern, the association between activity and DO was non-significant ($r_s = 0.08$, $N = 6$, $p = 0.55$). Regardless, the range of mean activity from both years tended to increase as DO declined (Figure 1.3).

Discussion

We tested whether two abundant species of the Hood Canal, Washington, benthic community exhibited patterns of movement consistent with hypoxia avoidance by quantifying their local and regional movement patterns relative to the onset of low oxygen conditions during two hypoxic summer events. The detailed data on depth distributions suggests that Dungeness crab habitat might be restricted by hypoxia, as both the mean depth and the range of depths declined as hypoxia strengthened in the southern site. However, contrary to our expectations, we found little evidence in support of regional-scale movements of mobile species from the hypoxia-impacted southern site into the more oxygenated areas in northern Hood Canal, which may suggest that behavioral responses to hypoxia are generally local.

The reduced depth ranges of Dungeness crab were consistent with the hypothesis that vertical expansion of hypoxia leads to a compression of habitat availability. Although other abiotic (*e.g.*, habitat quality) and biotic (*e.g.*, prey) factors not captured in this study could be influencing individual behavior, the overall shift of Dungeness crab into shallower habitat is consistent with numerous studies of hypoxia in other estuarine and semi-enclosed system (*e.g.*, (Bell and Eggleston 2005; Eby and Crowder 2002; Hazen et al. 2009; Pihl et al. 1991). These shifts are potentially meaningful in that the majority of crab fishing effort in Hood Canal occurs within the maximum depth range (19-59 m) of crabs during late summer (Froehlich, unpublished data). Particularly concerning, overlap with fishing gear may have been more concentrated during 2010 when hypoxia strengthened in mid-summer during the height of recreational crabbing season. We hypothesize that an increased overlap can possibly enhance fishing mortality for legal-sized males, as well as intensify handling mortality and sublethal effects of injuries for females and sub-legal males (Barber and Cobb 2007). Indeed, the 2009 female Dungeness crab displayed a similar range compresses as the 2010 male crabs in our study (Figure 1.2b). Sublethal damage from handling can negatively impact crustacean growth, reproductive fitness, and predator avoidance (*see* Murphy and Kruse 1995), which in turn can influence population abundances (Parsons and Eggleston 2007).

We also found elevated activity (no. of depth changes week^{-1}) of Dungeness crab associated with lower DO levels, which may also provide some evidence of an increased susceptibility to crab pots. The behavior of the crabs was indicative of acute avoidance rather than a burial response from chronic hypoxic stress (Bell et al. 2009; Bernatis et al. 2007; Das and Stickle 1993; Das and Stickle 1994; Lowery and Tate 1986; McGaw 2003). However, feeding state of Dungeness crab may also contribute to the level of activity, causing unfed crabs to be more active than postprandial individuals (Bernatis et al. 2007; Bernatis and McGaw 2004; Bernatis and McGaw 2005; Curtis and McGaw 2012). In fact, Dungeness crabs have been documented actively foraging in hypoxic areas (Bernatis et al. 2007). If this is the case in Hood Canal, we postulate that increased activity may contribute to increased vulnerability of crabs to baited crab pots. However, further investigation into the nature of these vertical movements and how that translates into capture probabilities is needed to test this hypothesis.

Movement into shallow hypoxia refuges reduces the amount of available habitat (Craig 2012; Craig and Crowder 2005; Eby and Crowder 2002) and increases densities of mobile species that could result in variety of other indirect ecological effects (Eby et al. 2005). In southern Hood Canal, these effects can potentially be pronounced because of the small percentage of total refuge habitat available during typical summer water conditions (Essington and Paulsen 2010). Habitat compression can enhance overlap of predators and prey, causing increased predation mortality (Eggleston et al. 2005; Lenihan et al. 2001; Long and Seitz 2008). Cannibalism is common in Dungeness crab (Fernandez 1999), so range compression of large, potentially cannibalistic crabs into shallow waters might enhance overlap of shallow-dwelling juvenile crabs with antagonistic adults (Aumann et al. 2006; Eggleston et al. 2005). As a result, if the movements we observed are in fact representative of community level shifts they could potentially induce effects on population productivity through a reduction in juvenile survivorship (Botsford 1981; Higgins et al. 1997).

Although only one Dungeness crab and English sole traversed over the two study regions from south to north (20 km), indicating a regional response is plausible, the majority of the southern tagged species exhibited little directed movement into the northern, less hypoxic site. Negative effects from shifts in community and habitat structure beyond the immediate area of a hypoxic disturbance have been documented in other estuaries (Lenihan et al. 2001). However, the extent of the hypoxic influence in the Hood Canal appears fairly concentrated within the southern hypoxic region with neither the crab nor sole dispersing into the northern refuges of the estuary. Although not definitive, other field experiments involving crabs suggest a limited propensity to navigate away from hypoxia on a large scale (Bell et al. 2003; Bernatis et al. 2007). In addition, several other studies have also reported more localized responses of benthic macroinvertebrates and fish to hypoxia (Craig 2012; Eby 2004; Eby and Crowder 2002; Pihl et al. 1991). In Hood Canal, management efforts could be centralized around the southern hypoxia-impacted region where mobile taxa are seemingly more vulnerable to the effects of hypoxia. However, in order to understand the full magnitude of hypoxia's impact in Hood Canal, more detailed information regarding the local responses of multiple species, similar to that of Dungeness crab in this study, is needed.

More investigation into the finer scale movement patterns would also be needed due to some detection loss and the small nature of our species sample sizes. Indeed, tag detection rates did decline over time, which could be an indication of undetected movement within or between regions that were not captured by our array(s). In addition, the attrition rate could be the result of predation mortality. It is unlikely the decline in detection rates was due to tag obsolescence as the manufacturer stated tag battery-life (> 200 days) (VEMCO: http://www.vemco.com/products/transmitters) well exceeded the length of both studies.

Although our sample sizes were typical of a telemetry study, there may also be biases associated the small number of individuals. While we were unable to explore these possible confounding factors due to time and cost constraints, specific movement paths could be investigated further by actively tacking (VR100) tagged individuals over a continuous period of time.

In addition to the decline in detection rates, without high resolution depth data, the localized response of English sole to hypoxia was less clear and also requires further research into their vertical movement patterns as well as Hood Canal's local hydrodynamics to understand the full extent of hypoxia driven shifts. English sole are moderately hypoxia tolerant (Boese 1988) and do not appear to avoid low DO conditions along the Oregon coast hypoxic gradient (Keller et al. 2010). That being said, Essington and Paulsen (2010) reported sizable density reductions of English sole across all depths greater than 30 m in hypoxia impacted regions of Hood Canal. Such findings suggest English sole could be utilizing depths below 30 m and possibly other unknown oxygenated refuges. Thus, the current knowledge of English sole behavior suggests they are (1) hypoxia tolerant, or (2) they are taking advantage of local oxygen spaces. If the latter is true, then more information concerning the spatial and temporal variability of hypoxia itself is critical to understanding and predicting the effects of hypoxia on biological communities. The large inter-annual differences in timing, intensity, and extent of hypoxia in Hood Canal suggests that the phenomenon is patchy in time and space. Therefore, the impacts of hypoxia on English sole cannot be fully understood without more detailed knowledge of their movements and the dynamics of oxygen conditions in the area.

This study improves upon the current understanding of hypoxia impacts in Hood Canal. The positive correlation between vertical shifts of Dungeness crab and hypoxia into shallower water in the southern site has important management implications concerning susceptibility to fishing and other ecological processes that could dictate growth and mortality. In addition, our findings provide some evidence that more focus on the localized patterns of conditions in conjunction with organismal responses to DO levels in Hood Canal, particularly those of English sole, is needed. Similarly, our results suggest management could potentially concentrate harvest restriction efforts on the more hypoxia-impacted region of the southern areas instead of the entire Hood Canal. However, the localized patterns might not hold true in the presence of additional extrinsic disturbances, such as degradation of refuge habitat (Lenihan et al. 2001) and increase in predators (Froeschke and Stunz 2012). Thus, information regarding changes in habitat, population connectivity within and among Puget Sound basins, and shifts in communities and food web interactions is pertinent to fully understanding the effects of hypoxia in complex estuarine ecosystems.

Table 1.1 Comparison of candidate statistical models predicting maximum and mean crab depth. All 2010 models with main fixed effects [week, 2010 north or south site (region)], an interaction term (week:region), and random effects of each individual crab. As 2009 study was restricted to the southern region, week is the only fixed effect in the model.

2010 Max Depth Model	AICc	ΔAICc
Intercept+Week+Region+Week:Region	2020	0
Intercept+Week+Region	2040	20
Intercept+Week	2044	24
Intercept+Region	2072	52
Intercept	2076	56

2009 Max Depth Model	AICc	ΔAICc
Intercept+Week	903	0
Intercept	918	15

2010 Mean Depth Model	AICc	ΔAICc
Intercept+Week+Region+Week:Region	1082	0
Intercept+Week+Region	1089	7
Intercept+Week	1086	4
Intercept+Region	1103	21
Intercept	1101	19

2009 Mean Depth Model	AICc	ΔAICc
Intercept+Week	527	0
Intercept	534	7

Table 1.2 Directional horizontal movement proportions for each crab and flatfish group based on the total displacement calculated form the final minus the initial individual mean geographic weekly centroids.

	2010			2009	
	North Region	South Region		South Region	
	Crab	Crab	Sole	Crab	Sole
North	0.76	0.31	0.07	0.33	0.10
South	0.12	0.31	0.86	0.50	0.80
No Displacement	0.12	0.38	0.07	0.17	0.10

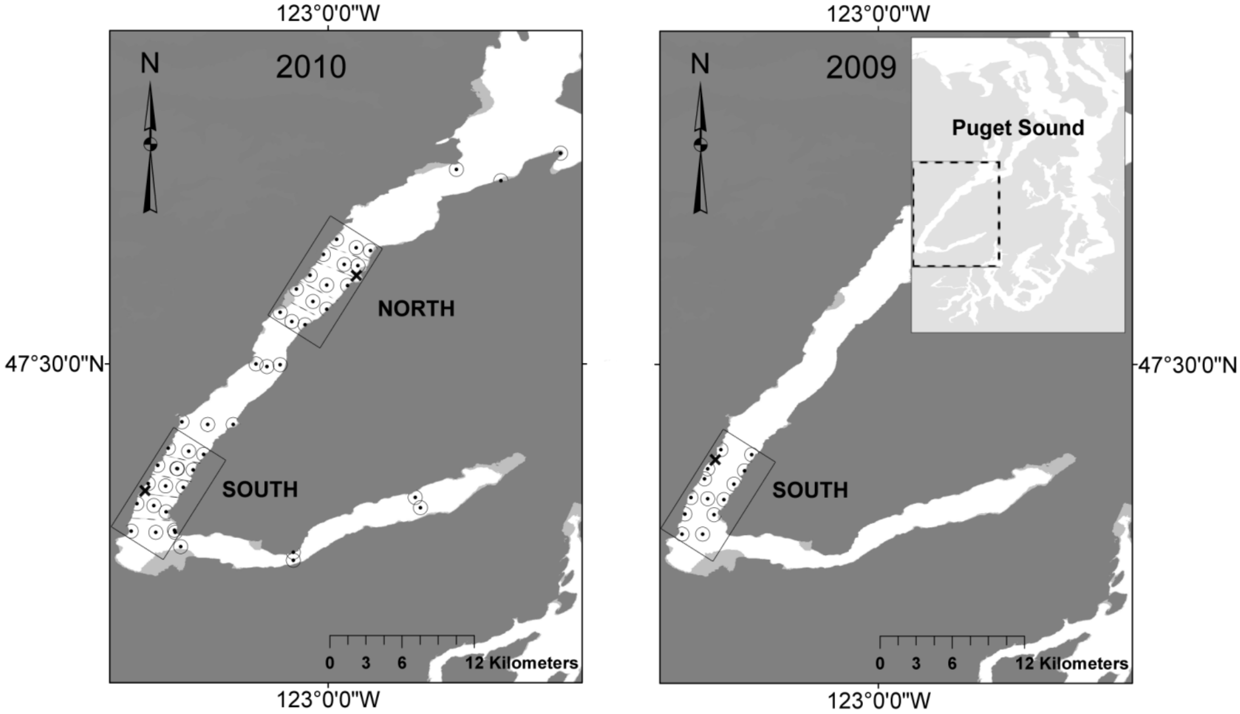

Figure 1.1 Specimen capture/release locations (black Xs) and passive acoustic receivers (solid points) in Hood Canal, Washington, USA in 2010 and 2009. Outer circles represent the 90 % detection range (350 m). Rectangles enclose the central north and south study sites. Dotted lines indicate mobile tracking transects.

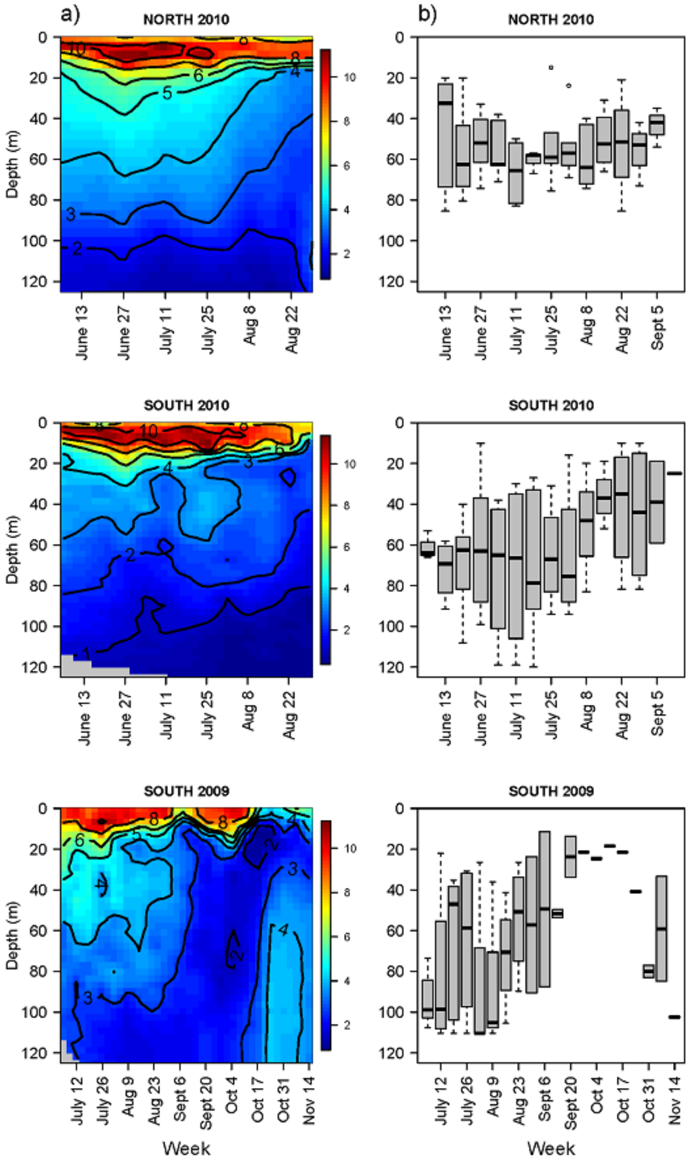

Figure 1.2 **a)** Interpolated average dissolved oxygen (mg L^{-1}) conditions of the 2010 north and south focal study sites and 2009 pilot study. Each graph depicts the contour (*black lines*) and gradient (*color*) of DO as depth and time increase. The gray color represents unsampled depths. Interpolation was performed in R using the interp function, which applies a bilinear interpolation to the data. In 2010, the hypoxic (DO < 2 mg L^{-1}) layer in the northern region remained deep, while low DO in the southern region rapidly decreased in depth over time. In 2009, the deteriorated oxygen levels appeared later in the summer (September), but quickly established within a shallow range of the water column. **b)** Range of maximum weekly depth (m) for all crabs in 2010 and 2009. Each graph shows the median (*black line*) and the upper and lower bounds of the crabs detected each week. The 2010 northern crabs remained above the hypoxia layer, while the southern crabs where in sync with the extending hypoxia layer and moved into shallower depths. The 2009 southern crabs exhibited a similar shoaling pattern. The x-axis on all graphs corresponds to the first day of each week interval.

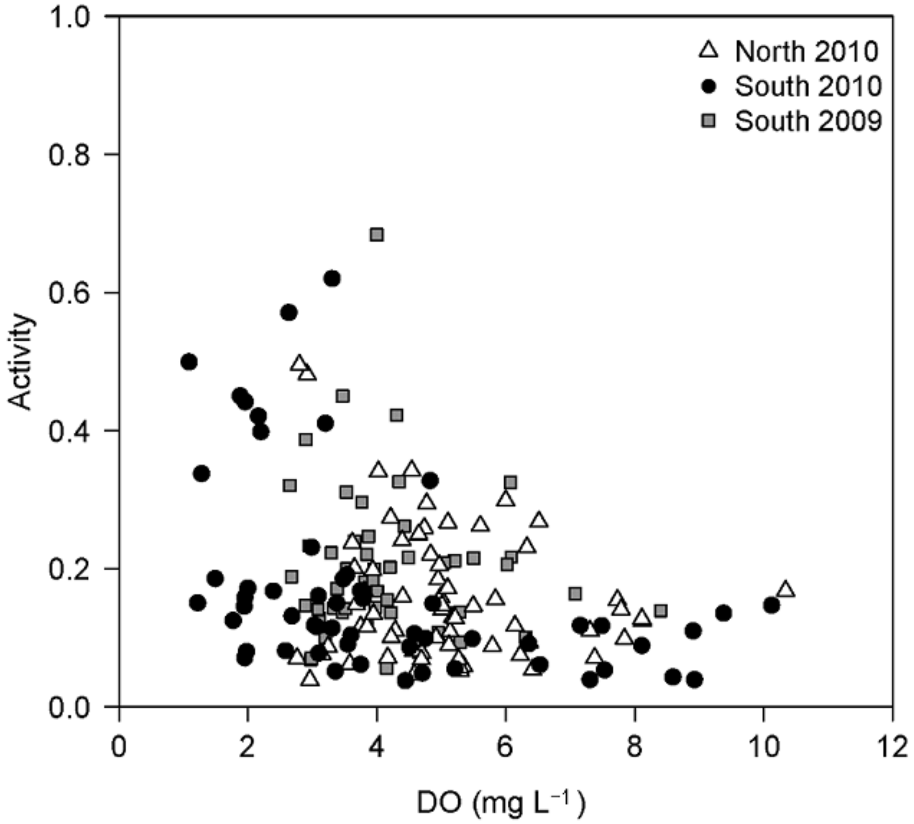

Figure 1.3 Individual activity (no. of depth changes week^{-1}) versus mean weekly dissolved oxygen (mg L^{-1}) of each crab over both years. *White triangles* correspond to the north 2010 crabs, *black circles* represent the south 2010 crabs, and *dark gray squares* depict the south 2009 crabs.

Chapter 2

Spatial and temporal variation in nearshore macrofaunal community structure in a seasonally hypoxic estuary[2]

Introduction

Low dissolved oxygen (DO) levels are a major environmental stressor on estuarine and coastal ecosystems (Diaz 2001). Depleted DO conditions, typically referred to as hypoxia, have been documented as one of the most important threats to these highly productive and essential marine systems (Diaz 2001; Diaz and Rosenberg 1995; Diaz and Rosenberg 2008). Due to the close proximity of human development to coastlines, coastal and estuarine environments are particularly prone to anthropogenic-induced hypoxia (Diaz and Rosenberg 2008; Rabalais et al. 2010). As a result, investigation into the scale and potential long-term effects of hypoxia is critical for the effective protection and management of these environments, particularly in highly sensitive areas, such as nearshore habitats.

The impact of low DO conditions on nearshore estuarine communities is not well understood. Nearshore, subtidal (≤ 30m) environments provide complex and diverse habitat conducive to high levels of primary productivity that supports an abundance of marine species (Beck et al. 2003). Nearshore abiotic (e.g., cobble) and biotic structures (e.g., algae) offer nursery habitat for larval and juvenile fishes and invertebrates (Gunderson et al. 1990; Simenstad et al. 1982; West 1997). While these habitats may not appear to be vulnerable to hypoxia, which manifests in deep water below the mixed layer, lower DO waters from offshore areas may be advected higher into the water column by wind, tides, and saltwater intrusion from the ocean, potentially influencing the adjacent nearshore communities (Breitburg 1990; Breitburg 1992c).

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Yet, the community level responses to low oxygen impacts in the shallower, nearshore regions of hypoxic estuaries are not well known.

While severe hypoxic conditions can induce mortality (e.g., fish-kills), non-lethal impacts on species occur above the lethal limit of hypoxia. The hypoxia threshold has previously been defined as DO less than 2 mg L^{-1}, related to a generalized lethal level of low DO leading to the collapse of fisheries (Diaz 2001; Rabalais et al. 2002). However, mounting evidence supports hypoxia impacting organisms at higher levels, particularly for mobile species that can potentially avoid mortality inducing DO conditions (Breitburg 2002; Buchheister et al. 2013; Diaz and Rosenberg 2008; Paerl et al. 1998; Vaquer-Sunyer and Duarte 2008). Thus, it seems the conventional '2 mg L^{-1}' is insufficient to capture the full extent of hypoxia impacts (Vaquer-Sunyer and Duarte 2008), particularly as it relates to the sublethal ecological consequences.

Hypoxia tends to appear as an intermittent or seasonal disturbance in estuaries (Diaz and Rosenberg 2008), resulting in the temporary, non-lethal reduction of mobile macroinvertebrate and fish densities in the hypoxia impacted depths; a behavioral response documented in numerous hypoxic systems (Das and Stickle 1994; Diaz and Rosenberg 1995; Eby et al. 2005; Petersen and Pihl 1995; Pihl et al. 1991). Commonly, mobile fauna seek refuge in more oxygenated habitat, which reduces the direct exposure to severe hypoxia, but could have important secondary ecological effects (Craig 2012; Craig and Bosman 2013; Eby et al. 2005; Froehlich et al. 2014). For instance, movement of organisms into more localized nearshore habitat could concentrate densities of fish and invertebrates, resulting in an increase in predator-prey overlap (Aumann et al. 2006; Long and Seitz 2008) and/or potentially induce density-dependent growth effects (Campbell and Rice 2014; Craig et al. 2007; Craig and Crowder 2005; Eby et al. 2005). Depending on the severity of conditions, lower oxygen levels can also displace physiologically sensitive organisms from the area and support other, more tolerant species (Dauer 1993; Sperling et al. 2013; Wu 2002); this typically appears as a reduction in fish and more sensitive species and persistence of a few tolerant invertebrates (Diaz and Rosenberg 1995; Vaquer-Sunyer and Duarte 2008; Wu 2002). Thus, identifying the extent of a multi-species level response to low DO is important for understanding the population and community level implications of hypoxia.

Exposure to low DO conditions shapes species composition through shifts in distribution and mortality (Nilsson and Rosenberg 1994; Rosenberg et al. 1992; Seitz et al. 2009; Vaquer-Sunyer and Duarte 2008). However, the temporal and spatial scale at which these changes occur is often in question (Eby 2004), particularly as it pertains to the nearshore, subtidal community. Community change in hypoxia-impacted estuaries can be temporary, lasting days or weeks (Buchheister et al. 2013; Eby 2004; Wu et al. 2003). However, more persistent or frequent low oxygen levels precipitate more permanent alterations in assemblages (Conley et al. 2009; Diaz and Rosenberg 1995; Matabos et al. 2012). The extent of these changes thus influences the state and function of the system, including local food web dynamics and energy flow (Baird et al. 2004; Campbell and Rice 2014; Eby 2004; Seitz et al. 2009; Sperling et al. 2013). Consequently, investigation into the most productive portions of an estuary is particularly relevant to appreciate the full extent of ecological implications of hypoxia. The scale of change also provides insight into potential long-term, lasting effects of hypoxia, such as prolonged reductions in species diversity (Eby 2004). However, the response of the system depends greatly on the geomorphology and hydrological dynamics of the estuary.

Hood Canal, a fjord-like estuarine basin in Puget Sound, Washington State, USA, commonly experiences extended periods of hypoxia (Newton et al. 2007). Hood Canal (110 km) comprises the western arm of Puget Sound, supporting commercially important species such as Pacific salmon (*Oncorhynchus* spp.), Dungeness crab (*Metacarcinus magister*), and spot prawn (*Pandalus platyceros*), as well as a diverse array of other marine fish, mammals, and seabirds. Hypoxia is a prominent feature in Hood Canal that has been documented since the 1950s (Newton 1995). A combination of a narrow sill at the northern opening and the deep bathymetric structure of the estuary reduces tidal currents and hydrological exchange rates from north to south (Newton 1995). Dissolved oxygen in the deep, southern reaches of the estuary declines throughout the summer months and expands vertically through the water column, typically reaching nearshore depths of 20–30 m (Newton 1995). In addition, low DO upwelled from the ocean can also contribute to reduced oxygen levels (Cope and Roberts 2013). Notably, future deterioration of oxygen conditions due to a combination of climate (e.g., increasing temperatures) and direct anthropogenic influence (e.g., septic nutrient loading) is a major concern (Cope and Roberts 2013; Fagergren et al. 2004; Newton 1995; Warner et al. 2001). In fact, low DO conditions can persist in the southern region of Hood Canal due to inadequate mixing, potentially creating a more permanent low DO environment compared to northern areas (Newton et al. 2007).

Recent evidence suggests deeper dwelling mobile species in Hood Canal exhibit seasonal distributional shifts relative to hypoxic conditions (Essington and Paulsen 2010; Froehlich et al. 2014), while hypoxia influence on the shallower, nearshore environment is less clear. Essington and Paulson (2010) reported a significant reduction in deep (> 30m) demersal fish densities and notable declines in macroinvertebrates, with little support for persistent reductions among the mobile fauna. In addition, a recent study by Froehlich et al. (2014) found that tagged Dungeness crab and English sole (*Parophrys vetulus*) remain in the southern hypoxic region, with

Dungeness crab moving into more shallow, less hypoxic habitat in concert with the more severe, vertically extending low DO conditions. Although these studies strongly suggest a seasonal pattern of distributional and community assemblage changes associated with the expansion of hypoxia, it is uncertain if other mobile species tend to shoal into the nearshore and/or move farther north into normoxic waters.

To investigate the sublethal influence of hypoxia on the nearshore community we used visual surveys of macro-benthic fauna from underwater video to document the abundance of conspicuous fishes and macroinvertebrates. The aim of the study was to evaluate whether hypothesized community differences - consistent with predictions based on physiological tolerances of organisms - would exist between hypoxia-impacted and less impacted areas of Hood Canal. We compared nearshore, subtidal communities of two regions: one in the south where deep-water severe hypoxia is common and one in the north that typically has higher oxygen conditions (Newton 2007). We tested the following hypotheses that evaluate the importance of DO as a nearshore environmental driver: **(1)** regional community assemblages are significantly different between high and low DO areas; specifically, **(1a)** the southern, low DO sites are primarily composed of more hypoxia tolerant invertebrates and fewer fish species compared to northern, higher DO sites and/or **(1b)** community structure in the southern, low DO region changes temporally (weekly), while the higher DO northern areas remain relatively constant; **(2)** DO and sampling period are main predictors for presence of the most abundant nearshore species; **(3)** hypoxia tolerant species display a distinct DO-tolerance threshold compared to more sensitive species. Inclusion of the temporal (week) component was used to assess changes in the south that would be consistent with organisms shifting into nearshore habitat as hypoxic conditions expanded vertically in the water column. Although our paired design does not permit strong inference regarding the long-term effects of DO exposure on the nearshore community, by combining our paired observations with testable hypotheses we were able to gauge whether the results were consistent with or counter to expectations emerging from our hypotheses.

Methods

Study design

We monitored conspicuous, mobile species along nearshore depth strata of Hood Canal during the summer months of 2010. Sampling spanned June –September and featured a paired study design consisting of two study regions, one in the southern, hypoxia-impact area of the estuary and the second approximately 13km to the north (Figure 2.1). Within both study areas, four sites were selected and surveyed each week (Figure 2.1). The northern study area was selected as a reference setting to compare southern patterns and discern potential differences associated with hypoxia from phenological shifts unrelated to low DO conditions (Essington and Paulsen 2010; Froehlich et al. 2014). We assessed the effect of hypoxia by evaluating changes in density and species composition at the northern reference sites and the southern impact sites before and during the expansion of severe hypoxia into shallower depths.

Drop-camera surveys

We used a camera-based visual survey to count visible fish and mobile macroinvertebrates at depths ranging from 10 – 30m. A downward facing drop-camera, fitted with parallel lasers at a known distance (5.5cm), was deployed using a downrigger device. The camera provided a live feed to a monitor onboard a vessel and transmitted the video to a recorder for future detailed analysis. We ran transects along isobaths at fixed depth intervals. The vessel was positioned at the start of a transect, and we used the surface currents (0.2-0.6m s^{-1}) to drift slowly along the transect (mean transect distance ± SD = 116 ± 67m). Waypoints and depth were recorded every minute using an on-board GPS unit and depth sounder, respectively. The depth of the drop camera was continually adjusted so that it was positioned roughly one meter off the bottom. A transect was complete after five minutes of drifting, or when the depth had shifted by more than three meters. This has proven to be a highly effective way to rapidly sample large areas for conspicuous species, including crabs, geoducks (siphons), sea cucumbers, flatfishes, sea stars, and anemones (Essington, unpublished data). Moreover, it required no direct disturbance or mortality, and was thus preferable to trawling or other capture-based surveys. There was sufficient light penetration to permit surveys at depths down to 30m. This maximum depth exceeded the expected threshold depth beyond which severe hypoxia is common. Thus, we could potentially detect seasonal changes of species in shallower (< 20m) compared to deeper (> 20m) areas in the hypoxia impacted region, with potentially more consistent densities and composition in the less impacted region. A total of four drop camera stations in each study site consisting of three sampling transect depths (10, 20, 30 ± 3m) were sampled for 10 weeks during the summer.

Post-processing of the videos was conducted to determine species densities. First, taxonomic level and counts of conspicuous mobile species were documented for each transect. If individuals were unable to be identified at the species level, groups were assigned (e.g., Order) or the mobile taxa were cited as “unknown”. Key substrate type and epibenthic structures were also documented during video analysis. Consolidation of the most frequent compositional features resulted in seven distinct habitat types consisting of combinations of sand, cobble, algae, sea whips, sea pens, and/or bedrock that typically differed with depth and occasionally site. To estimate the area surveyed by the camera, we calculated the average field of view (FOV) from the camera and the distance of each associated transect. A virtual instrument (VI) in LabVIEW

10 was created to measure the FOV of the camera for each transect using snapshots from the transect video. Every 10 seconds a snapshot was taken and imported into the VI. The LabVIEW program was constructed to detect the fixed laser points then calculate the FOV width (m) for each snapshot given the known distance of the lasers (5.5cm). The FOV snapshot widths were then averaged. Length of each transect was calculated using ArcGIS 10.1 software.

Water quality monitoring

All weekly field efforts included water quality monitoring at four fixed, nearshore stations that included both regions and the western and eastern shorelines in Hood Canal (two north, two south). Temperature (^{0}C), salinity (PSU), and dissolved oxygen (mg L^{-1}) profiles through the water column (casts) were measured using a Sea-Bird 19 plus SEACAT profiler, fitted with a dissolved oxygen sensor. All water quality measurements were conducted between mid-June to the beginning of September to coincide with the period prior to the first appearance of severe hypoxia in deep water expanding vertically in southern Hood Canal. Coefficient of variation (CV) of the three environmental conditions was assessed in order to directly compare the measurements between the study regions.

Analysis

As no single approach could address all of our hypotheses, we used separate methods to investigate each hypothesis and compared the similarities and discrepancies of the results for a more complete understanding of the influence of hypoxia on nearshore, subtidal species composition. For the first hypothesis (1a and 1b), relative abundances were assessed and then tested for significant compositional differences between high and low DO areas and across time periods using multivariate statistics. The DO tolerances of the most abundant and significant species were then evaluated using a literature review search. To address the second hypothesis, we tested the ability of DO to predict the presence of the common species relative to other potentially influential covariates, such as sampling period. For the third hypothesis, we then separated species into discrete low oxygen ‘tolerant’ and ‘sensitive’ groupings to identify approximate DO-tolerance thresholds where the occurrence of the species’ groups appeared to change. Using the same species groupings, we further investigated the magnitude of temporal community change using a generalized linear modeling approach. Lastly, we evaluated how commonly oxygen levels were below the DO-tolerance thresholds relative to the long-term, seasonal DO estimates.

Evaluating regional community differences and species DO tolerances

Differences in weekly regional community structure were analyzed using non-metric multidimensional scaling (NMDS) with a Bray-Curtis dissimilarity measure; a common and robust ordination technique to evaluate count data (Eby 2004; McRae et al. 1998). NMDS is a non-parametric technique that seeks an ordination in which the distances between all pairs of objects are in rank-order agreement with their dissimilarities in species composition, resulting in a graphical representation of relationships and patterns in multidimensional space (Quinn and Keough 2002). The measure of fit was determined by evaluating Kruskal’s stress value using two and three dimensions, as well as assessing the scree plot. Ideally, stress values are less than 0.15 (Clarke 1993). Separate Analysis of Similarity (ANOSIM) was also used to test the significance of the dissimilarities (Clarke 1993; Clarke and Warwick 1994) relative to region and sampling period (week). However, this approach can only evaluate one predictor at a time, ignoring potentially important interactions between covariates. While all unidentifiable organisms were removed for the NMDS analysis, aggregate groups of eelpouts, gobies, gunnels, sculpins, poachers, perch, and rockfishes, as well as unknown crabs, seastars, right-eyed flatfishes, and left-eyed flatfishes were analyzed due to sparse species-level data. Species' correlative significance to the NMDS ordinations were also assessed. Prior to analysis, all densities were averaged over sampling site (n = 4) for each depth and sampling week for each species relative to the respective region. The data were then log-transformed, standardized, and analyzed using the *vegan* package in R (Dixon 2003; RCoreTeam 2013).

We identified the most abundant and significant species (n=16) and performed a literature search to identify DO ranges and tolerances (if applicable) for each. When analysis was constructed on an aggregate of multiple species, the literature review was directed at the most common taxa that could be identified to species level. For the group "sculpin", we searched for DO tolerances for blackfin (*Malacocottus kincaidi*) and buffalo (*Enophrys bison*) sculpin. For the group "goby" we referenced blackeye goby (*Rhinogobiops nicholsii*) and for the eelpout species we used blackbelly eelpout (*Lycodopsis pacifica*). The DO ranges and tolerances, along with descriptive text, were taken directly from the primary literature and open-access data sources. The scientific articles were found via Web of Science and Google Scholar using all combinations of the following search terms: 'species name (common and scientific)', 'dissolved oxygen', 'hypoxia', and/or 'threshold'. However, several species, particularly the Echinoderms, had no peer-reviewed publications pertaining to DO tolerance. As a result, we included ranges reported from the open-access data forum Ocean Biogeographic Information System (OBIS; http://www.iobis.org/) for all species as baseline criteria. We summarized the results as qualitative levels (low to high) of 'Overall Tolerance'. Organisms were classified as 'high tolerance' when the minimum range was less than 1mg L^{-1} and/or tolerance level was reported. Given the various taxonomic groups, the cut-off of 1mg L^{-1} was selected to ensure a more clear division between more and less tolerant species. We considered organisms that can persist well below the lethal DO concentration level of most species (Vaquer-Sunyer and Duarte 2008) more likely to reflect the highest tolerance to hypoxia. Experimental documentation of tolerance was considered the most robust form of information. Otherwise, observational occurrence within hypoxia and range were taken in unison to determine overall tolerance, but with less certainty.

Assessing DO and other variables as predictors of community composition

To evaluate a larger extent of potential confounding predictor variables to explain and support the patterns revealed by the previous analyses, a random forest analysis was employed to ascertain the most important factors that predicted the probability of presence of the same 16 species (Table 2.1). Presence-absence was used as a more robust approach to determine types of species contributing to the community structure(s) (Wilson 2012). Random forest is a statistical data-mining, non-parametric approach that uses iterative recursive sampling and ‘out-of-bag’ bootstrapping (i.e., predicting data not in the bootstrap sample) to construct binary partitions of predictor variables. By fitting many classification trees (n = 1000) to the dataset, random forest combines the predictions from all the trees in order to calculate error rate for each observation and ultimately rank each predictor by mean decrease in accuracy (i.e., variable importance) (Breiman 2001; Cutler et al. 2007). This technique provides a high degree of accuracy for selecting the ‘best’ predictor(s) and has the ability to deal with a large number of variables, correlated data, and complex interactions that other statistical techniques (e.g., ANOSIM) cannot manage (Cutler et al. 2007; Strobl et al. 2009). To avoid possible biases associated with our variables that differed in scale of measurement and number of categories, we conducted the analysis using the *cforest* function from the *party* package in R (RCoreTeam 2013; Strobl et al. 2007; Strobl et al. 2009). We chose a classification approach with a binary response variable (i.e., ‘Present’ and ‘Absent’). A total of 10 predictors were considered in all models: region (north or south), DO, temperature, salinity, depth stratum, week, sampling site, shoreline (east or west), slope, and habitat type. Slope was calculated as the vertical change in depth (20m) divided by the horizontal distance between 10 to 30m depth for each sampling site (n=8). The distances were measured using ArcGIS 10.1. To ensure the predictor rankings were robust, random forest models were run ten separate times and importance values were averaged to construct the final covariate rankings for each species. The overall performance of each predictor variable given all species (n =16) was based on three metrics: mean rank, number of times ranked number one, and number of times ranked in the top five.

Measuring DO-tolerance thresholds and magnitude of temporal change

Based on the findings garnered from the literature review, a broken-line regression analysis was performed to determine DO-tolerance thresholds. Broken-line models are regression models that determine a piecewise linear relationship between a response and predictor variable(s), revealing two or more distinct slopes and breakpoint(s) (also referred to as ‘changepoint’ or ‘joinpoint’) (Muggeo 2008). This approach is commonly used in ecology to identify threshold values where the effect of a covariate changes (Muggeo 2008; Toms and Lesperance 2003). In order to detect distinct responses relative to physiological tolerance to depleted oxygen levels, the focal species (n=16) were divided into two groups based on the results of the literature review. Species classified as having ‘high’ to ‘moderate’ overall tolerance to low DO were assigned to the ‘tolerant’ group (n = 8), while species characterized by lower overall tolerances were placed into the ‘sensitive’ group (n = 8). Squat lobster (*Munida quadrispina*), Dungeness crab, spot prawn, long-rayed star (*Stylasterias forreri*), shortspined sea star, (*Pisaster brevispinus***),** sunflower star (*Pycnopodia helianthoides***),** giant California sea cucumber (*Parastichopus californicus*) and English sole were analyzed as the tolerant group, while the remaining eight species of invertebrates and fishes were analyzed separately as the sensitive group (Table 2.1). Species occurrence was modeled separately for tolerant and sensitive groups as a binary response variable using generalized linear models (GLMs; *binomial* family, *logit* link function), that included DO as the main effect. The broken-line analysis was performed using the package *segmented* in R (Faraway 2006; Muggeo 2003; Muggeo 2008; RCoreTeam 2013).

To disentangle the temporal response at the community level, we further evaluated the degree of regional community change relative to DO tolerance. Using the same tolerance groups (n=16) from the literature and breakpoint analysis, we ran separate binomial (*logit* link) GLMs for the 'sensitive' and 'tolerant' groups. The GLMs assessed the relationship between weekly presence-absence of species and time using all combinations of week, region, depth, and a week x region interaction term. We employed Akaike Information Criterion corrected (AICc) in order to measure the strength of each candidate model (Burnham and Anderson 2002). Models with AICc values within two units of the smallest AICc model were considered equivalent (Burnham and Anderson 2002). Using AICc selection allowed us to discern significant differences between southern and northern community responses that could not be gleaned from the previous analyses (i.e., ANOSIM and random forest). The GLMs were implemented in R (RCoreTeam 2013).

Comparing DO-tolerance thresholds to long-term water quality trends

We discerned potential long-term ecological implications by comparing the DO-tolerance breakpoints to long-term, seasonal DO patterns in Hood Canal. We first acquired shallow cruise time-series data of our two study regions from the Northwest Association of the Networked

Ocean Observing Systems (NANOOS) and the Hood Canal Dissolved Oxygen Program (HCDOP; http://nvs.nanoos.org/CruiseHcdop). HCDOP measured water quality 2-5 times a month in specified locations throughout Hood Canal for eight years (2003-2011). We used the most pertinent and complete set of data, which spanned from 2005 to 2011 at depths of 10, 20, and 30m and included DO (mg L^{-1}) from the north (Hamma Hamma, WA) and south (Potlach, WA) study areas (Figure 2.1). The DO was averaged for each combination of depth, year, and season (spring, summer, fall, winter) and then compared to the DO-tolerance threshold results (± 95% CI) from the breakpoint analysis.

Results

Weekly dissolved oxygen levels showed marked differences in the vertical extent and intensity of low oxygen conditions between the study regions, while temperature and salinity were regionally similar. The northern and southern nearshore temperatures (CV north = 0.21; south = 0.20) and salinities (CV north = 0.06; south = 0.06) were less variable and regionally equivalent compared to the distinct DO conditions (CV north = 0.34; south = 0.49). During the first week of the study, the north and south exhibited similarly high DO levels at 10m (Figure 2.2). Conversely, the DO levels in the south at 20 and 30m started out slightly reduced compared to the north (Figure 2.2). As the summer progressed, the extent of deteriorated DO conditions in the southern area expanded vertically into the nearshore water column, declining to or near severe hypoxic levels (2 mg L^{-1}) at all three depths by early September. While the northern DO conditions also declined, the oxygen never fell below 3mg L^{-1} and actually increased at 10m by the end of the study (Figure 2.2).

Consistent with our hypothesis that regional community assemblages are significantly different, evaluation of the relative densities show higher abundances of invertebrates in the southern region and fewer fish compared to the north (Figure 2.3). More specifically, Decapoda, Forcipulatida*,* and Aspidochirotida contributed 63% and 96% of the relative densities in the northern and southern areas, respectively. The most common fish orders (Pleuronectiformes and Scorpaeniformes) were less abundant than the invertebrates, but were an order of magnitude more prevalent in the north (26%) than in the south (2%). Fish also tended to be most abundant in the shallower depths (10m), while the opposite pattern was exhibited by the invertebrates in both regions (Figure 2.3). The most abundant invertebrates included squat lobster (55% south; 4% north) and spot prawn (15% south; 16% north), while English sole (0.4% south; 5% north) and sculpin (0.6% south; 5% north) were two of the most abundant fish taxa in both regions.

While using NMDS and ANOSIM revealed that the regional community assemblages were significantly different (R ± CI = 0.143 ± 0.03, $p < 0.001$), we also detected the seasonal compositional change in both regions (Figure 2.4). The best NMDS ordination included three dimensions (K = 3), with an acceptable stress value of 0.146. The fish species were heavily associated with the northern ordination and several influential invertebrates (e.g., squat lobster) were more associated with the southern region (Figure 2.4). There was also a significant weekly change in community composition (R ± CI = 0.25 ± 0.06, $p < 0.001$), with earlier periods (June-July) appearing more similar than later in the summer (August-September) for both regions (Figure 2.4). Although both regions exhibited change in composition, indicative of seasonal variation in estuarine productivity, we expected the south to exhibit a greater degree of community change relative to the north that could not be quantified by ANOSIM, but was explored further using other methods.

The DO limit table of the most abundant and significant 16 species (11 invertebrates; 5 fish) revealed a tolerance pattern similar to that of the regional patterns (Table 2.1). The five ‘high’ and ‘moderate to high’ species tolerant of low oxygen were primarily associated with the southern reaches of Hood Canal (Table 2.1). Similarly, most of the remaining fish and invertebrates revealed comparable patterns to the results from the abundance and NMDS results, supporting the hypothesis that low DO may be a primary driver structuring nearshore communities in Hood Canal. One exception among the fishes was English sole, which was assigned a relatively high tolerance level (moderate to high), but was primarily associated (density and ordination) with the northern study area. Another exception was mottled star (*Evasterias troschelii*), which was abundant in the south, but was classified with a ‘low to moderate’ tolerance with a stronger uncertainty (Table 2.1).

Accounting for more explanatory variables, the random forest analysis provided moderate support for our second hypothesis, that DO is an influential variable predicting species presence (Figure 2.5). Depth was the best predictor, averaging a rank of 3.5, with ranks ranging from 1 to 9 across all 16 species, and a ranking of at least 5 for 13 of the 16 species. Consistent with NMDS/ANOSIM results, region was on average the second best predictor of species presence-absence (mean rank = 3.8). Temperature also ranked highly in all three metrics (mean rank = 3.8; No. 1 = 2; Top 5 =13). DO was the best predictor for Dungeness crab and squat lobster and overall had intermediate ranking (mean rank = 5.1; No. 1 = 2; Top 5 = 9). Both habitat (mean rank = 5.9) and salinity (mean rank = 5.6) were among the top five for eight of the species, but neither were selected as the most important predictor for any species (Figure 2.5). Notably, site (mean rank = 4.9) ranked in the top five for nine species, but was only the most important predictor for the flatfish species (Figure 2.5). While slope was also an important predictor of flatfish occurrence, overall the variable ranked as one of the least informative predictors for all other species. Similarly, week and shoreline also performed poorly. The inconsistency in the effect of week between random forest and NMDS/ANOSIM suggests that the change in assemblage over time was likely driven by a few, less abundant species not included in the random forest analysis.

The broken-line analysis revealed distinct breakpoints and responses for the two tolerance groups. The presence of tolerant species slightly declined until 3.77mg L^{-1} (± 0.32), when their occurrence dramatically increased, more than doubling at the lowest oxygen level (1.8 mg L^{-1}). Conversely, presences of species in the sensitive group started to decline at relatively high DO levels, with a breakpoint of 6.96mg L^{-1} (SE ± 1.2). However, the sensitive breakpoint contained a high level of uncertainty compared to the estimate for tolerant species due to the inclusion of potentially more tolerant organisms (i.e, Echinoderms) that were data poor, specifically mottled star. While the same response pattern persisted (Figure 2.6), post-hoc addition of mottled star to the tolerant group (n=9) drastically reduced the error around the slightly lower sensitive (n=7) estimate (5.62mg L^{-1} ± 0.51) and increased the precision of the tolerant threshold (3.77mg L^{-1} ± 0.27). As a result, the post-hoc thresholds were considered more reliable and thus used for further evaluation and inference. In addition, as DO declines the 95% CIs of predicted presence for both groups greatly reduces after the breakpoints are surpassed (Figure 2.6). The smaller confidence intervals suggest a higher level of precision for the responses below the estimated DO-tolerance threshold.

While the NMDS analysis suggested community assemblages changed in both regions over time, regression analysis of the separate tolerance groups showed a larger initial difference and divergence in species occurrences in the south compared to the north relative to tolerance (Figure 2.7). Although there was some support for the full model (ΔAICc < 2) for each tolerance group, the best model in both instances only included week, region, and depth as the main effects

(Table 2.2). Overall the north displayed higher initial probabilities of sensitive species compared to tolerant species presence. Only near the end of the study (week 12; August 23rd) did tolerant species surpass the occurrence of the more sensitive group at all depths (Figure 2.7). Conversely, in the south, sensitive species had the lowest probabilities of presence throughout the study period, while the tolerant species had the highest. Notably, this difference became more than three times greater in the south [mean ΔP(presence) ± SD = 0.36 ± 0.04] than in the north (0.12 ± 0.04), as the occurrence of the sensitive species declined and tolerant species increased (Figure 2.7).

On average, long-term regional oxygen patterns were significantly ($p < 0.001$) lower in the south than the north. In the southern area, the mean seasonal DO tended to be near the DO-tolerance threshold level for tolerant species (3.77mg L^{-1}), but in the north the conditions were generally above the level across all seasons except Autumn (Figure 2.8). The southern nearshore DO levels also fell below the DO-tolerance threshold for sensitive species (5.62mg L^{-1}), while the mean northern conditions tended to exceed the threshold estimate, apart from Autumn (Figure 2.8).

Discussion

We assessed the role of sublethal hypoxia in the nearshore environment of Hood Canal by evaluating hypothesized differences in community composition over time and space. Although we found little evidence in support of multi-species distributional shifts occurring seasonally in response to late-summer intrusion of low DO water, there were significant differences in species assemblages between the southern hypoxia-impacted and the northern normoxic regions. In addition, the regional differences were relatively consistent with species' DO tolerances, and DO as a predictor variable performed moderately well in describing the presence of the same species. We also detected distinct threshold responses to DO for both tolerant and sensitive taxa, and a stronger change in species presence in the south compared to the north. These results provide insight into potential long-term effects of low DO driving differences in nearshore community patterns, even at levels that are not at the traditional, more severe hypoxic concentration ($< 2\text{mg L}^{-1}$).

Several lines of evidence support the hypothesis that low dissolved oxygen plays a role in shaping nearshore, subtidal communities and potentially increases the vulnerability of some species to other physical stressors. Comparisons between northern and southern regions revealed significant differences in community structure, with the south primarily composed of invertebrates and fewer fish. We also found that DO performed moderately well in explaining the presence of 16 nearshore species and the DO ranges and tolerance levels of the species reasonably mimicked the regional community differences. Moreover, the magnitude of the difference in presence of tolerant versus sensitive species was significantly larger in the south throughout the study period. Although severe hypoxia is usually most severe near the bottom of fjords, the impact of sublethal hypoxic levels may not be restricted to the deep dwelling community. As a result, lower oxygen conditions in southern Hood Canal could make species more vulnerable to other external stressors, such as temperature (Breitburg 2002; Diaz and Rosenberg 1995; Vaquer-Sunyer and Duarte 2011), which also appeared to be an important driver, though less regionally distinct, for many species in this study. Indeed, a meta-analysis of marine benthic organisms' survival relative to low DO and warmer temperatures revealed an average 74% decrease in survival times and 16% increase in median lethal DO concentration (Vaquer-Sunyer and Duarte 2011). This is particularly important for nearshore, subtidal environments ($\leq$ 30m) that tend to be warmer (Hood Canal mean temperature ± SD = 12.0^0C ±

2.6) than deeper parts of the estuary (9.7^0C ± 0.1), thus potentially magnifying the effects of lower oxygen levels for some species (McBryan et al. 2013). Large nearshore restoration and preservation programs in Puget Sound and other hypoxia-impacted estuaries are tasked with monitoring, identifying, and protecting key and at risk nearshore habitat (Batiuk et al. 2000; Simenstad et al. 2011). Knowledge that certain areas within the estuaries may be more sensitive to other stressors because of the presence of lower DO could prove important in future conservation planning, particularly in the face of climate change.

We also showed evidence of DO-tolerance thresholds consistent with other hypoxia studies and detected dramatically different responses between the two tolerance groups. The two distinct thresholds identified in this study corresponded to the relative 'sensitivity' of 16 species. Less tolerant species steadily appeared less frequently after 5.62mg L^{-1}, providing support that fish abundances and diversity tend to decline sooner due to generally higher sublethal DO ranges (Breitburg 2002; Diaz and Rosenberg 1995; Vaquer-Sunyer and Duarte 2008). Conversely, the presence of tolerant species only fell slightly with declining DO until 3.77mg L^{-1}, and then exhibited a rapid increase thereafter. While oxygen levels between 3-6mg L^{-1} have been put forth as more 'universal' definitions of hypoxia by numerous others (Breitburg 2002; Buchheister et al. 2013; Paerl et al. 1998; Vaquer-Sunyer and Duarte 2008), the magnitude of the increase in tolerant species was somewhat unexpected. Although hypoxia is known to alter composition by eliminating sensitive organisms while supporting the proliferation of a few tolerant species due to physiological, behavioral, and/or spatiotemporal recruitment adaptions (Dauer 1993), perhaps the large increase in the presence of tolerant organisms in this study was magnified by the reduction in the higher trophic level predators (i.e., fish) (Dinning and Metaxas 2013; Matabos et al. 2012; Sperling et al. 2013).

The southern region had a significantly different nearshore, subtidal community than the north, which could be a more permanent structure given the overall lower oxygen conditions that persist in the south. In hypoxic ecosystems, key features identified as regulating the communities include the severity and duration of hypoxia (Buchheister et al. 2013). The diminished turnover of the southern reaches of Hood Canal causes prolonged and more frequent episodes of low oxygen to occur. More specifically, the southern nearshore region of Hood Canal tends to hover around 4mg L^{-1} year around (Figure 2.8), which is potentially low enough to perpetuate a stark difference between regional species composition. The persistent, but not conventional hypoxic, low oxygen conditions could be altering competitive advantages of nearshore organisms, shifting the community composition towards more hypoxia-tolerant inhabitants (Eby 2004; Peterson et al. 2000). Indeed, the south displayed a consistently larger divergent response in species presence compared to the north throughout the season (Figure 2.7). However, longer periods of nearshore observation at additional sites are needed to provide stronger evidence in support of this hypothesis.

While temporal changes in community composition were detected in this study, there was little evidence of seasonal, distributional shifts. We detected significant temporal shifts in species composition in both regions, with a greater magnitude of change in the southern region. However, the northern region did not show an increase in sensitive species that would support a strong regional shift, nor did we detect changes in regional species presence-absence at depth that would suggest a local shoaling response. Essington and Paulson (2010) found some support for regional shifts in species densities from south to the north. However, that study evaluated the deeper epibenthic community (> 30m), which consisted of a different suite of species, particularly sea stars. In addition, the environmental conditions (salinity, temperature), hydrodynamic forces (Valle-Levinson 2011), and habitat structure (Beck et al. 2003) are considerably different above and below the pycnocline, potentially explaining the disparities between the two studies. Moreover, our study does not negate the potential of regional (south to north) distributional shifts occurring in the deeper benthos. Similarly, Froehlich et al. (2014) reported evidence of tagged Dungeness crab shoaling into nearshore habitat (i.e., local distributional shifts); this was not substantiated by this study. It is possible shoaling events are more concentrated and aggregation 'hot-spots' exists outside the sites sampled. However, this seems unlikely due to the already limited area (< 0.25 km^2) unaffected by severe hypoxic conditions in the south (Essington and Paulsen 2010). In fact, Essington and Paulsen (2010) suspected nearshore shoaling would not be sufficient to explain the density reductions reported in their study. More likely, individual species shift into shallower habitat, but these results are not born out when looking at a broader community.

Although we provide evidence in support of low DO influencing the nearshore, subtidal community, we acknowledge several limitations of the study. While the paired design allowed us to compare several sites in- and outside low oxygen regions of the same system, due to logistical constraints this was limited to only two areas of the estuary. In addition, the sampling sites themselves were not entirely homogenous, confounding some of our results. This was especially true for the flatfish species (English sole and Pacific sanddab), which were predominantly found in the north (site 3; Figure 2.1) near an alluvial plane (i.e., flat, soft bottom). This also highlights the importance of considering specific site qualities, which we tried to capture with some of the random forest covariates (e.g., slope, habitat type). However, we found, overall, structural features were not as influential as DO for predicting species presence-absence. Nevertheless, a larger number of sampling sites would strengthen the robustness of our findings. In addition, there are some biases associated with our sampling method, as there are with most sampling techniques. Visual surveys are not optimal for depicting cryptic species, and evasive behavior can bias observations of all sampling approaches; yet, these biases would ultimately be shared among all sample sites. However, due to the relatively quick and steady movement of the drop-camera and lack of motorized disturbance, we believe evasive behaviors were minimal. Thus, our study was a less invasive, non-destructive approach to investigate differences of non-cryptic macroinvertebrate and fishes across locations. Lastly, although the species DO tolerance patterns captured by the literature review were fairly consistent with our other regional findings, the qualitative assessment did not account for potential local adaptions to low DO conditions. In fact, organisms that encounter hypoxia more frequently may be more tolerant than those less likely to experience such conditions (Sagasti et al. 2001). In addition, there was greater uncertainty about DO-tolerance levels for data-poor species (Table 2.1). However, more species-specific information would most likely strengthen our tolerance comparisons.

This study provides new insight into the possible influential role of low oxygen conditions on the nearshore, subtidal environment of estuarine systems. The regional differences between the northern and southern nearshore communities of Hood Canal provide evidence of an ecological response to persistent low DO levels in the southern portion of the estuary. Given the relatively chronic nature of the overall lower oxygen conditions in the south, the region could be less resilient to other stressors, including warming temperatures. However, Hood Canal is not unique when considering the synergistic or additive effects of environment stressors. Numerous estuarine and semi-enclosed systems are experiencing more frequent and persistent events of hypoxia (Diaz and Rosenberg 2008). Throughout these ecosystems the nearshore environments provide important nursery habitat, and thus more information concerning the influence of low

DO could help focus future conservation and restoration decisions on high-impact areas. More specifically, monitoring and regulatory efforts could be centered on nearshore areas or sites deemed at higher risk or critical to species remaining in the hypoxia-impacted region(s). This project also highlights the lack of baseline physiological information and understanding of a number of fish and macroinvertebrate species, including exploited organisms (e.g., red rock crab). Better understanding of the nearshore, subtidal species and community structure relative to hypoxia can provide insight into the state and stability of estuaries as landscapes and climate continue to change.

Table 2.1 Dissolved oxygen limit table of the 16 most abundant and significant (ordinal correlation) nearshore species. Citations with '*' report on taxonomic groups that are not species specific. Overall tolerance levels with a '?' indicates a higher level of uncertainty due to lack of data.

Common name	**Scientific name**	**DO Range**	**DO Tolerance**	**Overall Tolerance**	**Relative Abundance (north; south)**	**Ordination Correlation Significance**
Long-rayed star	*Stylasterias forreri*	0.5-6.6 ml/L[a]	*more tolerant than fish & crustaceans[b]	High?	1.3%; 3.1%	$p < 0.01$
Spot prawn	*Pandalus platyceros*	0.9-6.9 ml/L[a]	minimum lethal ~1 ml/L[c]; 3.5 to 4.0 mg/l (below this metabolism declines)[d]	High	16.2%; 15%	$p < 0.001$
Squat lobster	*Munida quadrispina*	0.8-4.6 ml/L[a]	0.1 to 0.15 ml/L, well adapted to hypoxia[e,f]; exposed to < 0.5 mg/L[g]; no association between density & DO[h]	High	15%; 55.1%	$p < 0.001$
Dungeness crab	*Metacarcinas /Cancer magister*	2.6-7.5 ml/L[a]; 4.9-6.6 mg/L[i]	1.2 mg/L> DO > 0.6 mg/L[i]; >3.3 mg/L[i]; 0.4 to 0.9 ml/L[k]	Moderate to high	3.2%; 3.4%	$p < 0.01$
Giant California sea cucumber	*Parastichopus californicus*	0.32 - 6.7 ml/L[a]	*more tolerant than fish & crustaceans[b]; > 3.2 mg/L[d]; < 2.5 mg/L mortality[l]	Moderate to high	3.1%; 4.0%	$p < 0.001$
English sole	*Parophrys vetulus*	1.1-6.6 ml/L[a]	condition not sig. different btw 0.4-1.0 ml/L[k]; moderate degree of hypoxia tolerance (1.09 ml/L)[m]	Moderate to high	4.5%; 0.4%	$p < 0.01$
Sunflower star	*Pycnopodia helianthoides*	2.6-6.8 ml/L[a]	more abundant >1 ml/L[k] ; least abundant < 0.5 ml/L[k]; > 0.5 ml/L[n]; present in hypoxic conditions[n]	Moderate	9.8%; 5.3%	$p < 0.001$
Shortspined sea star	*Pisaster brevispinus*	3.9-6.6 ml/L[a]	*more tolerant than fish & crustaceans[b]; present in hypoxic conditions[o]	Moderate	1.6%; 0.9%	$p < 0.01$
Sculpin spp.	*Malacocottus kincaidi; Enophrys bison*	a.0-6.6 ml/L[a]	*subtidal sculpin less tolerant than intertidal sculpin[p]	Low to moderate?	4.5%; 0.6%	$p < 0.001$
Mottled star	*Evasterias troschelii*	3.0-7.3 ml/L[a]	*more tolerant than fish & crustaceans[b]	Low to moderate?	2.8%; 3.0%	$p < 0.01$
Striped nudibranch	*Armina californica*	2.4-6.5 ml/L[a]	absent ≤ 0.8 ml/L[k]	Low	3.6%; 0%	$p < 0.01$
Pacific sanddab	*Citharichthys sordidus*	2.6-5.5 ml/L[a]	condition sig. better in DO > 1ml/L[k]; can move off the bottom[q]	Low	3.9%; 0.2%	$p < 0.001$
Eelpout spp.	*Lycodopsis pacifica*	2.3-5.5 ml/L[a]		Low?	1.2%; 0.2%	$p < 0.001$
Goby spp.	*Rhinogobiops nicholsii*	3.3 - 6.4 ml/L[a]		Low?	3.1%; 0.4%	$p < 0.05$
Kelp crab	*Pugettia producta; Pugettia richii*	6.6 ml/L[a]		Low?	1.1%; 1.0%	$p < 0.001$
Red rock crab	*Cancer productus*	3.2-6.6 ml/L[a]	Emersion (<12hrs)[r]	Low?	8.2%; 1.7%	$p < 0.001$

Ocean Biogeographic Information System (OBIS)[a]; Vaquer-sunyer and Duarte (2008)[b]; Jamieson and Pikitch (1988)[c]; Whyte and Carswell (1982)[d]; Burd (1985; Burd 1988)[e]; (Burd and Brinkhurst 1984)[f]; Dinning and Metaxas (2013)[g]; Matabos et al.(2012)[h]; Bernatis et al. (2007)[i]; Airriess and McMahon (1994)[j]; Keller et al. (2010)[k]; Yu et al. (2012)[l]; Boese (1988)[m]; Peters(2007)[n]; Gilles (2012)[o]; Mandic et al. (2009)[p]; Love (1996)[q]; McGaw et al. (2009)[r]

Table 2.2 Comparison of candidate models predicting probability of presence over time relative DO tolerance. The 'best' models are depicted in *bold*.

'Sensitive' group	AICc	ΔAICc
Intercept+Week+Region+Depth+Week:Region	1562	1
Intercept+Week+Region+Week:Region	1580	19
Intercept+Week+Region+Depth	**1561**	**0**
Intercept+Week+Region	1579	18
Intercept+Week+Depth	1581	20
Intercept+Region+Depth	1568	7
Intercept+Week	1598	37
Intercept+Region	1586	25
Intercept+Depth	1587	26
Intercept	1605	44
'Tolerant' group	AICc	ΔAICc
Intercept+Week+Region+Depth+Week:Region	2843	1
Intercept+Week+Region+Week:Region	2854	12
Intercept+Week+Region+Depth	**2842**	**0**
Intercept+Week+Region	2853	11
Intercept+Week+Depth	2915	73
Intercept+Region+Depth	2852	10
Intercept+Week	2962	84
Intercept+Region	2863	21
Intercept+Depth	2925	83
Intercept	2935	93

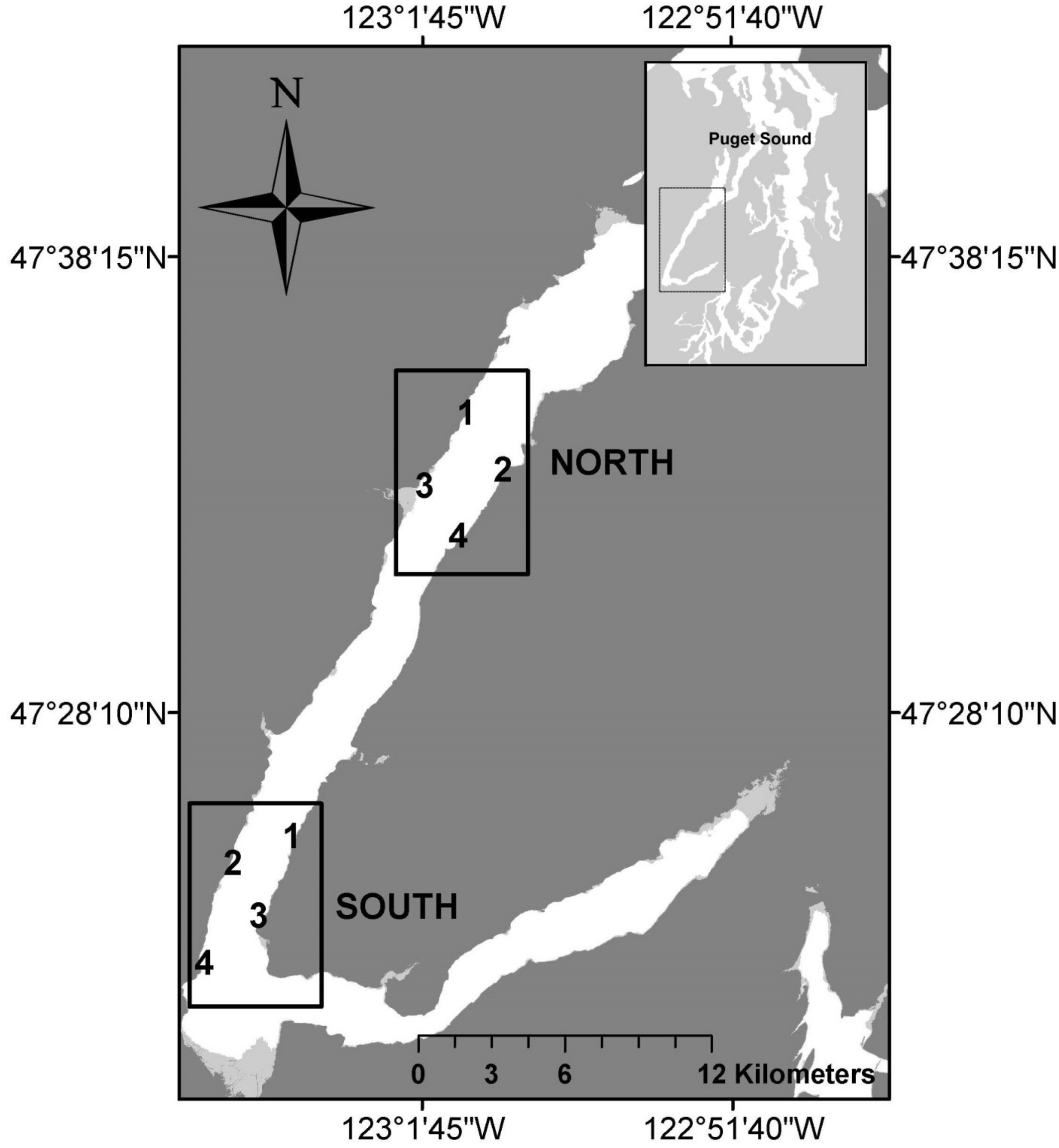

Figure 2.1 Drop-camera locations (*numbers*) in Hood Canal, Washington, USA in 2010. *Rectangles* enclose the central north and south study regions. Five minute transects at 10, 20, and 30 meters depth were conducted at each drop-camera site each week (10 weeks; June 23rd – September 1st). Land is depicted in *dark gray*, intertidal area (< 10m) is represented with light *gray*, and water in *white*.

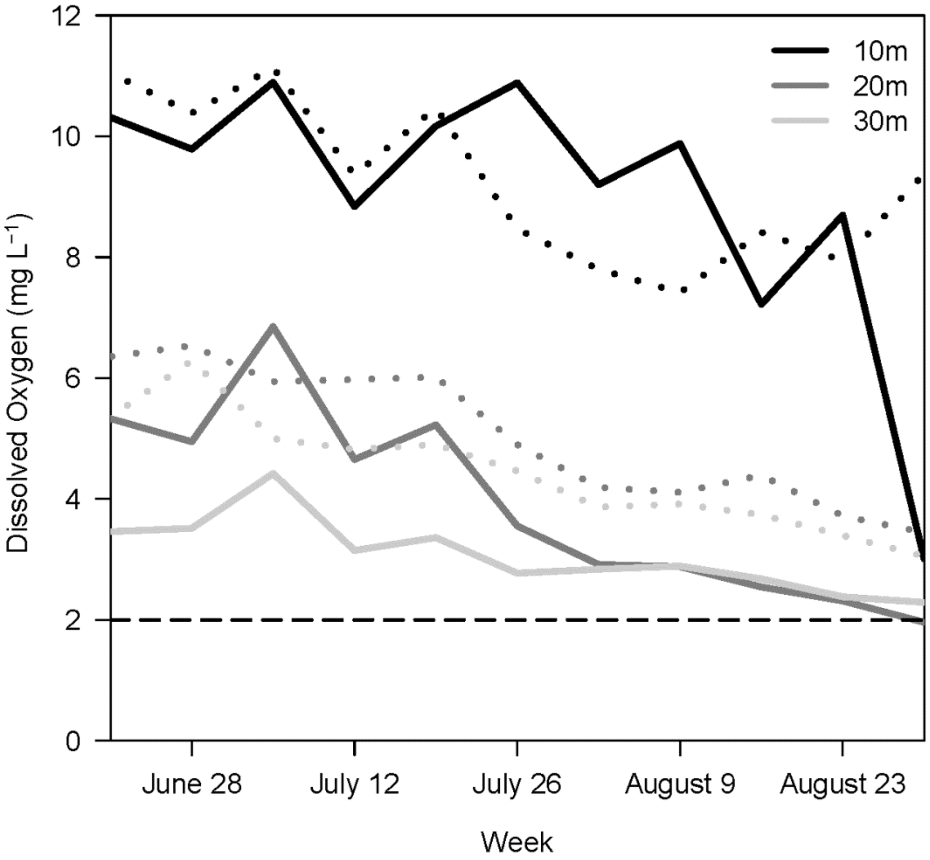

Figure 2.2 Mean dissolved oxygen (milligrams per liter) profiles at 10 (*black*), 20 (*dark gray*), and 30 meters (*light gray*) in the north (*dotted lines*) and south (*solid lines*) study areas. The *black, dashed line* corresponds to the 'traditional' hypoxia threshold of 2mg L^{-1}.

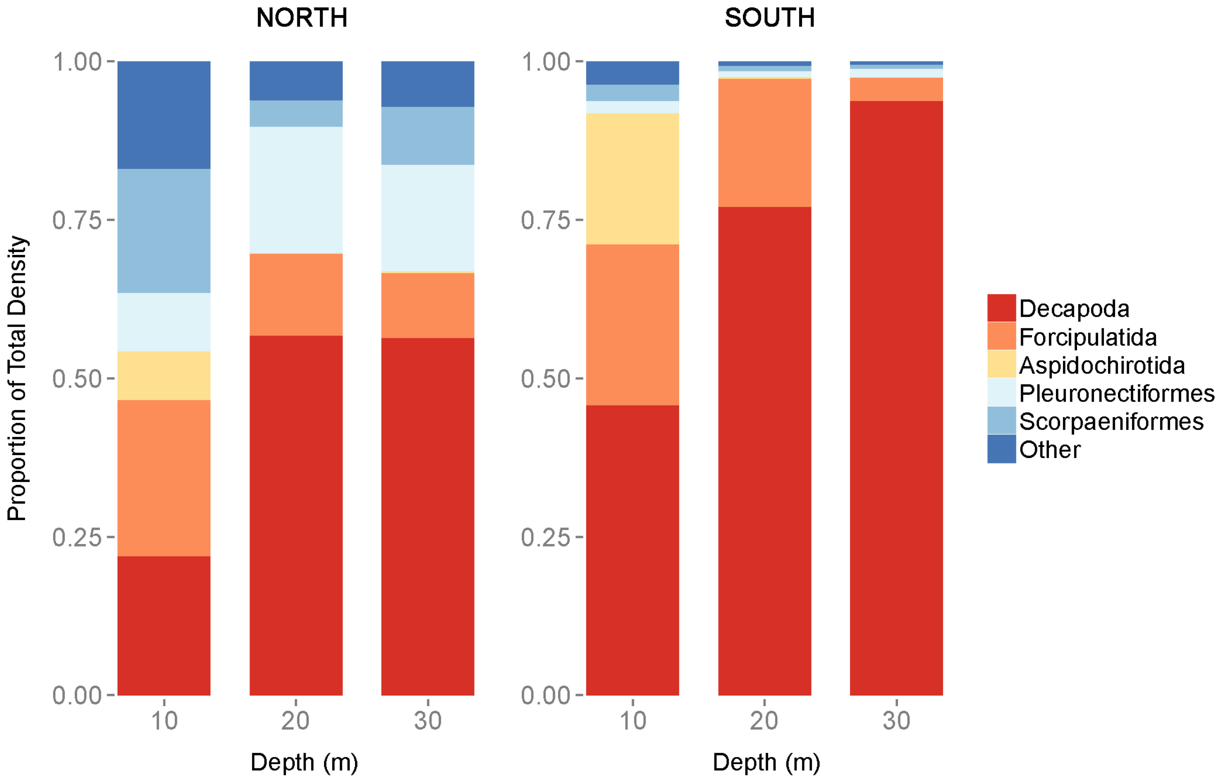

Figure 2.3 Proportions of total relative density from the north and south study areas at 10, 20, and 30 meter depths. Proportions divided into Order level contributions.

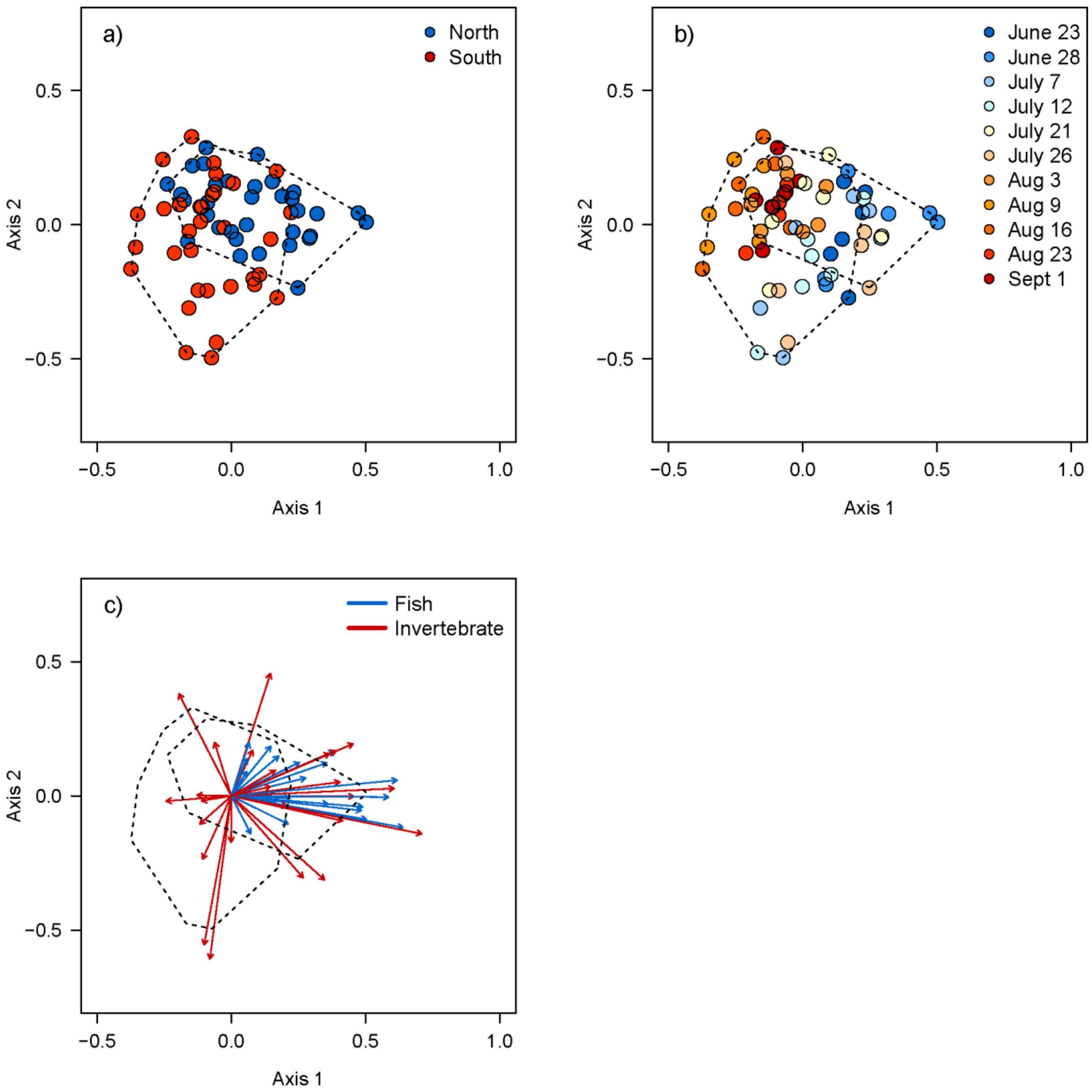

Figure 2.4 Nonmetric multidimensional scaling analysis (NMDS) of log-transformed and standardized average species abundance data. With a 3D ordination (stress = 0.146), the distances between all pairs of objects are in rank-order agreement with their dissimilarities in species composition. **a)** Significantly different (R ± CI = 0.143 ± 0.03, $p < 0.001$) north (*blue*) and south (*red*) assemblages. **b)** Significantly different (R ± CI = 0.25 ± 0.06, $p < 0.001$) mean weekly compositions; gray-scale colors correspond to each initial sampling week (n=10) date. **c)** All species represented in ordinal space, with fish depicted in *blue* and invertebrates in *red*. Each *arrow* represents a species and the magnitude of the *arrow* corresponds to the ordination correlation significance of the associated species. For reference, *dotted lines* in all graphs illustrate the regional ordination boundaries.

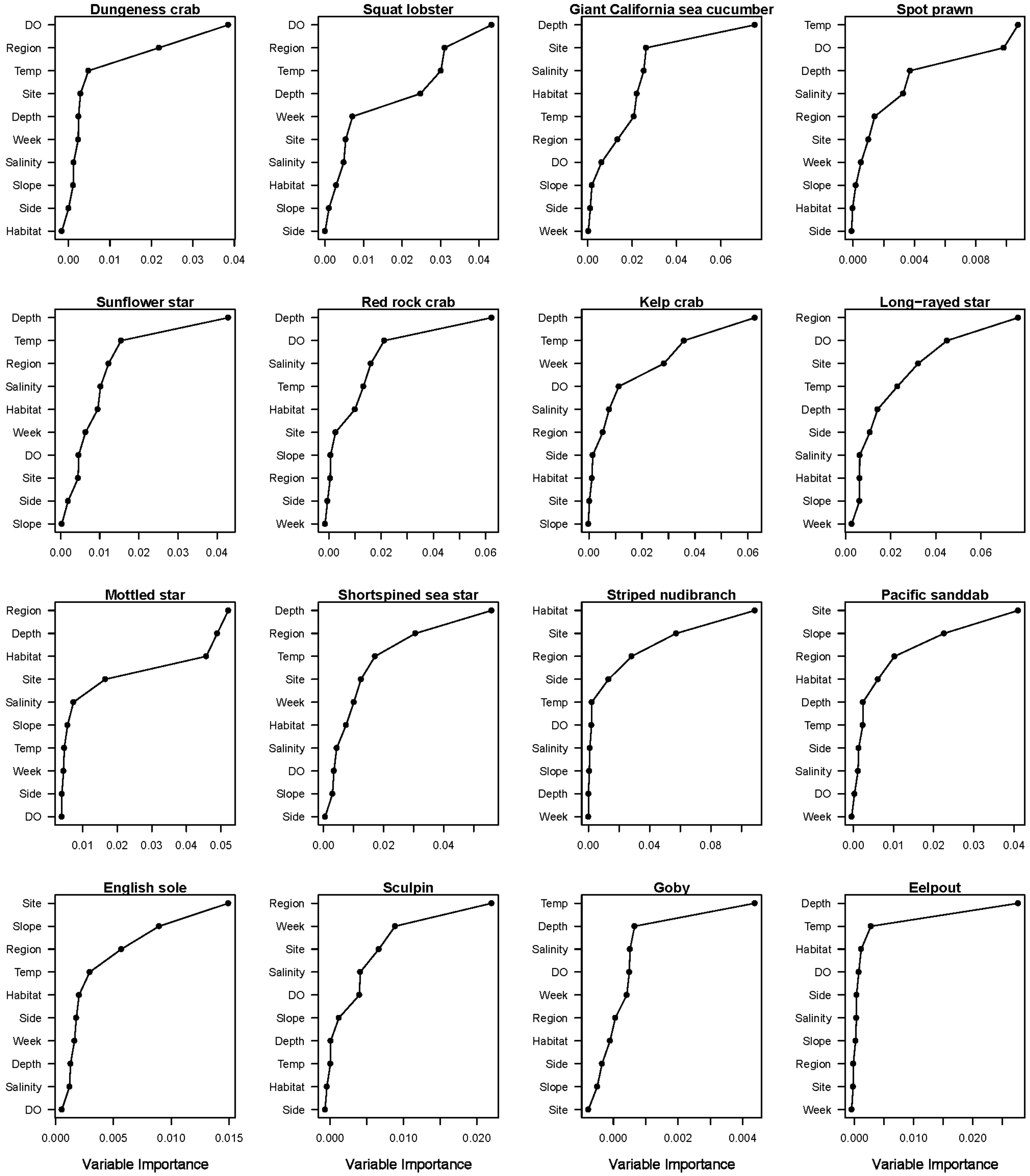

Figure 2.5 Random forest results of the presence-absence data from 16 of the most abundant and significant species. All 10 predictor variables are depicted on the y-axis and the level of the variables' importance on the x-axis. High levels of importance in describing the data receive larger numerical values and are relative to the respective dataset (i.e., cross-comparison of species is not meaningful).

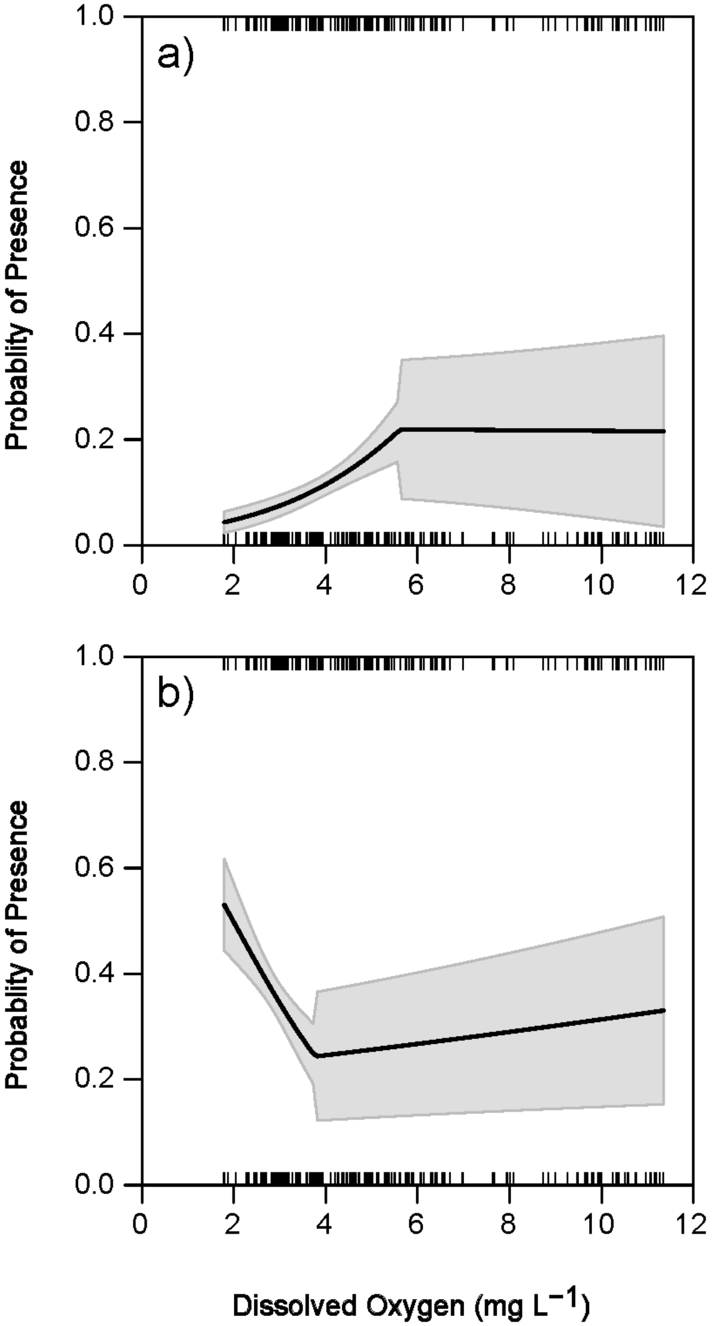

Figure 2.6 Post-hoc generalized broken-line analysis results for the (a) ‘sensitive’ species (n =7; breakpoint ± SE = 5.62 mg L^{-1} ± 0.51) and (b) ‘tolerant’ species (n = 9; 3.77 mg L^{-1} ± 0.27). The *black line* represents the model output of the mean probability of presence, with the associated 95% confidence intervals shown in *gray*. The *black tick-marks* depict the presence-absence data.

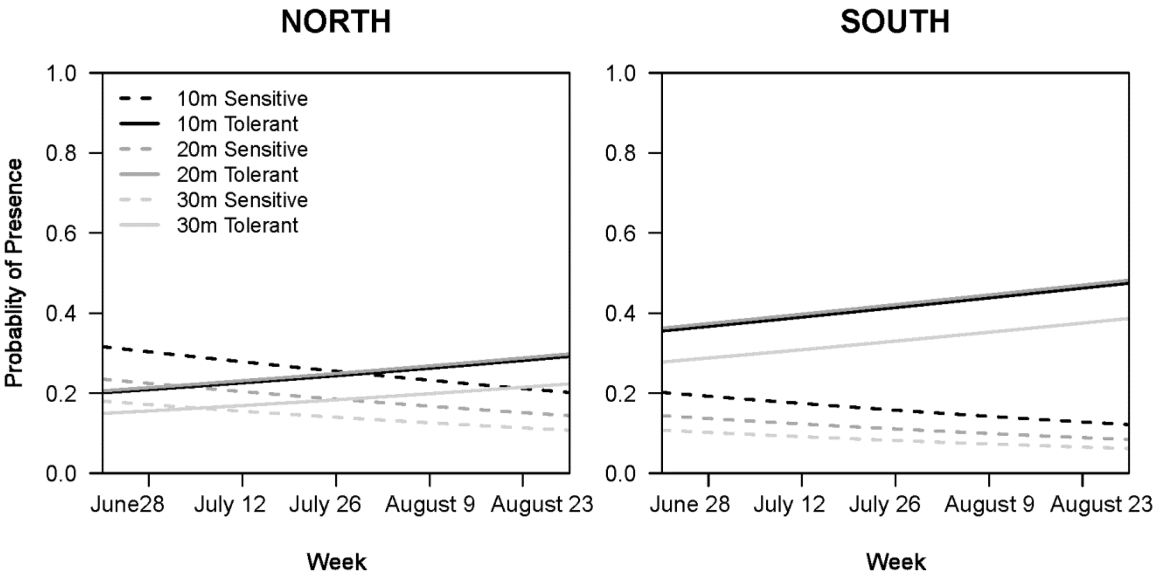

Figure 2.7 Probability of presence of hypoxia sensitive (*dashed lines*) and tolerant (*solid lines*) groups at 10 (*light* gray), 20 (*dark* gray), and 30 meters (*black*) over time in the northern and southern sampling regions.

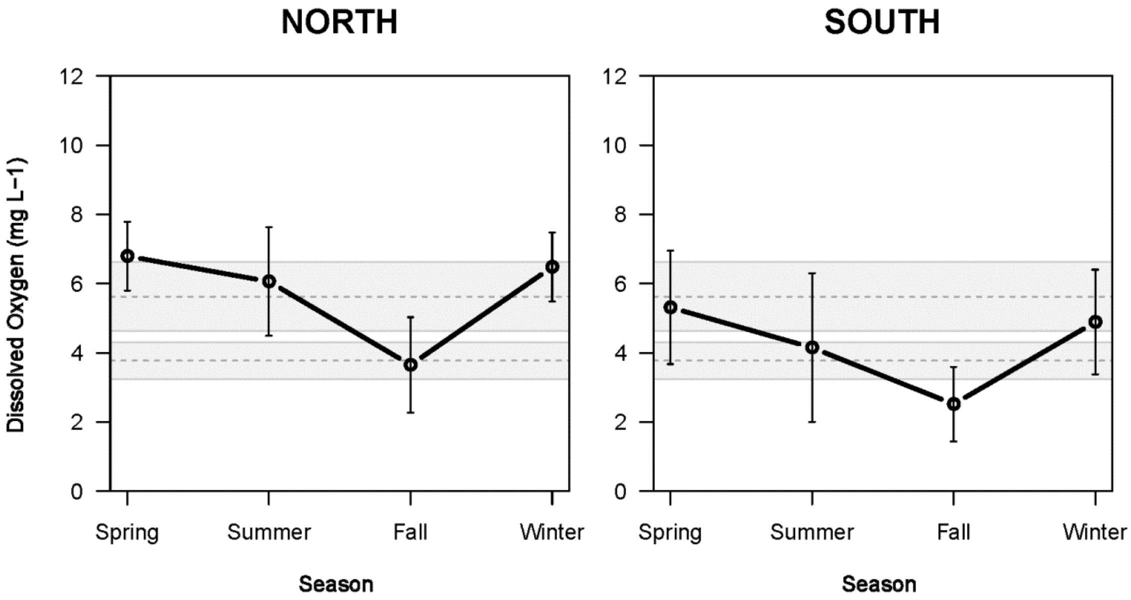

Figure 2.8 Long-term, seasonal patterns of dissolved oxygen for each study region. Each *point* represents the mean and standard deviation of dissolved oxygen each season over year (2005-2011) and nearshore depth (10-30m). The *gray dashed line* indicates the mean breakpoint estimates from the broken-line analysis, with the 95% confidence intervals (CIs) depicted as the *solid gray lines*. The 'sensitive' [mean threshold (95% CI) = 5.62mg L^{-1} (4.63mg L^{-1}, 6.62mg L^{-1})] and 'tolerant' [3.77mg L^{-1} (3.24mg L^{-1}, 4.31mg L^{-1})] thresholds are depicted in both graphs.

Chapter 3

Evaluating hypoxia-inducible factor-1α mRNA expression in a pelagic fish, Pacific herring *Clupea pallasii*, as a biomarker for hypoxia exposure[3]

Introduction

Low oxygen events are becoming more frequent, severe, and expansive in some of the most productive regions of our oceans (Diaz and Rosenberg 2008; Stramma et al. 2010). Low dissolved oxygen, hypoxia (typically defined as dissolved oxygen concentration less than 2 mg L^{-1}) is a particular threat to estuarine and semi-enclosed marine ecosystems with intermittent hypoxia (Diaz and Rosenberg 2008). Because hypoxia is most common in deep waters of estuaries, most investigation into the ecological impacts of hypoxia has focused on demersal and benthic species (e.g., (Diaz and Rosenberg 1995; Levin et al. 2009; Riedel et al. 2014). Yet, hypoxia also extends vertically into pelagic waters, but the impacts on pelagic organisms are not as well understood (Ekau et al. 2010; Zhang et al. 2009a). Given that the pelagic zone contains some of the most ecologically and commercially important species (e.g., forage fish), a deeper understanding of the implications of hypoxia is critical for improved conservation and management of these important ecosystems.

Indirect and/or sublethal effects are the most common biological response of organisms to hypoxia exposure in marine pelagic zones. Numerous studies have detected avoidance responses to hypoxia, resulting in habitat compression and/or reduced access to prey (i.e., zooplankton) (Longhurst 1967; Ludsin et al. 2009; Parker-Stetter and Horne 2009; Prince and Goodyear 2006; Vanderploeg et al. 2009b; Zhang et al. 2009a). However, depending on the system, species, and severity of the hypoxic conditions, pelagic fishes (e.g., Pacific herring) have also been documented inhabiting low oxygen habitats, presumably exploiting prey within the hypoxic layer

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[3] Full citation: Froehlich, H.E., S.B. Roberts, T.E. Essington. (in prep). Evaluating hypoxia-inducible factor-1α mRNA expression in a pelagic fish, Pacific herring *Clupea pallasii*, as a biomarker for hypoxia exposure.

(Parker-Stetter et al. 2009; Roberts et al. 2012a; Taylor et al. 2007; Zhang et al. 2009a). While these movements into low-DO water may provide foraging opportunities, exposure to reduced oxygen levels can negatively impact critical demographic rates, including growth, reproduction, and overall survival (e.g., disease, predator-avoidance) (Domenici et al. 2002; Domenici et al. 2007; Ekau et al. 2010; Pollock et al. 2007; Wu 2002; Zhang et al. 2009a). However, the extent and duration of exposure is often difficult to directly observe via standard monitoring frameworks (e.g. acoustics, capture sampling) without dependable biological markers (biomarkers) (Kodama et al. 2012a), in part because organisms may use these habitats for short durations.

In general, biomarkers are measurable changes in a biological response (e.g., gene expression) that helps elucidate levels of exposure to a particular stressor that then can be used to infer spatial and temporal patterns of a species relative to the environmental landscape. For instance, the use of species-specific biomarkers is common for monitoring pollutants in marine environments (e.g., Wang et al (2008)). One major advantage of a biomarker is the ability to detect sublethal impacts at low levels of stressor intensity. Moreover, implications about consequences of sublethal exposure can be made more robustly because the results gleaned from biomarkers can provide detailed physiological information about sentinel species. A useful biomarker needs a sufficient level of specificity to the stressor of interest, is easy to assay, and relatively unaffected by sampling procedures (Zhang et al. 2012). In addition, species monitored for biomarker levels should have a broad geographic range, be broadly distributed among habitats, and be sufficiently common and accessible to make monitoring less costly. That said, the use of biomarkers in monitoring is not a substitute for water quality measurements or population observations, but is an integrative technique to assist in evaluating exposure or avoidance.

Animals can often remain in low oxygen by increasing specific oxygen-dependent regulatory transcriptional proteins known as hypoxia-inducible factors (HIFs) (Bracken et al. 2003). HIFs can mediate oxygen efficiency and the suppression of metabolic rates, shifting energy production and function towards optimizing oxygen use and reserves (Bracken et al. 2003; Nikinmaa and Rees 2005). Laboratory studies have shown that organisms commonly increase HIF-1α gene and protein concentrations in response to low oxygen levels, and is the most ubiquitous isoform of the three HIF genes (HIF-1α, HIF-2α, and HIF-3α) (Bracken et al. 2003; Chen et al. 2012; Shen et al. 2010; Thomas 2007a; Thomas and Rahman 2009). In addition, because HIF-1α expression and subsequent degradation can be relatively rapid (hours to days), HIF-1α levels in tissues might serve as a robust and reliable proxy for proximate exposure to low DO conditions (Bracken et al. 2003; Thomas and Rahman 2009).

Field studies on benthic marine organisms have showed that HIF-1α mRNA levels become significantly elevated in tissues collected from hypoxic sites compared to specimens from normoxic sites (Kodama et al. 2012a; Thomas and Rahman 2009). However, the field component of these studies only spanned short time periods (days) during a single hypoxic event and exclusively focused on bottom-oriented species. Furthermore, a unifying DO threshold and temporal pattern of HIF-1α gene expression has not been established (Thomas and Rahman 2009). The biological signature may dampen (i.e., acclimation) (Law et al. 2006) or persist (i.e., continual expression to buffer deleterious hypoxic effects) (Thomas 2007a) over time depending on the species. Thus, it is uncertain how, or even if, HIF-1α mRNA levels can be used to reliably detect exposure or hypoxia avoidance in many species.

In Washington State, USA, several basins within the large marine ecosystem of Puget Sound experience seasonally hypoxic episodes. Composing the western arm of Puget Sound, Hood Canal is a 110 km fjord that typically exhibits hypoxic periods during the summer months when stratification restricts mixing throughout the water column (Newton et al. 2007). The factors driving the hypoxic conditions in Hood Canal include a bathymetric sill that reduces tidal currents and hydrological exchange rates from north to south, coupled with the oceanographic input of low oxygenated, upwelled waters (Cope and Roberts 2013; Newton 1995). As a result, bottom water DO levels in the southern regions decline throughout summer, while simultaneously hypoxia expands vertically from deeper depths into the pelagic, shallow reaches (20-30m) of the estuary (Newton 1995). Although hypoxia has been reported in Hood Canal since the 1950s, further deterioration of the oxygen conditions due to anthropogenic climate change and nutrient loading is a major concern (Fagergren et al. 2004; Newton 1995).

The aim of the present study was twofold: (1) develop a molecular assay to assess magnitude and temporal changes in HIF expression following acute hypoxia exposure; and (2) describe temporal and spatial patterns of acute hypoxia exposure using the assay for Pacific herring (*Clupae pallasii*), one of the most abundant pelagic fishes in coastal and estuarine ecosystems in the Northeast Pacific Ocean. Specifically, we used a hepatic transcriptome of Pacific herring to develop a qPCR assay for HIF-1α. We then tested the threshold and time-course of gene expression through laboratory, oxygen-treatment experiments and used those results to interpret expression patterns of herring liver tissue collected from Hood Canal. In the laboratory, we hypothesized that (1) under a threshold experiment, gene expression would be higher for hypoxia treated fish relative to controls and (2) under a time-course experiment, hypoxia induced gene expression would exhibit a dose-like response over time, and rapidly return to baseline levels once returned to normoxic conditions. In the field, we hypothesized HIF-1α mRNA expression would track the spatiotemporal patterns of Hood Canal by (1) elevating in the south compared to the north as hypoxia increased over the summer months (June – October) and (2) would increase overall under more severe hypoxic conditions. Although not a primary focus of this study, we also evaluated the effect of fish size for both the lab and field components, as previous work has suggested a relationship between hypoxia tolerance and size, with smaller individuals tending to be more susceptible to the impacts of hypoxia (Nilsson and Ostlund-Nilsson 2008).

Methods

Laboratory Experiments

Laboratory experiments were conducted in 2013 at the U.S. Geological Survey (USGS) Marrowstone Marine Field Station (MMFS), Nordland, Washington, U.S.A. All laboratory experiments used specific pathogen free (SPF) Pacific herring [mean fork-length (FL) ± SD = 129.0 ± 9.0 mm] reared at MMFS (stock no. SPF 12-2). The source location of the Pacific herring was Cherry Point, Washington. Prior to all experiments, fish were transferred to experimental tanks and allowed to acclimate for approximately 24 hours. Temperature was held relatively constant for all experiments (mean temperature ± SD = 12.8 ± 0.5^{0}C).

To determine the oxygen level that induces elevated HIF-1α gene expression, we conducted a *threshold experiment* with two treatment groups and one control. Each treatment group consisted of six Pacific herring. Hypoxic treatment groups were exposed to the lowest DO conditions (mean DO ± SD = 2.63 ± 0.40 mg L^{-1}), the second treatment to moderate levels (4.25 ± 0.31 mg L^{-1}), and the control groups kept at normal DO conditions (6.84 ± 0.08 mg L^{-1}); the range of DO levels corresponded to possible oxygen levels experienced by Pacific herring in their natural environment. In the flow-through system, DO levels were slowly reduced over a 2 hour acclimation period by bubbling nitrogen (N_2) gas into a separate reservoir (1600L), which flowed (ca. 4.5 L min^{-1}) into the treatment tanks (760L; Figure 3.1). All tanks were fitted with opaque, plastic covers placed at the water-air interface to maintain constant oxygen levels. In addition, a circulating-pump was placed inside the reservoir to homogenize the oxygen conditions throughout the container (Figure 3.1). DO and temperature conditions were continuously monitored and N_2 gas controlled through a data acquisition automated system that we developed using LabVIEW 2012 and Vernier DO and temperature probes (Figure 3.1). After 16 hours of exposure, individuals were euthanized (MS-222) and liver tissue extracted, placed in RNA*later*® (Life Technologies Corporation, Carlsbad, CA), and stored at -20^{0}C for further processing. The experiment was replicated a total of four times.

To test for a dose-like response of HIF-1α mRNA expression to hypoxia, we ran a *time-course experiment* where Pacific herring were exposed to hypoxia for several hours and then returned to normoxic conditions, while being sampled throughout. Similar to the threshold experiment, DO levels were slowly reduced (2hr) to the lowest oxygen level (mean DO ± SD = 2.31 ± 0.41 mg L^{-1}). Four herring were sampled at a geometric time interval from 0 to 16 hours after the initiation of hypoxic treatment; the same sampling procedures from the threshold experiment were applied. After 16 hour of exposure, DO concentrations in the treatment tanks were returned to normoxic levels (6.60 ± 0.28 mg L^{-1}) by increasing flow and aerating the tanks. At 12 and 24 hours after returning to normoxic conditions, four herring were then sampled from each tank as described above. Again, the time-course treatment was replicated four times and liver tissue extraction and preservation were the same. Regarding both experiments, any herring displaying loss of equilibrium were documented and immediately euthanized.

Field Sampling

Pacific herring were captured monthly from June – October in 2012 and 2013 using mid-water trawls. The site-based sampling design included two sites in the southern region that experience the strongest hypoxia (sites A and B), one site farther north in the main channel with light-to-moderate hypoxia (site C), and the reference, normoxic site in the far north (site D; Figure 3.2). Sites B, C, and D have depths ranging from ca. 100 to 200m, while the southern-most site (A) has a maximum depth of 70m. We expected differences in the timing, intensity, and vertical intensity of hypoxia among these sites, so measuring all four throughout two seasons allowed us to separate phenological effects unrelated to hypoxia from those related to hypoxia. In addition, all field observations were accompanied by synoptic monitoring of oxygen and temperature profiles using a Sea-Bird (SBE) 9plus SEACAT profiler, fitted with dissolved oxygen and pH auxiliary sensors.

We collected five Pacific herring at each site during each month over the two –year sampling period. Sampled fish were relatively similar in size, with mean FL =173.4 mm and a coefficient of variation of less than 10%. No herring were captured during the 2013 July collection period at site C. Acoustic surveys determined areas and depths to be sampled with a Marinovich mid-water trawl. Fish were emptied from the trawl into a well oxygenated holding tank, and five herring were haphazardly collected immediately, and euthanized using an overdose of MS-222. Herring liver tissue was extracted immediately after death, placed in RNA*later*®, and stored at -20°C for later genetic processing.

RNA isolation and cDNA synthesis

All liver tissue from laboratory experiments and field sampling was processed and cDNA synthesized. First, total RNA was extracted from herring tissue using TriReagent (Molecular Research Center, Cincinnati, OH), according to the manufacturer’s protocol. To eliminate any remnant genomic DNA, the RNA samples were treated with Ambion® DNA-free™ DNase (Life Technologies Corporation, Carlsbad, CA). Purified RNA was then quantified by optical density at 260nm using UV spectrophotometer (NanoDrop *ND-1000*). Quantitative PCR (qPCR) was performed on treated RNA to ensure absence of DNA carryover. Finally, cDNA was synthesized from the RNA (1ug) using a reverse transcription (RT) kit (Promega Corporation, Madison, WI). First 17.75ul of RNA and 0.5ul of oligo-dT primers were incubated at 70°C for 5min, then immediately placed on ice. For a 25ul reaction, 6.75ul of master mix containing 5ul of M-MLV RT Buffer, 1.25ul of 2.5mM dNTP, and 0.5ul M-MLV reverse transcriptase was added to the primed RNA. The solution was then incubated at 42°C for 1hr, heat inactivated at 95°C for 3min, and resulting cDNA stored at -20°C.

Primer development

In order to identify the target sequence a hepatic transcriptome (Roberts et al. 2012b) was annotated using the GenBank database and National Center for Biotechnology Information (NCBI)-Basic Local Alignment Search Tool (BLAST) algorithm (McGinnis and Madden 2004; Morgulis et al. 2008; Zhang et al. 2000). Use of the BLASTx algorithm identified the sequence as HIF-1 and its associated complexes (i.e., PAS domain and HIF-1α CTAD). The BLASTn program was then implemented to construct a dendrogram (tree) based on alignments to visualize and compare species with the most similar nucleotide sequences (homologs) to the herring HIF-1α sequence. Primers were then developed with NCBI Primer-BLAST (forward 5’-GTTGAGCAGCTTCCTCATGC-3’; reverse 5’-GGAGTCGGAGGTGTTCTACG-3’). The level of pairwise congruence of the herring sequence and primer region was then cross-compared to three sequenced isoforms (HIF-1α,-2α, and -3α)[4] from Zebrafish (*Danio rerio*) and Chinese sucker (*Myxocyprinus asiaticus*) to corroborate isoform specificity.

To confirm primer amplification size we ran conventional polymerase chain reaction (cPCR) in a 1.3% agarose gel. In a 25ul reaction, cPCR was performed using 12.5ul 2x Apex

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[4] *Danio rerio* HIF-1α (AY326951), HIF-2α (DQ375242), HIF-3α (AY330295); *Myxocyprinus asiaticus* HIF-1α (HQ432957), HIF-2α (HQ432955), HIF-3α (HQ432956).

Red (Promega Corporation, Madison, WI), 0.5ul of forward and reverse primer (10uM) each, 9.5ul of ultra-pure water, and 2ul of template cDNA. Cycling parameters for cPCR were as follows: (1) 95°C for 10min (2) 39 cycles of 95°C for 15s, (60-65)°C for 15s, then 72°C for 30s, and (3) 72°C for 8min. Products were compared using an annealing temperature gradient spanning 60-65°C. Similar methods were used to develop the assay for our normalizing gene, elongation factor-1α (EF-1α), a common housekeeping gene (forward 5’-AGAGCAATGTCAATGGTGAT-3’; reverse 5’-TCTCATCTACAAATGCGGAG-3’).

Quantitative PCR

For amplification and measurement gene expression, qPCR was performed in a 20ul reaction mixture using 10ul of 2x SSoFast^{TM} EvaGreen (Bio-Rad Laboratories, Inc., Hercules, CA), 0.5ul of forward and reverse primer (10uM) each, 8ul of ultra-pure water, and 1ul of cDNA template. PCR cycles were as follows: (1) 95°C for 30s, (2) 95°C for 5s, (3) 60°C for 5s, (4) plate read, (4) return to step two 39 more times, and (6) melt curve from 65°C to 95°C, at 0.5°C for 3s (+plate read). Amplification and melt curves were evaluated and relative fluorescence units (RFU) quantified using PCR Miner software (Zhao & Fernald 2005). Average gene expression was then calculated using cycle threshold (CT) and average efficiency (AE) from the equation, mRNA expression = $(1+AE)^{-CT}$. HIF gene expression was normalized by dividing by the corresponding EF-1α values (Zhao & Fernald 2005). All samples were run in duplicate.

Analysis

The laboratory threshold and time-course data were analyzed separately using a two-way analysis of variance (ANOVA), with a post-hoc Tukey’s test. We specifically evaluated the relationship of mean gene expression levels of HIF-1α to treatment (level of DO or length of exposure) and size of the fish [fork-length (mm)]. We used a level of 0.05 to determine significant differences among treatments and/or time periods.

The field data were analyzed by separately modeling the frequency of elevated gene expression in relation to key spatial, temporal, and biological variables. Given the complexity of herring behavior and environmental heterogeneity, evaluating occurrence (number of fish) of elevated expression rather than the mean expression provided an improved approach to capture patterns of HIF-1α elevation. Inferred by the laboratory results, we tested the probability of significant increases in mRNA expression - more than 1 standard deviation (sd) above mean normoxic expression levels - relative to several potential predictor variables. We assessed the frequency of elevated expression by using a generalized linear model, a binomial probability density function, and a logit link function to model the proportion of herring exhibiting high levels of HIF-1 α (Gelman and Hill 2007). The binomial model evaluated the relationship between the probability of elevated HIF-1α mRNA expression relative to sampling year (2012, 2013) or month (June - October), sampling site (A, B, C), fork-length of each fish (mm), and the mean trawl depth (m) at which the herring were captured. Monthly effects were analyzed separately for each year due to the strong interannual differences. We considered statistically significant covariates with p values less than 0.05. All statistics were performed in R (RCoreTeam 2013).

Results

Assay Development

The Pacific herring sequence (858 bp; Contig8790) (Roberts et al. 2012b) and associated primer pairs exhibited a high level of agreement with one isoform, HIF-1α. The putative herring HIF-1α sequence showed identities ≥75% and query coverage ≥73% with zebrafish, Chinese sucker, common carp (*Cyprinus carpio*), Crucian carp (*Carassius carassius*), goldfish (*Carassius auratus*), asp (*Aspius aspius*) stone moroko (*Pseudorasbora parva*), and Mexican tetra (*Astyanax* *mexicanus*). Overall, the nucleotide alignment tree depicts a strong likeness of the herring sequence with other HIF-1α bony-fish sequences (Figure 3.3). In addition, only HIF-1α of the zebrafish and Chinese sucker displayed high levels of identity with the herring sequence, while no significant similarities were found with the other isoforms (HIF-2α & -3α). Further inspection of the alignment within the primer region of the two species also showed strong similarities (Table 3.1). Relative to the primer region of the herring sequence, the zebrafish and Chinese sucker shared 80% and 84% identical base pairs, respectively. Lastly, cPCR confirmed the expected product length (129 bp) predicted from the primer pairs (Figure 3.4).

Threshold experiment

While the qPCR results showed expression of hepatic HIF-1α under all treatments, gene expression was significantly higher (F= 3.29, df =68, *p* = 0.0435) for fish exposed to the lowest oxygen conditions (2.63 ± 0.40 mg L^{-1}). Average HIF-1α mRNA expression of Pacific herring after 16hrs of hypoxia exposure was approximately two times higher than fish under moderate or control conditions (Figure 3.5). In addition, mean expression of the moderate and control groups were not significantly different. Similarly, there was no significant effect of size (F = 0.003, df = 67, *p* = 0.956). We note that six fish from the hypoxia treatment groups lost equilibrium and were euthanized an average of 8.2hrs into the experiment. We considered such events mortalities. Conversely, no moderate or control fish specimens perished prior to the end of any replicate trial.

Time-course experiment

Post acclimation, Pacific herring liver tissue showed a clear temporal increase in mean HIF-1α mRNA expression over the majority of hypoxic exposure, and a rapid decline after conditions were returned to normoxic levels. Relative to average control expression, HIF-1α gene expression did not significantly increase until 2hrs, and peaked at 8hrs, into the experiment (F = 2.31, df = 153, p = 0.0185; Figure 3.6). Although the 16hr HIF-1α gene expression levels were similar to levels seen in the threshold hypoxia treatment (ca. 2x the control), the time-course experiment revealed a significant decrease in overall expression. Once the Pacific herring were returned to normal oxygen conditions (i.e., recovery period), HIF-1α gene expression continued to decline (Figure 3.6). Given the low level of expression of the control and potential influence of the 2hr acclimation, mRNA levels greater than one standard deviation from the control mean appeared to be significantly above the normal expression variability; we thus used this as the defining measure for identifying significantly elevated expression. In addition, HIF-1α expression generally returned to baseline levels 24hrs after exposure (Figure 3.6). Again, there was no significant difference (F = 2.58, df = 152, $p = 0.110$) in mean expression relative to size. Similar to the threshold experiment, five fish suffered mortality an average of 6.0hrs from initiation of the experiment.

Field collection

Monthly dissolved oxygen profiles showed discernable differences in hypoxic distribution over the sampling sites and between the two study periods. Site A, the most shallow and hypoxia prone location, displayed higher oxygen levels at the beginning of the study in 2012 compared to 2013 (Figure 3.7). However, during both years oxygen conditions declined throughout the summer, becoming most depleted (ca. 2 mg L^{-1}) in October and August of 2012 and 2013, respectively. Site B, the deeper southern site, also had higher oxygen levels in 2012, but in both years these levels declined seasonally, reaching to hypoxic levels in 2013 (Figure 3.7). Site C, the deepest of the sampling sites, displayed the most similar pattern across both years, with higher levels of DO during both sampling periods that slowly declined and expanded over time (Figure 3.7). However, similar to all other hypoxia impacted sites, 2013 conditions were more severe than in 2012. It is important to note that the deepest portion of site C (160 – 200m) was not measured (Figure 3.7), but can experience severe hypoxia year around (Froehlich et al. 2015). Site D, the shallow northern reference, maintained better water quality over both study seasons compared to all other sites, consistently staying near or above 4mg L^{-1}. All Herring were captured in DO conditions greater than 2mg L^{-1}.

The probability of elevated HIF-1α mRNA expression was significantly different between site and year (Table 3.2). Despite expectation, the probability of elevated HIF expression was independent of sampling month, thus we focus on the year effect model. Counter to our hypothesis, the probability of elevated hepatic HIF-1α gene expression was greatest in the north site (C) to lowest in the south site (A; Figure 3.7). In fact, the majority of herring captured in the lowest oxygen conditions (2-3 mg L^{-1}), typically in site A, did not exhibit elevated levels (Figure 3.7). However, consistent with more severe hypoxic conditions inducing a response, the model coefficients showed, on average, herring in 2013 had three times higher probability of exhibiting increased HIF-1α expression compared to fish captured in 2012 (Figure 3.7). Akin to the laboratory results, size was not a statistically significant variable.

Discussion

Our findings provide, to our knowledge, the first evaluation of hepatic HIF-1α as a biomarker for hypoxia exposure in a pelagic fish species, Pacific herring. In the laboratory, we were able to establish the level of elevated gene response (>1sd of normoxic levels), exposure amplification (≥2hrs), and degradation rates (ca. 24hrs) of HIF-1α mRNA expression. We also uncovered some evidence of a hypoxic lethal-limit for the herring species. However, in field the spatial pattern of gene expression was counter to our expectations, with higher and more frequently elevated expression levels occurring in the moderate hypoxic site (C), and the lowest expression levels in the most impacted site (A). Yet, consistent with our hypothesis, herring from the more severe hypoxic year (2013) had a higher probability of evaluated mRNA levels, suggesting the fish will or have to enter lower oxygen conditions when water quality is more deteriorated.

In the laboratory, we detected increased levels of HIF-1α mRNA resulting from acute hypoxia exposure and verified reduction in expression upon returning to normoxic conditions. The hypoxia-experiments revealed a clear pattern of increased HIF-1α expression under oxygen conditions around 2mg L^{-1} (Figure 3.5 & Figure 3.6). Similar to studies on Eurasian perch (*Perca fluviatilis*), sea bass (*Dicentrarchus labrax*) and Chinese sucker, acute exposure (hours) to hypoxia can significantly increase HIF-1α gene expression in liver tissue (Chen et al. 2012; Rimoldi et al. 2012; Terova et al. 2008). In addition, mRNA expression returning to baseline levels with 24hr (Figure 3.6) is also consistent across multiple studies and species, tolerant and sensitive alike (Kodama et al. 2012b; Rahman and Thomas 2007; Rimoldi et al. 2012; Terova et al. 2008). Although there is a notable range of 'hypoxic' levels (0.4-4.3 mg L^{-1}), exposure times (1-24hrs), and sampling intervals between the studies due to species specific variability, the general pattern appears fairly ubiquitous. The 24hr reduction in HIF-1α expression is particularly informative, providing key insight into the timing of exposure in a field setting that cannot be gleaned from other common sampling methods alone (e.g., trawling). Based on these findings, hepatic HIF-1α gene expression could prove to be a viable and robust biomarker across numerous species.

Our laboratory experiments also provided some indication of the lethal-threshold of Pacific herring to hypoxia. For the forage fish species, 4 to 8hrs of exposure to low DO conditions (2.31

± 0.41 mg L^{-1}) showed the strongest molecular response (Figure 3.6). However, that similar time window also had the potential for mortality induced effects (i.e., loss of equilibrium), suggesting a lethal limit of hypoxia exposure. Moreover, expression of HIF-1α plummeted after 16hrs (Figure 3.6), most likely due to suppression of overall transcription and translation and not acclimation. Typically, when an organism becomes stressed and homeostatic conditions cannot be achieved, cellular energy is shunted towards cardiac and neural function and down-regulated in other, non-essential tissues (Gorr et al. 2010). However, given the highly mobile nature of pelagic fish, it seems unlikely Pacific herring in the wild would be exposed to such conditions. Nonetheless, the time course laboratory experiments allowed us to reveal the full scope of the HIF-1α mRNA response, establishing the baseline variability, and how that relates to hypoxia lethality. While other studies have reported HIF peaks and troughs with prolonged hypoxia exposure (Law et al. 2006; Rimoldi et al. 2012)*,* the fine scale association with lethal limits presented in this study is unique.

Consistent with one of our field hypotheses, we found evidence that more severe hypoxic conditions on an interannual scale increased the probability of elevated genetic expression of HIF-1α, and thus exposure to hypoxia. Specifically, Pacific herring sampled during the more intense 2013 hypoxia event had a greater probability of elevated HIF-1α expression compared to 2012 (Figure 3.7). These results suggest the herring are more willing and/or obliged to enter hypoxia when low oxygen conditions are more severe. Similar to responses reported for more benthic-oriented species, exposure is most likely context-dependent to the level and extent of the poor water quality conditions (Eby and Crowder 2002). As result, if DO levels continue to decline due to anthropogenic influences of climate change and nutrient loading (Newton 2007), Pacific herring, and potentially other estuarine species in the system, could be at risk for increased exposure and the associated biological ramifications (e.g., increased disease, depressed fecundity, reduced growth) (Ekau et al. 2010; Wu 2002).

While Pacific herring were captured within low DO waters (2-3 mg L^{-1}), the HIF-1α gene expression was not significantly elevated, suggesting a level of adaptation and/or avoidance with varying ecological implications. In fact, the lowest levels of Pacific herring HIF-1α mRNA expression were in the most hypoxia prone sampling sites (A and B; Figure 3.2). Based on the laboratory results, Pacific herring should show at least a 2 to 5 fold increase in expression when remaining in oxygen conditions between 2-3mg L^{-1} for 2 to 8 hours, respectively (Figure 3.6). While acclimation maybe occurring, similar low oxygen levels resulted in mortalities and depressed levels of expression after 16hrs of exposure in the laboratory. However, as the laboratory herring were not from the Hood Canal basin there is a higher potential for the field herring to be better adapted to hypoxic conditions. In fact, species that experience hypoxia more frequently may be more tolerant compared to naïve organisms (Sagasti et al. 2001). Alternatively, herring may be making brief forays into the low oxygen, potentially minimizing the detrimental impacts associated with the energetic cost to remain in poor water quality conditions. While other studies have documented fish utilizing the hypoxic layer (Parker-Stetter et al. 2009; Rahel and Nutzman 1994; Roberts et al. 2012a; Taylor et al. 2007; Vanderploeg et al. 2009a; Zhang et al. 2009a), the level of exposure is typically qualitatively assessed. Evaluation of HIF-1α provides a quantitative approach that shows whether the hypoxia exposure is severe or prolonged enough to elicit a molecular response known to impact key biological processes. In addition, the lack of elevated expression suggests Pacific herring may be more restricted to higher oxygen levels, either closer to the surface (Horppila et al. 2000; Ludsin et al. 2009; Taylor et al. 2007; Vanderploeg et al. 2009b) and/or deep below the hypoxic layer (Parker-Stetter and

Horne 2009; Vanderploeg et al. 2009b). Regarding the former avoidance case, which tends to be more common, residing closer to the surface could increase the risk of predation by visual predators, particularly during the day (Bohl 1980; Costantini et al. 2008; Vanderploeg et al. 2009a). Alternatively, restricted to the depths could keep the fish from its prey, which has been a reported occurrence for Pacific herring in Hood Canal (Parker-Stetter and Horne 2009). Lending more credence to an avoidance response, herring were never captured in conditions less than 2mg L^{-1}.

Although Pacific herring captured in low DO conditions did not show increased HIF-1α gene expression, we did detect significantly higher frequency of evaluated levels at sampling sites and times with only low-to-moderate hypoxia. During June 2012 and August 2013, oxygen conditions at site C were relatively high (ca. 4 mg L^{-1}) at depths <160m of the water column (Figure 3.7). However, unlike the other sampling locations, site C can be as deep as 200m, with continuous bouts of hypoxia. As a result, herring may be making brief forays into the deepest, hypoxic reaches of the site – for foraging or predator avoidance - causing the probability of elevated hepatic HIF-1α mRNA levels. Alternatively, due to the deep bathometry of site C, trawl tows tended to be deeper and, on average, run 10min longer than the other sampling sites. The extended nature of the site C tows could be unintentionally biasing the HIF-1α response. Regardless, the counterintuitive results highlight the difference between lab and field conditions and the importance of sampling over multiple sites and times.

While it is unclear the exact circumstances leading to the HIF-1α gene expression patterns in the field, it is certain that pelagic species behavior and responses are more variable than their demersal counterparts. The ability of forage fish to quickly move horizontally and vertically in the water column appears to result in different exposure and oxygen demand patterns than those reported for more benthic-oriented organisms (Vanderploeg et al. 2009a; Vanderploeg et al. 2009b). Species such as Atlantic croaker (Thomas and Rahman 2009) and mantis shrimp (Kodama 2012) consistently show high expression in hypoxic field sites, which we did not find for Pacific herring. However, more similar to our findings, Thomas and Rahman (2009) reported elevated levels of HIF-1α in a central normoxic location of their field study, which they attributed to movement of the fish from the adjacent hypoxic waters to a more oxygenated area. Although the increased flexibly of movement within the water column results in more a complex expression pattern, it does appear lower DO levels are affecting herring at the molecular level in some capacity.

Given herring exhibit increased HIF-1α gene expression under hypoxic exposure in the lab and elevated levels were detected in the field, there are several important sublethal implications to consider, including impacts on growth and reproductive function. As demonstrated in laboratory experiments, HIF-1α mRNA expression does not immediately increase under depleted environmental oxygen conditions. Rather, increased expression occurs when homeostatic oxygen levels can no longer be maintained (Bruick 2003). As a result, elevation in HIF-1α can relate to other important metabolic pathways being affected; one known relationships is appetite suppression. It has been reported that increases in HIF-1α expression also increases the hormone leptin, which suppresses appetite in order to conserve energy for more important survival processes (e.g., oxygen delivery and transport) (Chu et al. 2010). As a result, if conditions are severe enough, as was the case in the laboratory, there is a potential for reduction in growth and thus population level effects. Another important demographic process that has been directly linked to HIF-1α expression is reproductive function. Thomas and Rohman (2009; 2012) found that elevations in HIF-1α corresponded to improper gonadal function due to a reduction in essential hormones, aromatase and testosterone. Even more recently, a study found hypoxia can directly influence genes essential for regulating sex determination and differentiation (Cheung et al. 2014). As such, more frequent or prolonged exposure could perpetuate poor years of recruitment. Other factors that could also be of concern, but have not been studied relative to HIF-1α expression in fish, include disease and predator avoidance (Domenici et al. 2007; Snieszko 1974).

Although significant elevated expression was detected in the laboratory experiments and in the field, we found no evidence of size influencing HIF-1α mRNA levels. Other studies report smaller individuals having reduced metabolic capacities to handle low DO conditions (Nilsson and Ostlund-Nilsson 2008; Zhang et al. 2009b). Presumably, higher expression levels of HIF-1α would be necessary to accommodate the higher metabolic rates of smaller fishes. However, we were unable to detect significant differences of HIF expression relative to size. Thus, more targeted research is needed to establish, if any, the relationship of size and HIF-1α mRNA levels under hypoxic conditions.

While this is the first time HIF-1α mRNA expression has been used to evaluate a marine pelagic fish in a hypoxic environment, there are several limitations to this study. For one, the mining of public genomic resources, accompanied with laboratory validation, is a relatively novel approach to characterize mRNA expression (Riaz et al. 2011) and there is a level of uncertainty around the specificity of the gene and potential biological responses. As such, the results could be further strengthened by a formal genetic assay and sequencing of the three HIF isoforms. Moreover, we were unable to explain the patterns of the mean HIF-1α expression levels, suggesting other variables not considered in this study could be driving the overall expression levels in the field. In addition, mRNA expression is only a proxy for protein expression, the true active form of HIF. Thus, more in-depth molecular evaluation of Pacific herring could enhance understanding of the physiology of this important forage fish species.

This ecophysiological research offers a unique approach to understanding the influence of hypoxia on a pelagic marine species. We found acute exposure (2-8hrs) to DO between 2-3mg L^{-1} significantly increased the gene expression of HIF-1α, but simultaneously caused detrimental effects as time progressed. In addition, the 24hr reduction of HIF-1α was consistent with numerous other studies. In the field, while probability of expression increased during the more severe low DO event, the spatial pattern was counter to the spatial hypoxic gradient spanning south to north. These results show that while hepatic HIF-1α does not translate directly to the local hypoxic conditions, it did potentially capture broader spatial and temporal signals of hypoxia's influence. In addition, the size of the fish appeared to have no influence on the level of expression. Although the use of HIF-1α gene expression as a biomarker for hypoxia exposure provided quantitative measures and means to better infer potential biological implications, the complex behavior and energy demands of Pacific herring made field interpretation less clear than those of more benthic-oriented species. As a result, more in-depth evaluation of movement patterns and connectivity of Pacific herring in Hood Canal, and other basins of Puget Sound, is needed to fully understand the responses of this important forage fish species to hypoxia and other environmental stressors.

Table 3.1 Cross-comparison from NCBI Align Sequences Nucleotide BLAST of Pacific herring contig and sequenced HIF-1α mRNA of Zebrafish (*Danio rerio*) and Chinese sucker (*Myxocyprinus asiaticus*). Only the alignment of the contig primer region is depicted (highlighted in *black*).

Danio rerio HIF-1α

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Contig   2459  AGCTGGTTGGCCAGCTCGTAGAACACCTCCGACTCCTTGCCTCGGCG-GCTGCGAGCGGC  2517
               |||||||  || | ||||||||||||||| |||||||| || | ||| | | || || ||
Subject   365  AGCTGGTGTGCTAACTCGTAGAACACCTCAGACTCCTTTCCCCTGCGAGAT-CGCGCTGC   307

Contig   2518  ATCGCGGGACTTCTCCTTCCTGCGCTCGGAGCTCACCCTTTTCTTTTCAGTGGTAACACC  2577
               ||| | ||||||||||||||||||||| ||||||||||||||||||||||||  ||| ||
Subject   306  ATCCCTGGACTTCTCCTTCCTGCGCTCCGAGCTCACCCTTTTCTTTTCAGTGACAACTCC   247

Contig   2578  AGCATCCATGCCGGGGTGGAACCTATC--TATGTGTATGGTTTTC                 2620
               || |||||| || ||||| ||||  ||  |||||||  |||||||
Subject   246  AGTATCCATTCCTGGGTGAAACCACTCAGTATGTGTTAGGTTTTC                  202
```

Myxocyprinus asiaticus HIF-1α

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Contig   2459  AGCTGGTTGGCCAGCTCGTAGAACACCTCCGACTCCTTGCCTCGGCGGCTGCGAGCGGCA  2518
               |||||||  || ||||||||||||||||| |||||||| || | ||| || || || |||
Subject   345  AGCTGGTGTGCTAGCTCGTAGAACACCTCAGACTCCTTTCCCCTGCGACTTCGCGCCGCA   286

Contig   2519  TCGCGGGACTTCTCCTTCCTGCGCTCGGAGCTCACCCTTTTCTTTTCAGTGGTAACACCA  2578
               |||| ||||||||||||||||||||| |||||||||||||||||||||||| | || |||
Subject   285  TCCCTGGACTTCTCCTTCCTGCGCTCCGAGCTCACCCTTTTCTTTTCAGTGACAACTCCA   226

Contig   2579  GCATCCATGCCGGGGTGGAACCTATC--TATGTGTATGGTTTTCCCAGACC           2627
               | |||||| || || ||| |||| ||  ||||||| | ||||| ||| |||
Subject   225  GTATCCATTCCT GGGTGAAACCACTCAGTATGTGT-TAGTTTTCCAAACC            176
```

Table 3.2 Analysis of deviance table for model predicting probability of elevated (>1sd from reference mean) HIF-1α mRNA expression. Depicted are the main effects: sampling year (2012, 2013), sampling site (A, B, C), size of fish (mm), and mean trawl depth (m); terms added sequentially (first to last). P-values less than 0.05 were considered statistically significant.

	Df	**Deviance**	**Residual Df**	**Residual Deviance**	**P-value (>Chi)**
Intercept			136	141.43	
Year	1	4.8611	135	136.57	0.02747
Site	2	6.4596	133	130.11	0.03957
Size	1	1.5805	132	128.53	0.20869
Trawl Depth	1	0.07570	131	127.77	0.38427

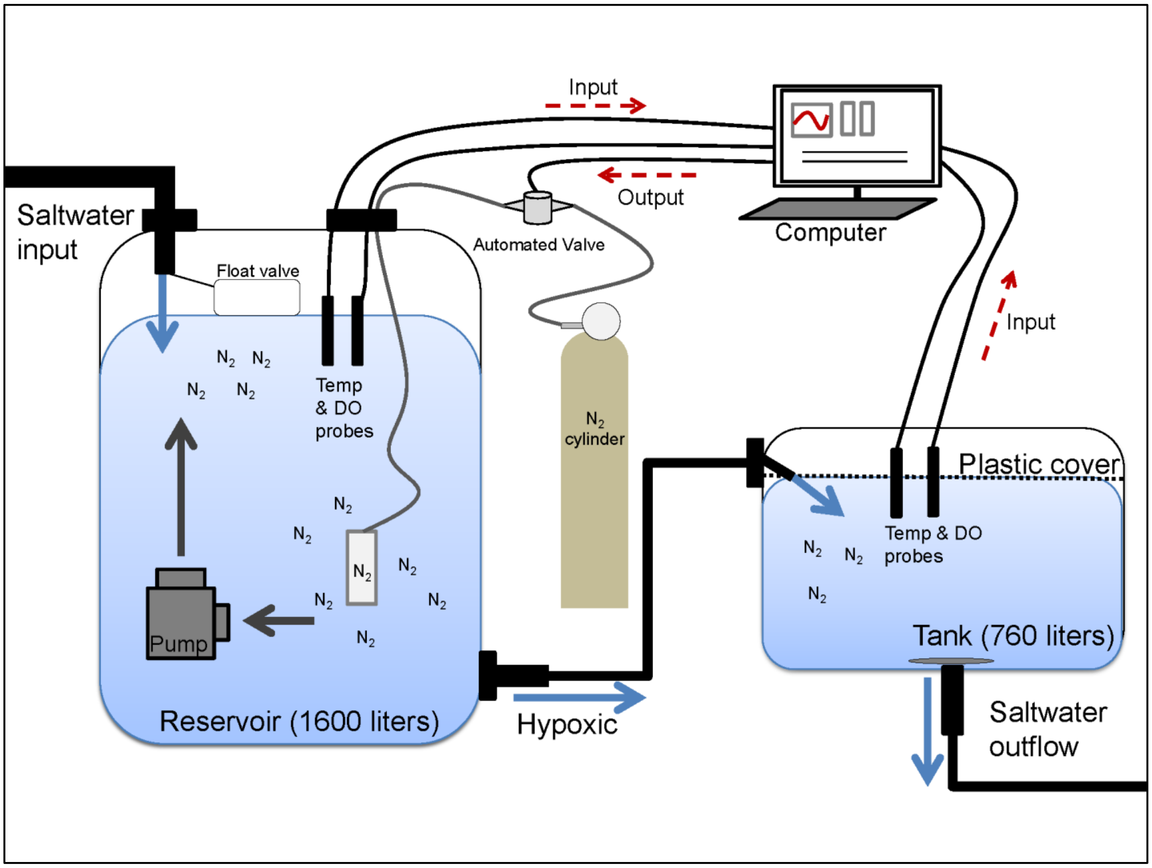

Figure 3.1 Flow-through laboratory set-up. Nitrogen (N_2) was regulated and flowed directly into the reservoir and homogenized with a submergible pump. The automated valve controlling the flow of N_2 into the reservoir responded to the temperature and DO conditions; both levels were monitored in the reservoir and treatment tank(s) with Vernier probes and LabVIEW 2012 software. Simultaneously, hypoxic water was flowed (ca. 4.5L min^{-1}) into the treatment tank(s). A plastic cover was placed on the treatment tank(s) to stabilize the oxygen conditions.

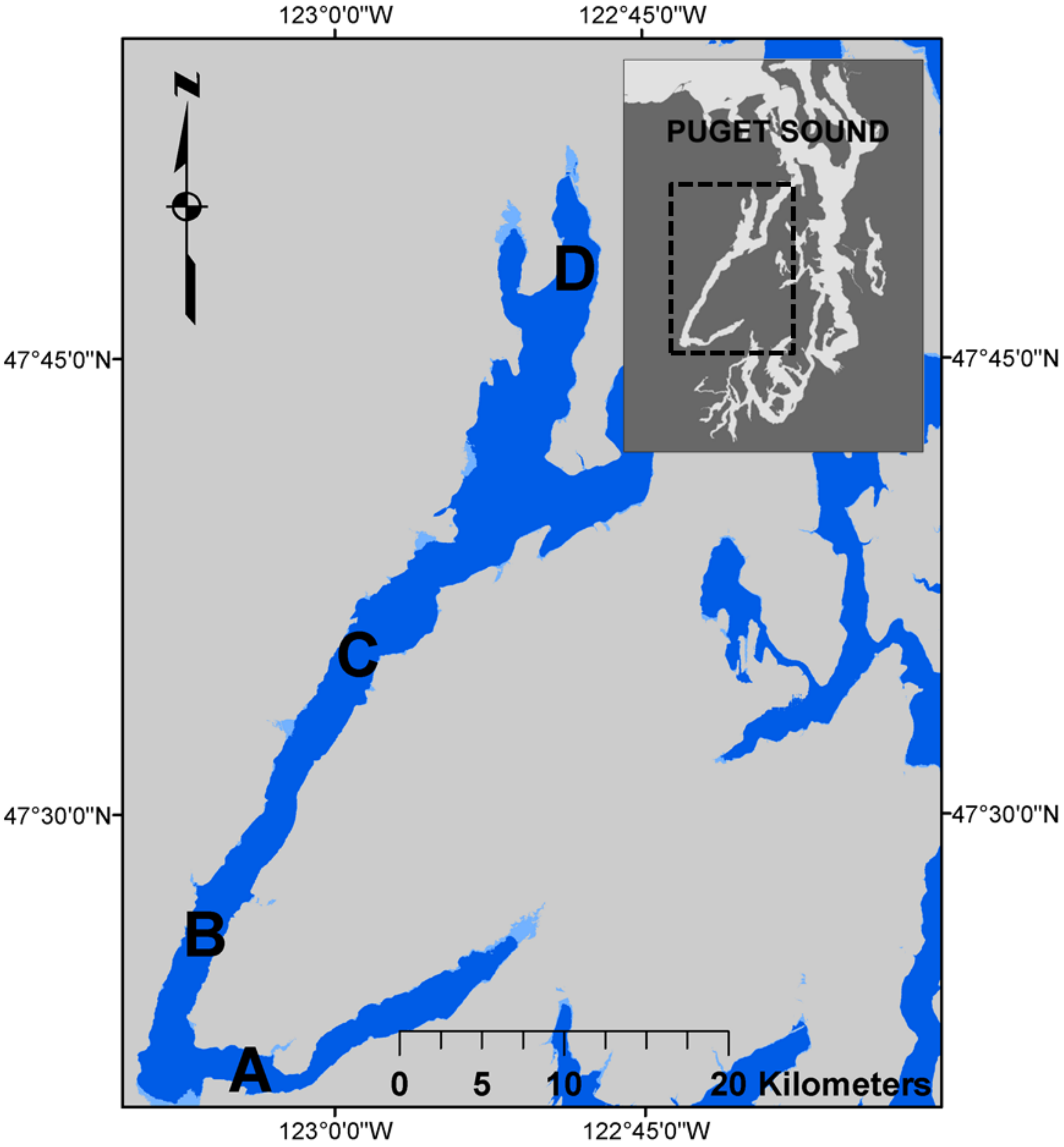

Figure 3.2 Study sites in Hood Canal, Washington. From June to September DO declines in the estuary, but southern sites (A, B) experience more rapid and intense low oxygen levels. By late summer, both southern sites have hypoxic and/or anoxic layers. Site C water quality also declines, but typically gets only moderately hypoxic. Site D, the reference sampling site, tends to have the most consistent and highest (≥ 4 mg L^{-1}) DO conditions.

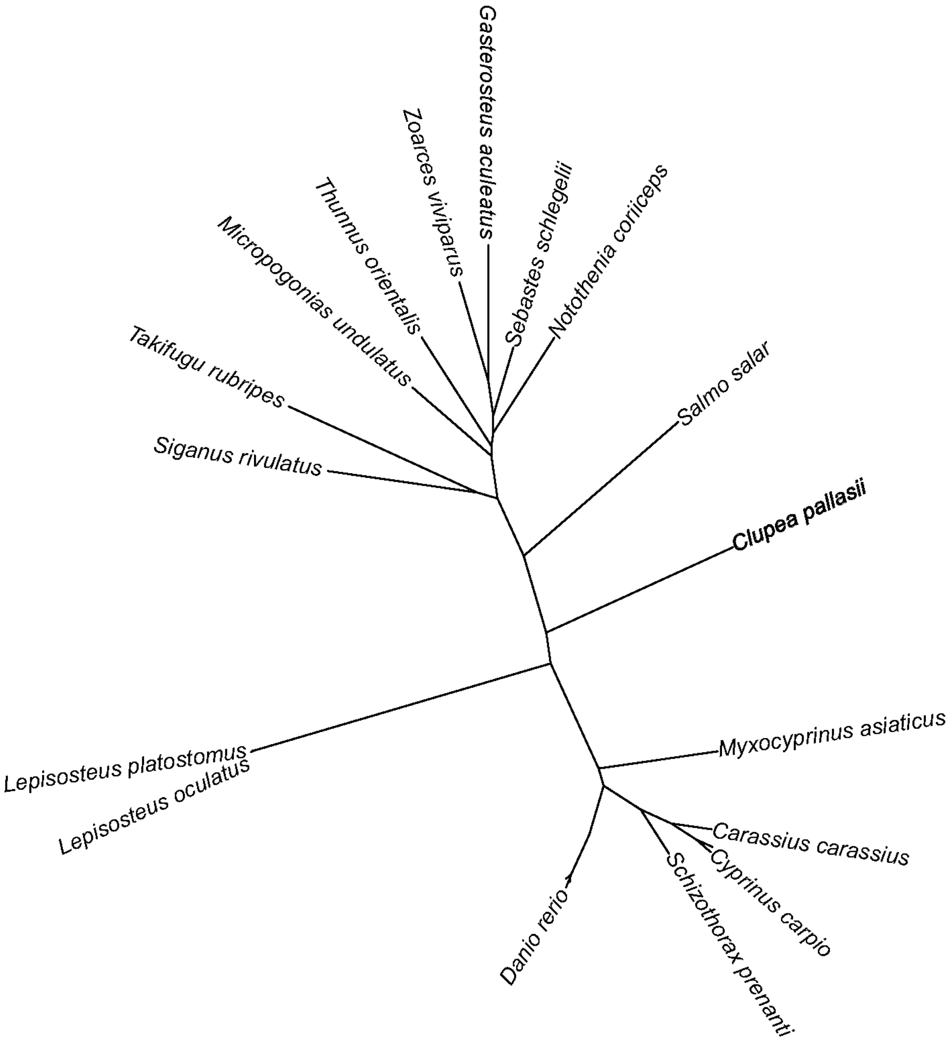

Figure 3.3 NCBI Align Sequences Nucleotide BLAST unrooted-tree depicting pairwise alignment of Pacific herring contig and GenBank sequences. The tree shows clusters of sequences relative to their similarity to the query sequence (i.e, *Clupea pallasii*). Length of branches between nodes indicate the level of similarity (shorter = more similar). Any fraction of mismatched bases between the contig and any aligned sequence greater than 0.25 were excluded from the tree. All species listed are boney fishes, with the Pacific herring (*Clupea pallasii*) sequence depicted in *bold*. [Accession numbers: *Carassius carassius* (DQ306727); *Cyprinus carpio* (EU144225; HM237077); *Danio rerio* (AY326951; BC165712; BX255914; NM_200233; XM_005160492); *Gasterosteus aculeatus* (NM_001267649); *Lepisosteus oculatus* (JQ31038); Lepisosteus platostomus (JQ31038); *Micropogonias undulatus* (DQ363931); *Myxocyprinus asiaticus* (HQ432957); *Notothenia coriiceps* (GU362089); *Salmo salari* (BT059247); *Schizothorax prenanti* (JQ031042); *Sebastes schlegelii* (KC918362); *Siganus rivulatus* (GU249151); *Takifugu rubripes* (XM_003962474); *Thunnus orientalis* (EU300942); *Zoarces viviparus* (DQ089695)].

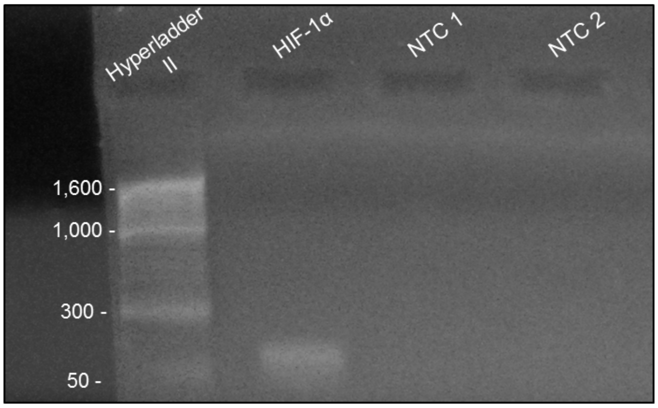

Figure 3.4 Results from conventional polymerase chain reaction (cPCR; annealing temperature = 62^{0}C) using electrophoresis (1.3% agarose gel). HIF-1α (product length = 129 bp) developed from the contiguous HIF-1α sequence of Pacific herring. Primer pairs resulted in the expected product output, and all non-template controls (NTC) were clean.

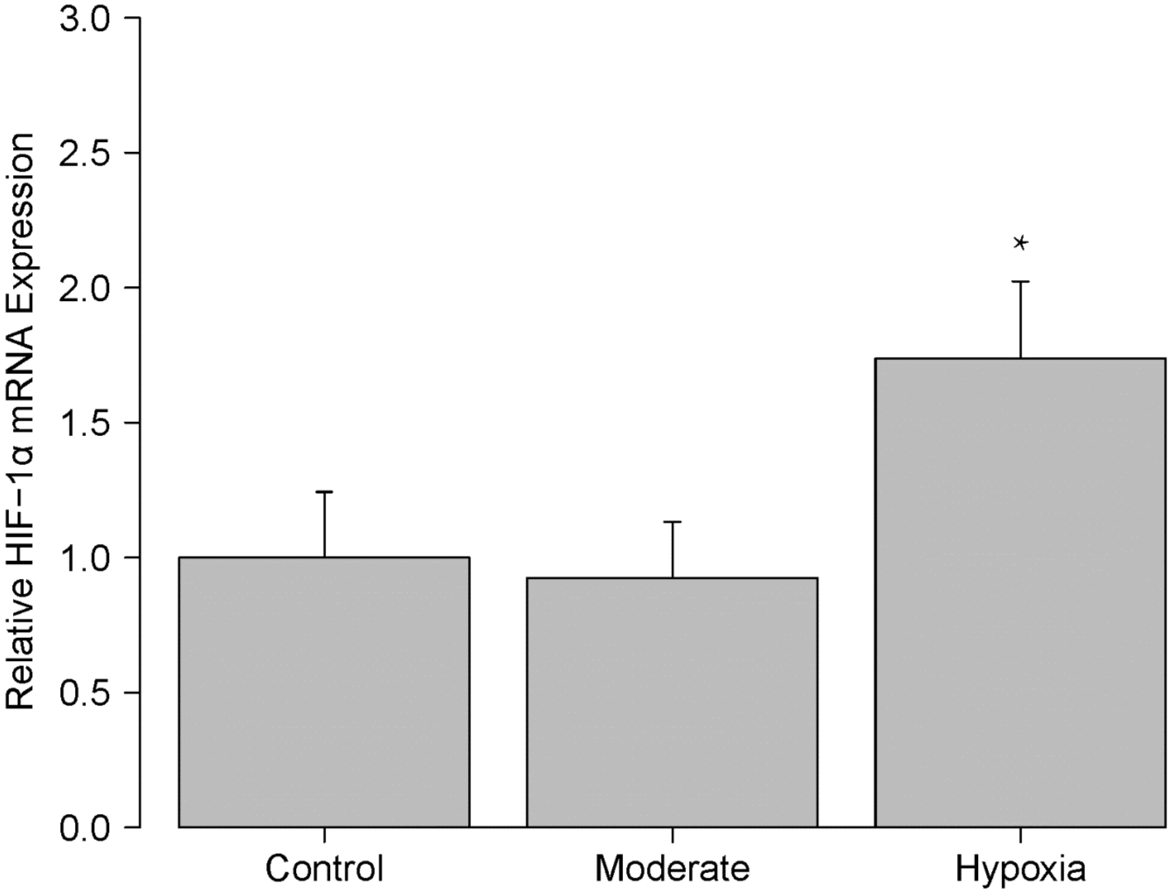

Figure 3.5 Relative mean (± SE) normalized HIF-1α gene expression of Pacific herring liver tissue from threshold experiments. Pacific herring were exposed to 16hrs of control (Mean DO ± SD = 6.84 ± 0.08 mg L^{-1}), moderate (4.25 ± 0.31 mg L^{-1}), and hypoxic (2.63 ± 0.40 mg L^{-1}) conditions. Each treatment consisted of six herring. The experiment was replicated four times. Expression levels are relative to the control. Differences in mean mRNA levels between the control treatment and other treatments were tested by using a two-way ANOVA and post-hoc Tukey's test, *$p < 0.05$. All qPCR samples were run in duplicate.

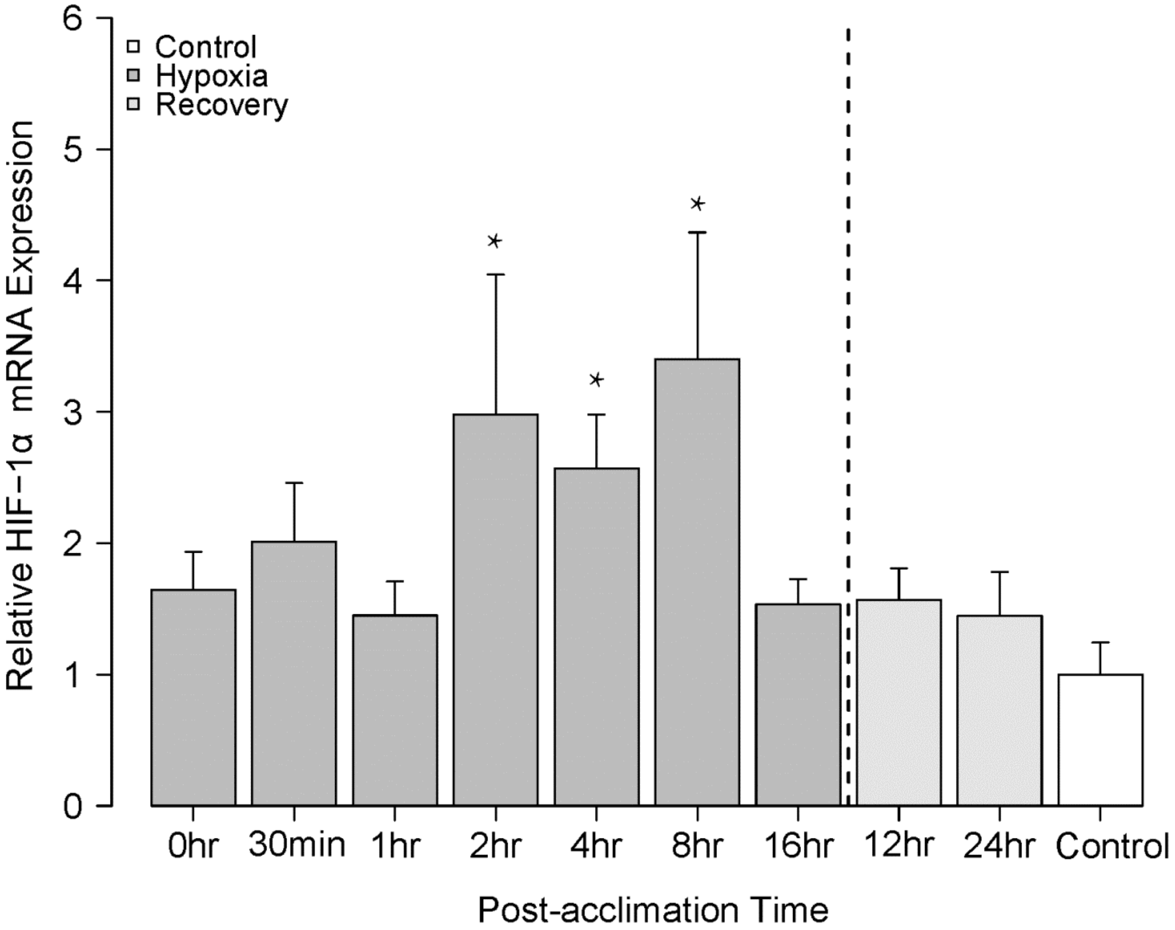

Figure 3.6 Relative mean (± SE) normalized HIF-1α gene expression of Pacific herring liver tissue from the time-course experiment. Four herring were sampled at a geometric time interval from 0 to 16 hours of hypoxia (mean DO ± SD = 2.31 ± 0.41 mg L^{-1}) exposure (*dark gray*). After 16hrs, oxygen conditions were returned to normal (6.60 ± 0.28 mg L^{-1}) and samples were taken 12hr and 24hrs post-hypoxia treatment (*light gray*). The experiment was replicated four times. 'Control', depicted in *white*, corresponds to the 16hr Threshold mean expression result. Expression levels are relative to the 'control'. Differences in mean mRNA levels between the normoxic treatment and other time-steps were tested by using a two-way ANOVA and post-hoc Tukey's test, *$p < 0.05$. All qPCR samples were run in duplicate.

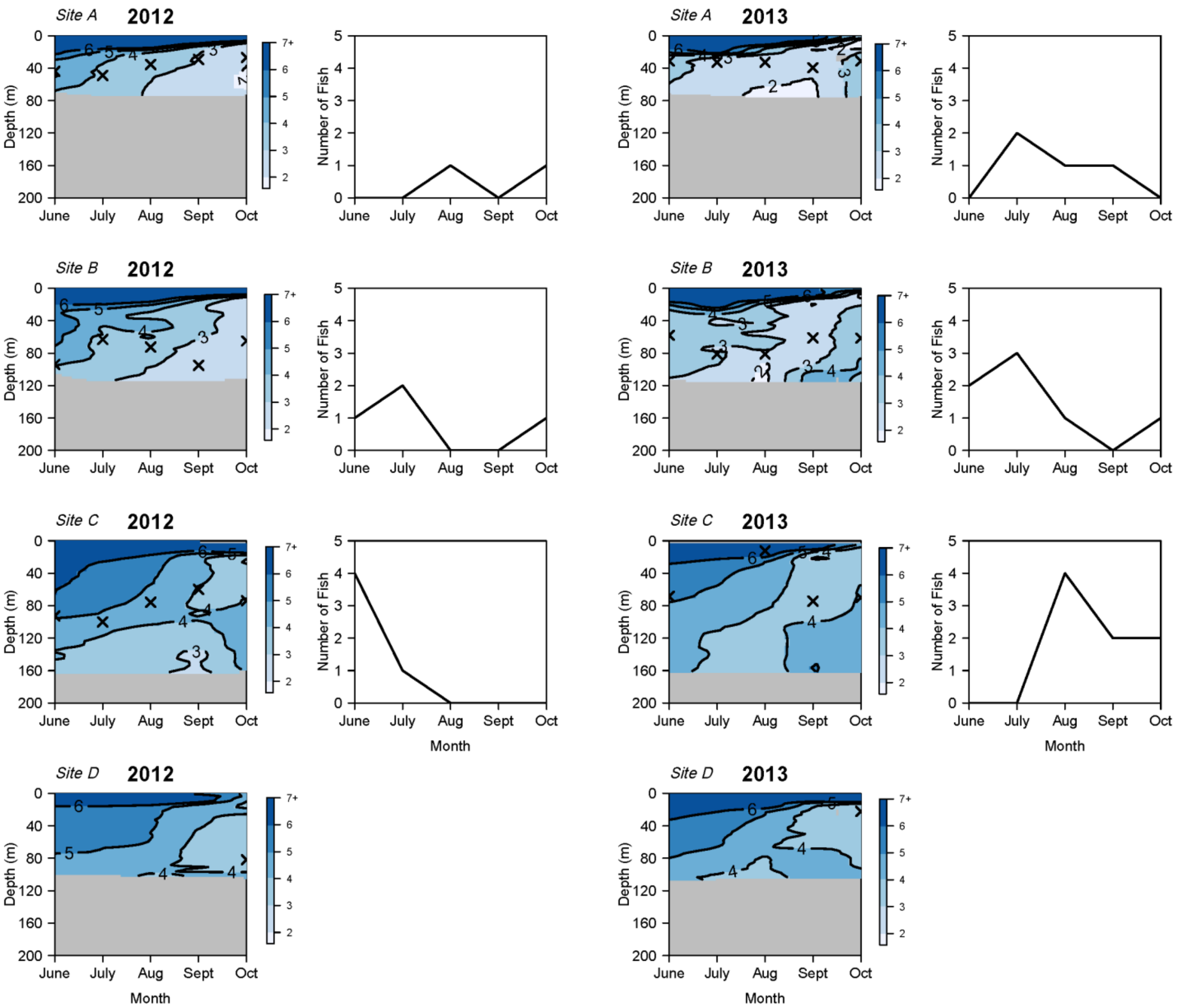

Figure 3.7 2012 and 2013 interpolated dissolved oxygen (mg L^{-1}) conditions and number of fish with significantly elevated HIF-1α gene expression over all sample periods (June-October) and sites (A, B, C, and D). Each DO graph represents a sampling site depicting the contour (*black lines*) and gradient (*color*) of DO as depth and time increase. The *black Xs* represent the mean trawl depth (m). The *gray* color represents the unsampled and/or maximum depths. Specifically, site C maximum depth is 200m, but was not measured. Interpolation was performed in R using the *interp* function, which applies a bilinear interpolation to the data. Number of fish exhibiting elevated mRNA expression (i.e., >1sd mean norm) is only shown for the three hypoxia prone sites, as site D (October) was the reference site.

Chapter 4

Investigating indirect hypoxia effects on Dungeness crab (*Metacarcinus magister*) harvest using management strategy evaluation[5]

Introduction

Estuaries are among the most productive and biologically rich systems on earth, providing essential habitat for numerous species and associated fisheries. However, these ecosystems often occur in close proximity to dense human populated areas and are thus under the threat of multiple human impacts (Halpern et al. 2008; McGranahan et al. 2007). In addition to exploitation, one of the primary threats is diminished dissolved oxygen (*i.e.,* hypoxia and anoxia), which is increasing in frequency, as well as spatial and temporal extent as a result of anthropogenic impacts, such as nutrient loading and climate change (Diaz and Rosenberg 2008). The exacerbation of low dissolved oxygen (DO) conditions has the potential to impact the productivity of local fisheries by altering the structure and biological interactions in aquatic ecosystems (Breitburg 2002; Breitburg et al. 2009; Diaz 2001). Hypoxia, traditionally defined as dissolved oxygen concentrations less than 2 mg L^{-1}, is now a common feature of estuaries and coasts worldwide (Diaz and Rosenberg 2008). Therefore, as oxygen conditions continue to deteriorate, understanding the direct (*e.g.*, mortality) and indirect consequences of hypoxia on exploited organisms is pertinent to the conservation and management of these ecosystems.

In general, local fisheries can be impacted by two main types of hypoxia related biological effects that can ultimately result in direct and indirect effects on target and non-target organisms. First, low oxygen conditions can cause mortality events, leading to immediate, and potentially persistent, decline and even collapse of harvested species (Diaz 2001; Rabalais et al. 2002). For example, hypoxia has a particularly high lethal impact on sessile organisms that lack

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[5] Full citation: Froehlich, H.E., T.E. Essington, P.S. McDonald. (in prep). Investigating indirect hypoxia effects on Dungeness crab (*Metacarcinus magister*) harvest using management strategy evaluation.

the capacity to seek out refuges (Breitburg 1992c; Diaz and Rosenberg 1995; Vaquer-Sunyer and Duarte 2008). Second, hypoxia can induce an avoidance behavioral response, typically documented as short-term reductions in densities of mobile fauna in hypoxia-impacted areas (Diaz and Rosenberg 1995; Eby et al. 2005; Petersen and Pihl 1995; Pihl et al. 1991). Although avoidance behavior reduces direct hypoxia exposure and mortality, distributional shifts can cause changes in ecological and fisher interactions with harvested species. For instance, avoidance can reduce catch in impacted areas (Mistiaen et al. 2003), cause fishers to increase effort (Craig 2012; Selberg et al. 2001), and potentially increase bycatch (incidental) morality (Arreguín-Sánchez 1996; Craig 2012; Hazen et al. 2009; Paloheimo and Dickie 1964; Winters and Wheeler 1985). However, there is limited understanding of how exploited populations respond to such indirect hypoxia impacts.

One of the most important recreational and commercial species in Washington State is the Dungeness crab (*Metacarcinus magister*) (Armstrong et al. 2010; Armstrong et al. 2003; Pauley et al. 1986). Dungeness crab range from Unalaska, Alaska to Santa Barbara, California (Jensen 1995) and are an abundant benthic species in the northwest (Pauley et al. 1986). In Puget Sound, crabbing regulations are divided among ten regions, including Hood Canal (110 km) – a seasonally hypoxic fjordic estuary (Figure 4.1). In 2013, over half-million pounds of Dungeness crab were harvested in Hood Canal alone (WDFW unpublished data). All regions follow the 3-S management strategy – size, sex, and season restrictions. Harvestable crabs must have a carapace width (CW) greater than or equal to 159 mm (ca. age 4) to help promote at least one successful mating event (mature around age 2), must be male, and harvest is closed during the peak of the spring moulting period (Armstrong et al. 2003). In Puget Sound, crabbing can occur during the summer and winter, with quotas and total summer catch dictating the length of the winter crabbing season. In addition, crab fishing is allocated evenly between the state and tribes. The primary forms of harvest in Hood Canal are recreational and tribal, with the latter encompassing commercial, ceremonial, and subsistence practices.

It is assumed that the life history traits of Dungeness crab – including high fecundity, low maximum age, early maturity and recruitment to the fishery, and high natural mortality – can sustain high levels of harvest (Kruse 1993), but there is growing concern by fishers and managers over the crabs harvested in Hood Canal. It has been estimated that upwards of 90% of legal-sized males can be harvested in one crabbing season (Armstrong et al. 2010; Gotshall 1978). While most of the regions' Dungeness crab landings have been relatively stable over the past decade, Hood Canal has exhibited less persistent trends with substantial declines between 2007 to 2010 (WDFW unpublished data). In 2010, a Washington State audit of the fishery listed the open-access recreational fishery – particularly illegal crabbing – and hypoxia as major risk factors to the crab species (Sonntag 2010). As a result, there is a current need to better understand the possible implications of the fishery and hypoxia on Dungeness crab persistence.

While Hood Canal can experience fish-kills associated with hypoxic events (Fagergren et al. 2004), recent findings appear to describe more common large scale hypoxia avoidance by several local species, including Dungeness crab. A study by Froehlich et al. (2014) found supporting evidence of tagged Dungeness crab remaining in the hypoxic region, but shoaling into shallower, less hypoxic habitat as the lower DO expanded vertically through the water column. Both of these studies suggest hypoxia is displacing Dungeness crab from the deeper reaches of the estuary during the summer months, coinciding with the height of crabbing season in the area. Thus, Dungeness crab may experience increased vulnerability to crabbing, of which the potential consequences on the fishery and crab population are not well understood.

Although previous research has shown hypoxia appears to influence the distribution of Dungeness crab, the sensitivity of the management strategy to such indirect effects likely depends on other biologic (e.g. reproductive impediment) and anthropogenic stressors (e.g., illegal crabbing). Currently, the entire estuary is closed to harvest only after severe hypoxic events (i.e., fish-kills). Although hypoxic conditions have not been directly linked to a declining crab population in Hood Canal (Newton 2008), the hypoxia-driven compression of Dungeness crab into shallow, oxygenated waters provides evidence of increased overlap of crabs with shallow (≤ 30 m) fishing gear (Froehlich et al. 2014). Indeed, studies have suggested regional fishery declines of estuarine Dungeness crab could result from the loss of critical habitat (Holsman et al. 2006) and potential overfishing of shoaling crabs (Selberg et al. 2001). In addition, an investigative model of the Fraser delta Dungeness crab population found (incidental) handling mortality can negatively affect maximum yield as exploitation rate increases (Zhang et al. 2004). While the dynamic nature of population variability and the multiple sources of that variation (*e.g.*, climate, predation, habitat quality) don't easily permit a direct approximation of hypoxic impact, modelling various hypoxia scenarios on crabbing and population abundance might provide some insight into the role of hypoxia in Hood Canal fisheries.

An effective modelling approach to assess impacts of catch control rules on harvested species is through a Management Strategy Evaluation (MSE). MSE is a model-based method that assesses the consequences of specific management strategies under scenarios and objectives of interest to elucidate, and ultimately communicate, key tradeoffs for a natural resource (Smith 1994; Smith et al. 1999). More specifically, MSE relies on simulation testing of management goals using performance metrics derived from an operating model (Sainsbury et al. 2000). This approach provides an avenue to 'test' the effectiveness of a policy by projecting the effects into the future, but avoids the obvious risk of actively adjusting actual policies. Moreover, we can investigate the possible effects of perturbations, such as hypoxia, and how robust the policy measures are to these perturbations (Walters 1986). A MSE approach has been used to assess numerous fisheries at the single species, multispecies, and ecosystem level (Butterworth and Punt 1999; Sainsbury et al. 2000). One key advantage of MSE is the method tries to capture the range of uncertainty and requires management to specify objectives and acknowledge necessary trade-offs; this can, and should, involve stakeholders (Butterworth and Punt 1999).

Although not spatially explicit, the intent of this simulation study was to explore the generalized relationship between hypoxia, Dungeness crab abundance, and harvest in the context of specific management interests and goals in Hood Canal, WA. Using a simplified age-structured population model, we investigate how hypoxia, crab and fisher behavior, and crabbing mortality interact and vary the catch and viability of the crab population. Although the operating model will represent a basic form of the underlying forces influencing Dungeness crab in the estuary, it can prove useful in making inferences about hypoxia and the efficacy of management harvest policies in Hood Canal and potentially other hypoxia prone estuaries (e.g., Chesapeake Bay) and coastal systems (e.g., California current). We hypothesize that while legal males are already heavily exploited every season, hypoxia increasing catchability may prove detrimental to the long-term management of the fishery when considering other demographic impediments, such reproductive size limitations, illegal crabbing, and incidental capture mortality. We are particularly interested in comparing the impact of indirect hypoxia effects on Dungeness crab under current (low) and potential future (high) crabbing effort scenarios.

Methods

An operating model was constructed to describe possible effects of hypoxia and harvest on Dungeness crab in Hood Canal and the associated consequences for the population under different scenarios. We first discuss identifying key co-managers' objectives and interests. Next, we describe the deterministic operating model and investigating steady-state characteristics under changes in key parameters of reproduction, illegal crab fishing, and incidental mortality. Finally, we present simulated time-course results when a catch control rule and stochastic processes are incorporated into the model under a set of hypoxia and crabbing scenarios. Figure 4.2 provides a conceptual framework of the model and how hypoxia is incorporated.

Co-managers

While developing an investigative operating model was a key element of this study, establishing the primary management objectives and concerns was the first critical step in effectively creating and testing a meaningful model. From the inception of this study Washington state and tribal co-managers were explicitly involved to establish important policy expectations, processes of interest, and key trade-offs that ultimately dictated the components of the operating model. The input from the co-managers gave a clearer conceptual understanding of the model content and assumptions. For instance, irrespective of hypoxia, exploring aspects of illegal catch and incidental mortality were an important consideration. Recruitment variation and reproductive impediments to the population were also of interest.

In addition to the expert opinions of the co-managers, we were also given access to Hood Canal landings records. The catch records spanned from 1996 to 2013 and contained the total catch of all tribal and recreational sources. The catch data were used to explore trends, determine possible reference metrics, and confirm values of our catch model outputs.

Deterministic Operating Model

The Dungeness crab population and harvest in Hood Canal were modelled using an age-structured model. Similar modelling approaches have been implemented for Dungeness crab previously (Botsford and Wickham 1978; Higgins et al. 1997; Toft et al. 2013; Zhang and Dunham 2013). The crab population (males and females) and catch were modelled at a weekly time-step with an age structure containing 10 discrete year classes (Armstrong et al. 2010; Pauley et al. 1986):

$$N_{s,a+1,t+1} = N_{s,a,t} e^{-(M_{s,a}+F_{s,r}+F_{s,i})} \qquad \text{Eq. 1}$$

$$C_t = \frac{N_{m,a,t} F_{m,r}}{M_{m,a}+F_{m,r}+F_{m,i}} \left(1 - e^{-(M_{m,a}+F_{m,r}+F_{m,i})}\right) \qquad \text{Eq.2}$$

where $N_{s,a,t}$, the number of male ($s = m$) or female ($s = f$) crabs at age a and time t, is equal to the abundance of crabs of age a from the previous time-step relative to the instantaneous weekly survival. Survival was modelled as a function of natural mortality ($M_{s,a}$) at each age for both sexes, crab fishing mortality due to retention ($F_{s,r}$), and incidental crab fishing mortality ($F_{s,i}$), e.g., handling mortality. By separating the fishing mortalities, we were able to explore the population implications of legal crabbing and accidental deaths due to capture. Similarly, legal (and illegal) catch (C_t) each week was calculated based on the sum of the same age specific mortalities.

The crab fishing mortality functions were based on three parameters:

$$F_{s,r} = u_{s,a} \Omega_{s,a} \qquad \text{Eq. 3}$$

$$F_{s,i} = u_{s,a}(1 - \Omega_{s,a})\delta_a \qquad \text{Eq. 4}$$

where, , $u_{s,a}$ represents the probability of a particular sex (s) being captured at age a time t, $\Omega_{s,a}$ the probability of a male or female being retained, and δ_a is the probability of incidental death for crabs that are not retained. The $u_{s,a}$ parameter was calculated as the product of total weekly crabbing effort (tribal and recreational no. pots) and catchability of either sex ($q_{s,a,t}$). We considered several scenarios for how catchability varies seasonally. First, we considered constant catchability, representing no hypoxia effects. Second, we incorporated eight possible hypoxia intrusion scenarios, represented as non-linear changes in catchability. More specifically, we used weekly hypoxic (DO < 2 mg L^{-1}) depth profiles spanning 2006 to 2013 provided by the Hood Canal Dissolved Oxygen Program (HCDOP) as possible alternative seasonal hypoxia conditions. Catchability was then calculated as scaled, log-transformed values of the minimum hypoxic depth over the crabbing seasons (Figure 4.3). Lastly, we accounted for effects of sexual dimorphism and crab pot escape-rings (diameter = 108 mm) by assigning a catchability of zero to males younger than 3 years and females younger than 4 years, while all other age classes were assumed equally catchable (Table 1).

The probability of retention ($\Omega_{s,a,t}$) after being captured could also be held constant or treated as a function of weekly catch rate of legal males (i.e., functional retention):

$$\Omega_{s,a,t} = \Omega_{max} e^{-c\left(\frac{C_t}{E_t}\right)} \qquad \text{Eq. 5}$$

with a maximum probability of capture retention (Ω_{max}) depending on exponentiated, scaled (c) weekly catch rate; thus, as availability of legal crabs declines – due to a reduced population and/or increases in effort – the probability of illegal crabbing increases. This relationship was introduced based on feedback we received from shellfish co-managers indicating that illegal take was inversely related to availability of legal-sized males. While the probability of retention for legal males ($a \geq 4$) was assumed 1, the above relationship was applied to sub-legal catchable males ($a = 3$). Although females appear to be rarely taken (WDFW unpublished data), we also explored the illegal take of older females ($a \geq 4$). Lastly, the incidental mortality parameter (δ_a) was held constant and only applied to catchable ages of both sexes. Increases in δ_a encompasses higher levels of incidental mortality from soft-shell captures, extended air exposure, prolonged soak times, and multiple capture events (Barry 1981; Kruse et al. 1994; Reilly 1983; Van Tamelen 2005; Yochum and Sampson 2015). Additionally, crabbing is assumed to happen prior to recruitment, so that our model year begins in June and ends in May of the following calendar year.

Recruitment ($a = 1$) was calculated based on a Beverton-Holt stock-recruit relationship. Males and females were modelled separately, and recruitment was based on egg production, male abundance, and both density-independent (α) and density-dependent (β) mortality. We assumed only males of age $a+1$ or greater could successfully mate with age a females (Butler 1960; Hankin et al. 1997; Smith and Jamieson 1991). We could then adjust the dependence of egg production (g_t) on male density through a single parameter, the effective reproductive ratio ($\Phi_{a,t}$), which allowed us to consider the sensitivity of management regimes to potential over-depletion of males that hampers reproductive output:

$$g_t = \sum_{i=a}^{n} N_{f,a,t} \text{Ƒ}_a \, \partial_a \, \Phi_{a,t} \qquad \text{Eq.6}$$

$$\Phi_{a,t} = \frac{\rho}{\eta + \rho} \qquad \text{Eq.7}$$

$$\rho = \frac{\sum N_{m,a+1,t}}{N_{f,a,t}} \qquad \text{Eq. 8}$$

with $N_{f,a,t}$ as the number of age a females at time t, $Ƒ_a$ as the fecundity of females at age a, ∂_a as the proportion of mature females at age a. The effective reproductive ratio ($\Phi_{a,t}$) is an asymptotic relationship that assumes a value between 0 and 1. The relationship depends on ρ, the ratio of all age $a+i$ males to age a females at time t, and a half-saturation constant (η). Crabs mate in a female post-moult embrace, that some suggest require the male to be larger than the soft-shell female (Butler 1960; Hankin et al. 1997; Smith and Jamieson 1991), which we simplify by using age as a proxy for size. Egg production was then related to steepness (h), instead of more detailed mortality inputs, due to the lack of data for each larval stage. We also assumed a small proportion (20%) of the estuarine crabs mature at age 2 and all individuals mature by age 3 (Armstrong et al. 2003; Butler 1960; Butler 1961a; Jensen and Asplen 1998; Tasto 1983; Wainwright and Armstrong 1993). Additionally, younger ($a < 3$) and older ($a > 5$) female crabs were assumed to exhibited reduced egg production (Botsford and Wickham 1978; Hankin et al. 1997). Older individuals experience reduced fecundity and have been omitted from other Dungeness crab models (Botsford and Wickham 1978; Higgins et al. 1997; Toft et al. 2013), but we included older crabs to explore a more complete representation of illegal crab fishing – particularly on older females. However, inclusion of $\Phi_{a,t}$ reduced the reproductive contribution of older females', making the population effects more similar to models with fewer age classes. All model parameterization and assumptions were based on primary literature, personal correspondence with co-managers, and sensitivity testing of the model (Table 1).

Equilibrium sensitivity analysis

Sensitivity testing of the deterministic model was conducted to explore the general relationships and patterns of the modelled Dungeness crab population and reveal important interactive effects of hypoxia with other processes on steady state yield. For ease of comparison we investigated catch versus fishing effort with combinations of parameters; specific attention was paid to changes in maximum catch and effect of increasing effort (no. pots) as a representation of recreational expansion, the open-access form of the fishery. Under constant catchability, we separately evaluated the consequences of constant and functional illegal retention ($\Omega_{s,a,t}$) and incidental mortality (δ_a). Similarly, the sensitivity of the model to the same changing conditions ($\Delta\Omega_{s,a,t}$, $\Delta\delta_a$) was assessed with non-linear, catchability over time, representing hypoxia effects (Figure 4.3). All sensitivity tests were conducted using high (h = 0.8), moderate (0.6), and low (0.5) steepness levels with and without an effective reproductive ratio ($\Phi_{a,t}$). One hypoxia intrusion scenario (2010) was used for all sensitivity comparisons.

Stochastic Model Scenarios

Harvest control and stochasticity were added to the model to evaluate temporal trends as they relate to hypoxia-catchability effects with incidental mortality and illegal crabbing. As is done in management of Puget Sound Dungeness crab, catch was controlled by a three year running average quota. More specifically, the catch quota – calculated as the 3 year running average of catch – prevents winter season crabbing if the quota was met or surpassed during summer. Seasonal crabbing effort (no. pots) was modelled to occur over a 20 week period, where commercial tribal and state recreational crabbing both occur during the ‘summer’ and may or may not happen during the ‘winter’ season based on the quota. As is the case in Hood Canal, recreational crabbing in the model was allowed to occur through the season(s), while commercial crabbing was restricted to five weeks per season. Stochasticity was incorporated via recruitment, representing environmental drivers on crab early life history survivorship. We assumed recruitment variability was corrected lognormal. To reduce the number of meaningful scenarios, values of σ (0.8) and h (0.6) that produced the most similar catch patterns seen in actual catch data were used for detailed comparisons. The eight possible hypoxia intrusion scenarios were randomly selected for each year for every model run.

Using low (maximum pots week^{-1} = 7,000) and high (20,000) levels of crabbing effort, we compared time-series projections of 50 years with some level of incidental mortality and illegal take under scenarios with and without hypoxia effects. As a conservative approach we included an effective reproductive ratio for all scenarios. While the sensitivity testing of equilibrium conditions showed the consequences of extreme increases in illegal catch and incidental mortality, the actual levels are unknown. As a result, levels were informed by primarily literature, surveys, and patrol records (Table 1); specifically, the average contribution (%) of total capture (i.e., combined legal catch, illegally retained, and incidental deaths) reflected documented levels. We also assumed illegal take was best represented as the functional illegal take relationship ($\Omega_{max} = 0.02$) and incidental mortality was assumed constant ($\delta_a = 0.005$) for sub-legal males ($a = 3$) and females ($a > 3$).

Five metrics were used to compare across the different hypoxia and crabbing scenarios. We specifically assessed changes in the level of population variability (CV_N), catch variability (CV_C), the respective proportion of catches less than 50% ($0.50\overline{C}$) of average yearly catch, the number of times catch declined by more than 70,000 crabs from the previous year ($\Delta C_t > 70k$), and overall minimum catch. Currently there are no formal metrics used to monitor the Puget Sound Dungeness crab fishery, so these metrics were developed based on the actual catch data patterns and input from co-managers. As a qualitative measure of model performance, the simulated metric values were compared to the same metrics from the Hood Canal catch data.

Results

Equilibrium catch vs effort relationships

When only accounting for natural mortality and legal crabbing, effort never limits the landings under all three recruitment levels – a current assumption of management practices that is not upheld under demographic restrictions, such as reproductive size limitations. Including an effective reproductive ratio into the model impacted maximum catch and the response of the population to higher levels of crabbing effort (Figure 4.4). Specifically, the biological restriction of females requiring older, assumed larger, males for successful reproduction causes notable decreases in catch relative to steepness, with the lowest catch returns under lower compensation (h=0.5; Figure 4.4).

Similar to the impacts of the effective reproductive ratio, inclusion of illegal crabbing or incidental mortality reduces maximum catch and the amount of effort needed to overfish the population (Figure 4.4). A high probability (0.8) of constant illegal crabbing of sub-legal males reduced maximum legal catch by more than half and magnified the impact of increasing effort. Conversely, incorporation of a functional response for illegal catch – driven by legal catch rate – buffers the effect of illegal retention at lower levels of effort, with a more threshold-like at high effort levels. However, low steepness paired with the effective reproductive ratio in both the constant and functional cases causes a more pronounced negative effect on returns (Figure 4.4). The addition of female illegal take did not result in significant changes to catch patterns. Incorporation of incidental mortality (without illegal retention) results in slightly stronger effects than illegal crabbing, but with similar patterns (Figure 4.4). Again, a lower steepness and an effective reproductive ratio strengthen the impact of the additional source of mortality (Figure 4.4).

Inclusion of non-linear catchability ($q_{s,a,t}$), representing non-lethal hypoxic influence in this model, increases catch at lower efforts and the sensitivity of the population to overfishing. Initially, increases in catchability of crabs benefits the fishery, escalating the possible maximum catch (Figure 4.5). However, growth of the recreational effort more quickly induces the population to become overfished (Figure 4.5). Again, inclusion of an effective reproductive ratio and lower steepness inputs results in more pronounced negative impacts on catch and population abundance.

Stochastic time-series

The combination of random hypoxia effects through changes in catchability and increasing levels of fishing effort reduced the average crab population and catch, and increased the number of incidental and illegal mortalities. Crab abundance declined with increases in effort and hypoxia-effects (i.e., non-linear catchabilities), with hypoxia compounding the negative impacts of fishing (Figure 4.6). Catch also declined, but only under the scenario that included hypoxia with high effort (20,000 pots week^{-1}). Conversely, hypoxia-induced increases in catchability resulted in a 2 to 3 fold increase of incidental deaths and illegally retained crabs (sub-legal and female) compared to the same effort levels with constant catchability (Figure 4.6).

Evaluation of the five metrics under hypoxic scenarios revealed increases in variability, greater number of lower catch occurrences, but fewer catch extremes. Population variability increased with effort and hypoxia-effects, with the largest CV (0.58) resulting from high effort and non-linear catchability – ca. 1.4 times greater than the low effort, no-hypoxia scenario (Figure 4.7). Increases in catch variability showed a similar, but more pronounced pattern, with a maximum CV difference of 0.42 between opposing scenarios (Figure 4.7). Similarly, $0.50\overline{C}$ also increased, but was relativity low. The magnitude of catch extremes (> 70k) increased under low effort with hypoxia and high effort without hypoxia, but declined with the most severe scenario; this suggests although variability increased, the reduction in the overall population abundance restricts the scale of change in catch over time.

Assuming the current conditions in Hood Canal are hypoxic with low crabbing effort (WDFW unpublished data), comparison of the simulated metric values to those of the actual catch records provides some support for the model and associated parameter values. The CV_C based on the catch records (0.38) falls between the simulated low effort metrics with and without hypoxia. The operating model also produced values of $0.50\overline{C}$ that were similar to those derived from the data. However, our model consistently underestimated the extent of change in catch ($\Delta C_t > 70k$) and overestimated minimum catch at low effort with hypoxia-induced catchability. As there are no population abundance estimates for Dungeness crab in Hood Canal, the CV_N values could not be compared.

Discussion

Although mortality is an important component of hypoxia effects on marine ecosystems, our MSE of Hood Canal Dungeness crab shows that hypoxia avoidance has the potential to cause substantial indirect impacts on catch, population abundance, and variability of exploited species. We found the 3-S management strategy most sensitive to the influence of hypoxia when other sources of demographic restrictions were considered. For example, addition of an effective reproductive ratio limiting the contribution of older females' input into the population decreased catch and the amount of effort necessary to overfish the population. Similarly, illegal crab retention and incidental mortalities from crabbing both reduced long-term catch levels, population abundance, and increased variability; outcomes magnified by the non-linear hypoxia dynamics. While our study is a general exploration of the possible population dynamics and harvest repercussions of Dungeness crab in a hypoxic environment, a simulation approach such as this underscores areas of concerns, gaps in the understanding – both in the ecology and management – and need for data to reduce these uncertainties.

Accounting for changes in catchability as an artifact of hypoxic, nearshore intrusion resulted in declines in abundance and increases in variability of the population and catch, particularly at higher levels of crabbing effort. Hypoxic shoaling has been reported off coastal systems (Chan et al. 2008; Craig 2012; Grantham et al. 2004) and other hypoxic estuaries (Craig 2012; Mistiaen et al. 2003; Pihl et al. 1991; Selberg et al. 2001), but how that translates to the fisheries in not well understood. Aggregations of species can cause artificially high catches, which can potentially lead to undetectable reduction in biomass and sudden collapse (Craig 2012). Increases in variability through environmental forcing and/or fishing can also impact the resilience of marine populations, reducing robustness to perturbations and increasing the uncertainty of recovery in light of a collapse (Anderson et al. 2008; Hsieh et al. 2006; Hutchings 2001; Kuparinen et al. 2014; Shelton and Mangel 2011). The results from this simulation convey that there is a limit to the number of pots and potential for overfishing when considering environmental heterogeneity and impacts of crabbing beyond legal catches.

Inclusion of biologically meaningful reproductive attributes of the population has important repercussions for the sustainability of the crab fishery. The incorporation of an effective reproductive ratio showed how the Dungeness crab population could be more sensitive to weaker recruitment events, additional sources of mortality (e.g., illegal crabbing), and/or environmental perturbations, such as hypoxia (Figure 4.4). Evidence in the primary literature and through personal correspondence supported the incorporation of a reproductive size-hindrance due to the pre-moulting embrace of females that needs to occur prior to copulation (Butler 1960; Butler 1961a; Hankin et al. 1997; Smith and Jamieson 1991). Conversely, others suggests that there is such a small percentage of large females (>155 mm) that it doesn't impact the population (Hankin et al. 1997) and that large females can successfully mate (Dunn and Shanks 2012). In fact, there is some support for the latter in Hood Canal where large females have been found with sperm plugs (Point No Point Treaty unpublished data), an indication of a successful mating event (Oh and Hankin 2004). Due to the uncertainty surrounding the reproductive limitations of Dungeness crab, we chose to explore the potential sensitivity of catch and the population to this biological impediment. Yet, it is also important to note that our reproductive function was an age-based proxy and could be explored in more detail with a size-based model.

We also found illegal take can jeopardize catch levels and the population's ability to withstand increases in crabbing, but may be buffered by fishers' reluctance to retain illegal crabs when catch rates of legal crabs are high. Certainly, illegal fishing can contribute to the overexploitation of harvest species and threaten the sustainability of fisheries (Agnew et al. 2009); this is particularly concerning if such practices scale with effort, as it did in our model (Figure 4.6). Based on our simulations, take of just the catchable sub-legal males can have negative repercussions. However, equivalent levels of female illegal retention, in addition to sub-legal take, did not result in significant changes to catch patterns. This suggests that much of the population is dependent upon the younger age classes ($a < 4$) reproducing, which do account for the majority of the population abundance. That said, we are not advocating for retaining females, as they may act as some level of buffer to the population during weaker recruitment events (Chesson 1984; Longhurst 2002; Warner and Chesson 1985), particularly in the face of climate change. Additionally, incorporation of functional illegal retention based on legal catch rates appeared to help mitigate some of negative consequences at lower crabbing effort, but did result in a more sudden potential for collapse with an expanding fishery (i.e., more crab pots). In Hood Canal, illegal crabbing can take the form of harvesting sub-legal and female crabs, crabbing on no-take days/seasons, and/or exceeding the total allowable catch of legal crabs. Our model only accounted for illegal female and sub-legal retention. It's unclear how pervasive illegal take is within the area as the capacity to enforce the 3-S management strategy is extremely challenging at the recreational level.

Although inclusion of incidental capture mortality into the model reduced maximum yield and increased sensitivity of the population to crabbing effort, there is uncertainty concerning the magnitude of the additional mortality. Incidental mortality in our model once again scaled with effort and very generally encompassed any mortality due to handling injury, extent of air exposure, multiple capture events, and prolonged soak times (Barber and Cobb 2007; Barry 1981; Kruse 1993; Murphy and Kruse 1995; Van Tamelen 2005; Yochum and Sampson 2015). The contribution of the different forms of incidental death are unknown and likely differ between recreational and commercial practices (Yochum and Sampson 2015). In addition, growth, as it relates to moulting increments and timing, is inherently linked to the probability of incidental mortality. Zhang et al. (2004) used complex length-based modelling and measurements to evaluate the effects of Dungeness crab moulting related mortality – natural and handling – on yield in the Fraser Delta, Canada. Similar to our results, the authors found that even low levels of handling mortality can reduce long-term yield as effort increases. That said, more detailed information regarding moulting and mortality rates in Hood Canal would provide more accurate estimates for modelling future scenarios.

Our model was designed to explore the generalized relationship of hypoxia and Dungeness crab harvest in Hood Canal and thus made several simplifying assumptions. One key parameter that dictates the relative influence of the other parameters in the model is catchability, $q_{s,a,t}$. We selected a range for q that corresponded to catch levels reported in the primary literature and management reports (Table 1). However, a less uncertain estimate requires a formal stock assessment, which is not performed for any Dungeness crab fishery. In addition, we assumed that natural mortality, which encompasses predation, did not vary with changing catchability. We did include some level of age-specific mortality (i.e., younger age classes experience higher mortality), but hypoxia induced mortality of younger life-stages, specifically megalopae, was not directly modelled. Peak megalopae recruitment occurs between June and July in Hood Canal (Fisher and Velasquez 2008) when summer hypoxia could be a mortality factor, as reported for other invertebrate larvae (Eerkes-Medrano et al. 2013; Vaquer-Sunyer and Duarte 2008); this is particularly pertinent for inland Washington megalopae, which appear to aggregate in deeper (ca. 160m) waters than their coastal counterparts (ca. 25m) (Jamieson and Phillips 1993). Our model also lacked a spatial context. Indeed, the northern region of Hood Canal is less susceptible to severe hypoxia intrusion and could reduce the impact of the indirect hypoxia effects investigated in this simulation study (Froehlich et al. 2014; Froehlich et al. 2015). Spatially we also assumed Hood Canal Dungeness crab is a closed population. While adult crabs appear to exhibit restricted movement (Froehlich et al. 2014) and the population may rely on self-recruitment (Dinnel et al. 1993), more genetically-based information is needed. Aspects such as these could be explored in more detail, but the intent of this study was to demonstrate overall harvest patterns and identify key gaps in the understanding of the Hood Canal Dungeness crab.

Using an MSE approach, this study offers new insight into possible interactions of the non-lethal effects of hypoxia and harvest of an estuarine macroinvertebrate. Models are essential tools for evaluating fishing and environmental impacts of data-poor species, like the Hood Canal Dungeness crab. Critical to the development of the operating model and scenarios of interest was the collaboration with Washington state and tribal co-managers. Using the managers' expertise, we incorporated model components that helped us to explore multiple aspects of the fishery relative to hypoxia influence, including an effective reproductive ratio, illegal retention, and incidental mortality. While reducing the reproductive input and adding other sources of mortality reduced catch, increased variability, and made the population more susceptible to overfishing – as is the case for many fisheries – including the hypoxia impacted, non-linear catchability exacerbated these negative consequences. Thus, non-lethal impacts of environmental forcing may make the population more sensitive to an expanding fishery. This is a critical consideration in that many estuaries and coastal ecosystems are facing more severe and frequent hypoxic events due to anthropogenic pressures, such as climate change (Diaz and Rosenberg 2008). With this in mind, although the 3-S management strategy appears effective, models such as ours highlight the uncertainty and potential threats to harvested organisms, particularly data-poor species.

Table 4.1 List of model parameters, range of values, and associated literature or personal correspondence sources.

Parameter	Value	Source
M_a = mortality at age *a*	Age 1-2 (juv.) = 0.8 yr^{-1} Age ≥ 3 (adults) = 0.3 yr^{-1}	(Butler and Hankin 1992; Higgins et al. 1997; Zhang et al. 2004)
$Ƒ_a$ = fecundity at age *a*	Age 1 = 0 Age 2 = 1 million Age 3-5 = 2 million Age ≥ 6 = 1 million	(Botsford and Wickham 1978; Higgins et al. 1997; Rasmuson 2013; Toft et al. 2013)
∂_a = proportion of age *a* mature females	Age 1 = 0 Age 2 = 0.2 Age ≥ 3 = 1	No direct estimates of proportions mature; (Armstrong et al. 2003; Butler 1960; Butler 1961a; Jensen and Asplen 1998; Tasto 1983; Wainwright and Armstrong 1993)
$\Phi_{a,t}$ = Effective reproductive ratio	0-1	(Butler 1960; Butler 1961a; Hankin et al. 1997; Smith and Jamieson 1991); Jensen correspondence
η = half-saturation constant for 'effective reproductive ratio'	0.1	
h = steepness	0.5-0.8	(Zheng and Kruse 2003; Zheng et al. 1995)
EPR = eggs-per-recruit	630,222 eggs	Calculated separately based on natural mortalities, fecundities, and proportion mature
Ro = unfished number of age 1 recruits	7,000,000 crabs	Sensitivity testing; (Zheng and Kruse 2003; Zheng et al. 1995)
$\Omega_{s,a,t}$ = probability of capture retention at age *a*	Males Age 1-2= 0 Age 3 = max 0.02 Age ≥ 4 = 1 Female Age 1-3 = 0 Age ≥ 4 =max 0.02	Co-managers unpublished data and personal correspondence; scales with legal catch rate
δ_a = probability of incidental death at age *a*	Age 1-2 = 0 Age 3-10 = 0.005	(Kruse et al. 1994; Reilly 1983; Tegelberg 1970; Yochum and Sampson 2015); Correspondence with co-managers
$q_{s,a,t}$ = catchability at age *a*, time *t*	Male Age 1-2 = 0 Age 3-10 = 0.0003-0.0074 Female Age 1-3 = 0 Age 4-10 = 0.0003-0.0074	Toft et al. (2013); co-managers correspondence
E_{rec} = state recreational effort	3000;16000 pots $week^{-1}$	Co-managers unpublished data and personal correspondence
E_{com} = tribal commercial effort	4000 pots $week^{-1}$	Co-managers unpublished data

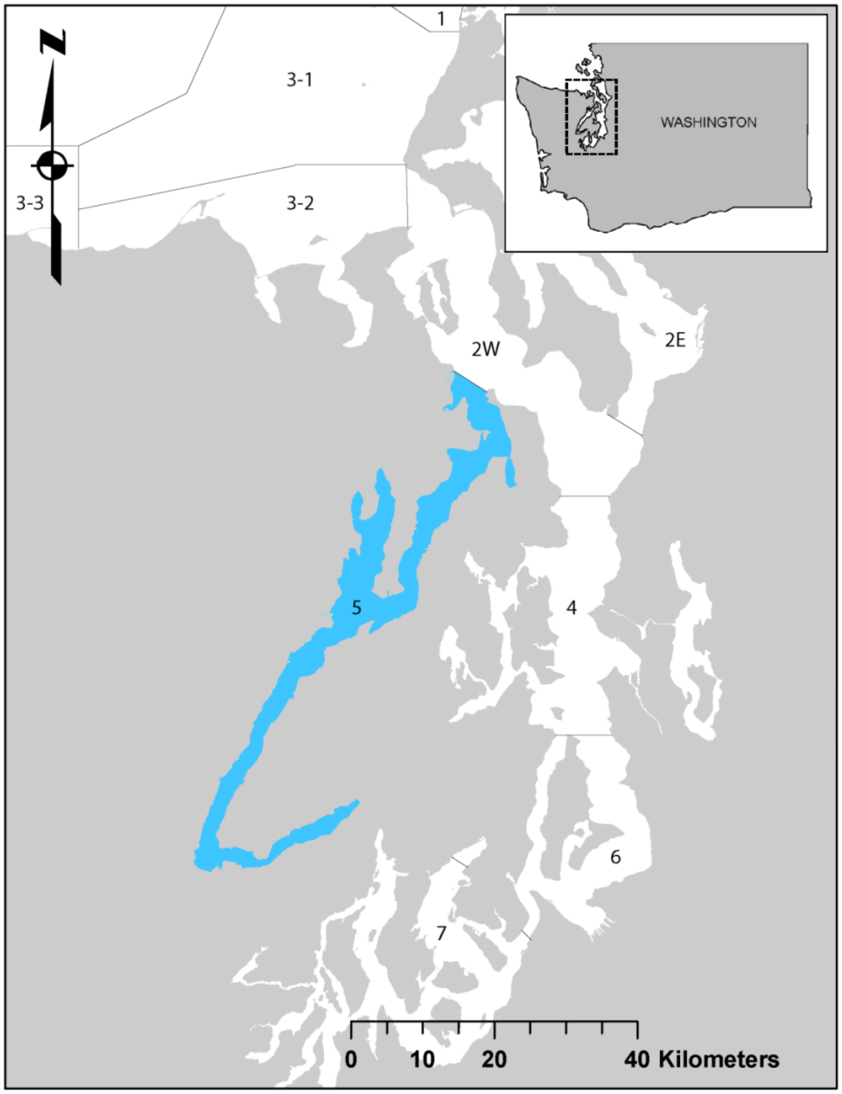

Figure 4.1 Harvest regions of Puget Sound, Washington, U.S.A. Hood Canal (Region 5) highlighted in *blue*. Land is depicted in *gray*.

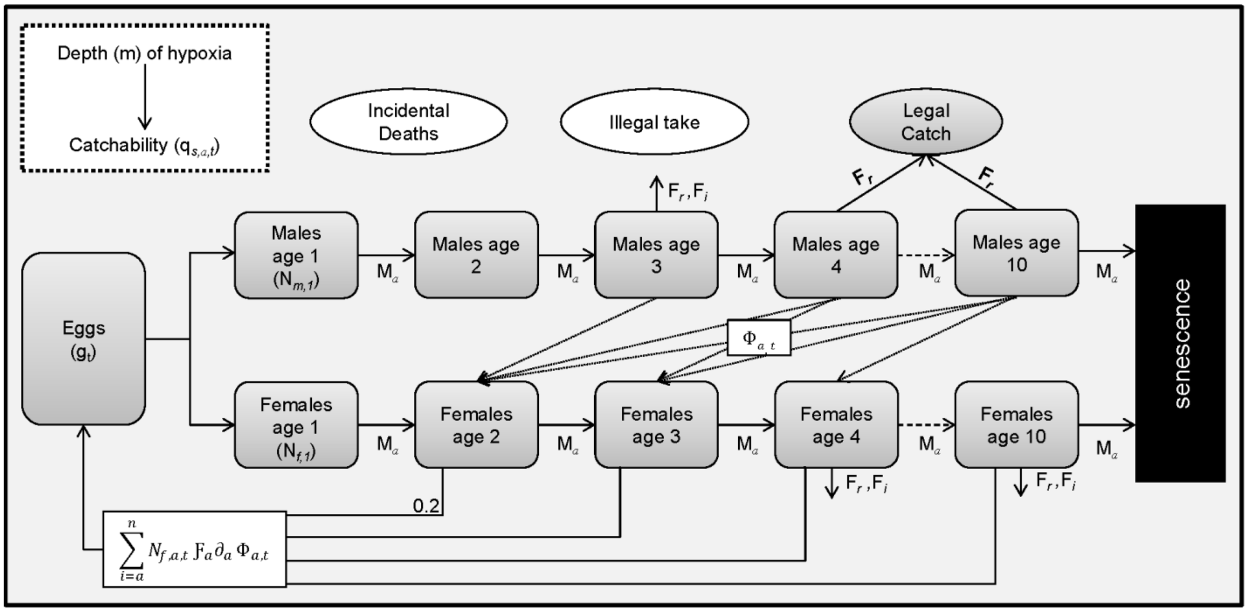

Figure 4.2 Framework of the deterministic age-structured operating model including three sources of mortality (natural M_a, fishing (retention) F_r, and incidental mortality F_i), an effective reproductive ratio ($\Phi_{a,t}$), and catchability of both sexes ($q_{s,a,t}$) as the factor changing due to hypoxia expansion (higher q) and contraction (lower q) - not explicitly modelled (*dotted-line box*). Egg production (g_t) is a function of the number of mature (∂_a; 20% age 2 mature) females ($N_{f,a,t}$) in the population, fecundity ($Ƒ_a$), and, if induced, the effective reproductive ratio.

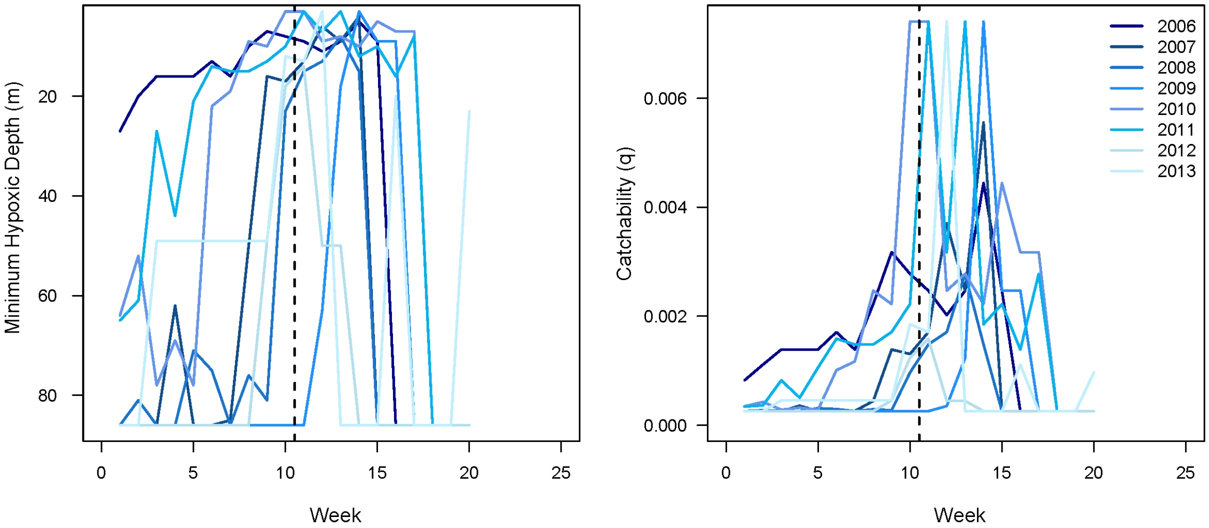

Figure 4.3 (*left panel*) Eight years (2006-2013) of minimum hypoxic (DO ≤ 2 mg L^{-1}) depth (m) (ORCA: http://orca.ocean.washington.edu/) and associated (*right panel*) non-linear catchabilities (q), calculated as adjusted, log-transformed values of hypoxic depths (m). ‘Summer’ crabbing occurred the first 10 weeks, while ‘Winter’ crabbing (if the quota was not met or exceeded) occurred in the latter 10 weeks. The *dashed line* depicts the differentiation between crabbing ‘seasons’ in the model.

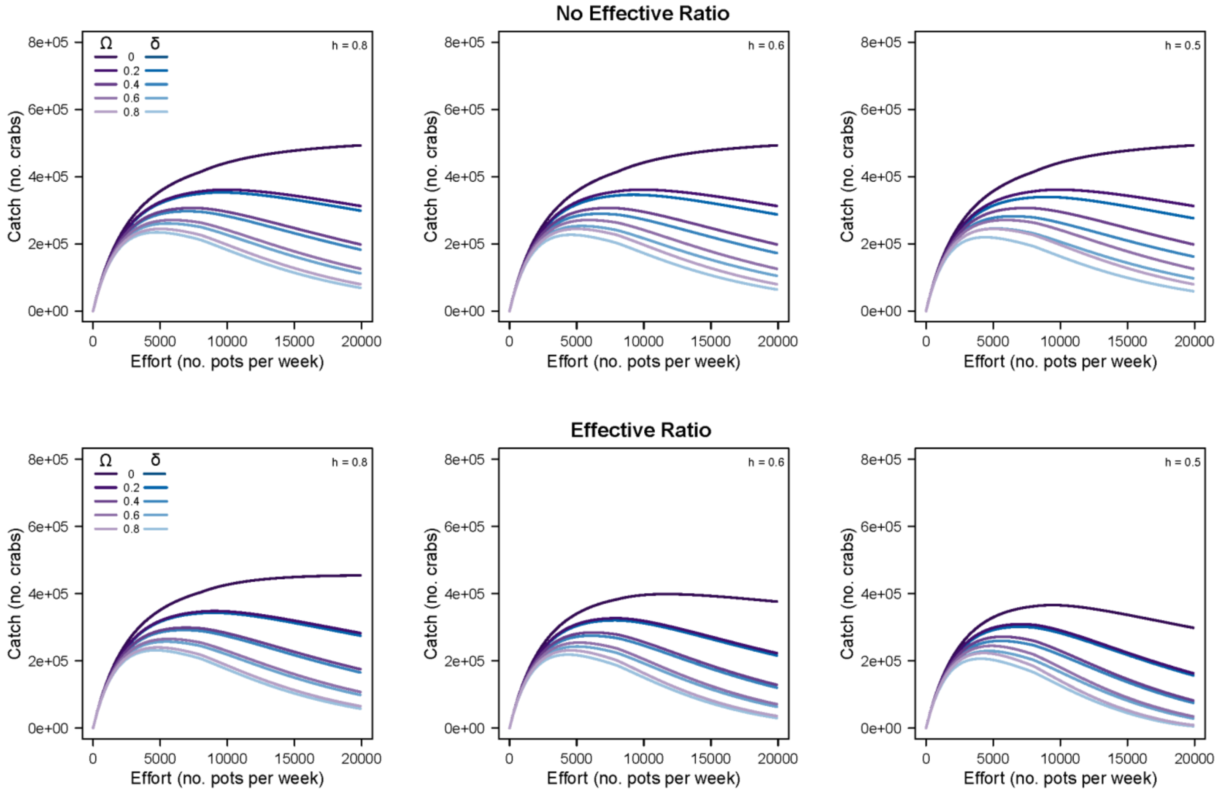

Figure 4.4 Increasing the probability of constant illegal retention (Ω - *purple*) or incidental morality (δ - *blue*) over three steepness (h) levels (0.8, 0.6, 0.5), referenced in the upper right hand corner of each graph. Graphs depict sensitivity results with (*top*) and without (*bottom*) an effective reproductive ratio ($\Phi_{a,t}$).

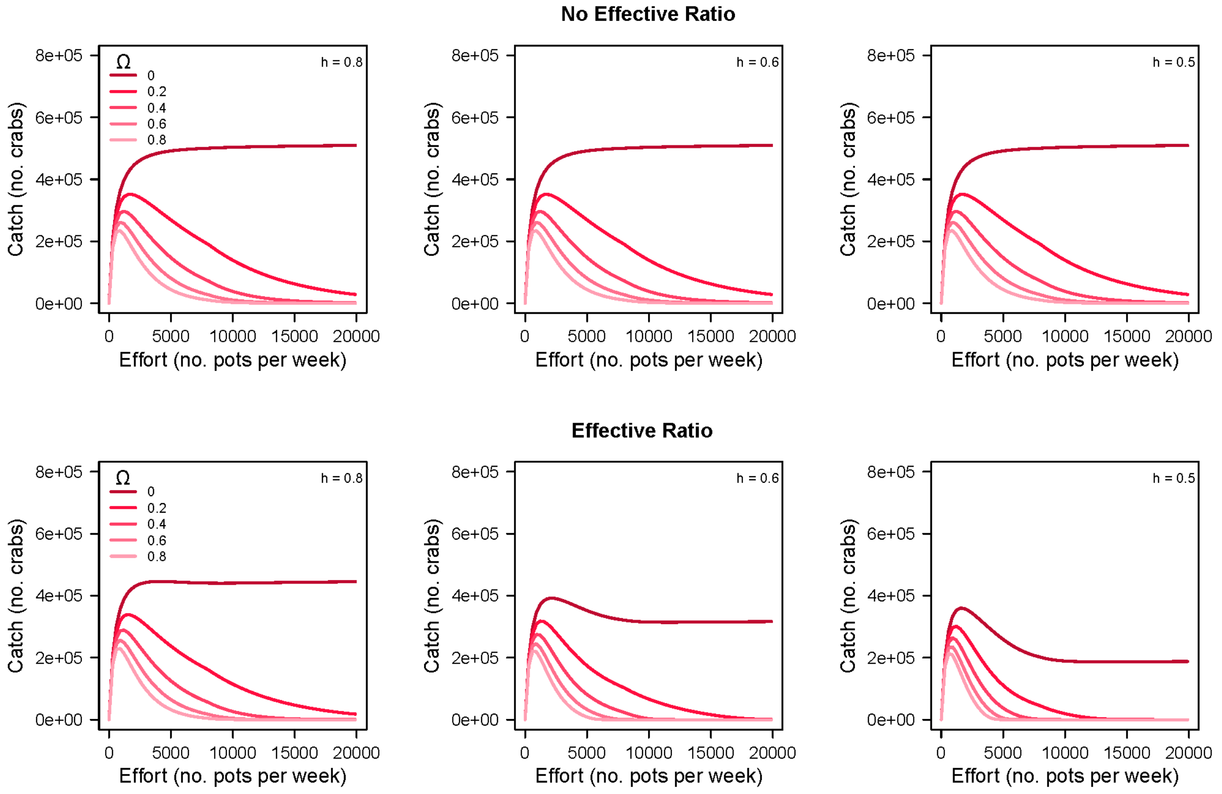

Figure 4.5 Increasing constant illegal retention (Ω) with hypoxia driven changes in catchability under three steepness (h) levels, denoted in the upper right hand corner of each graph. Graphs depict results with (*top*) and without (*bottom*) an effective reproductive ratio. The 2010 hypoxia event was used in the sensitivity comparisons.

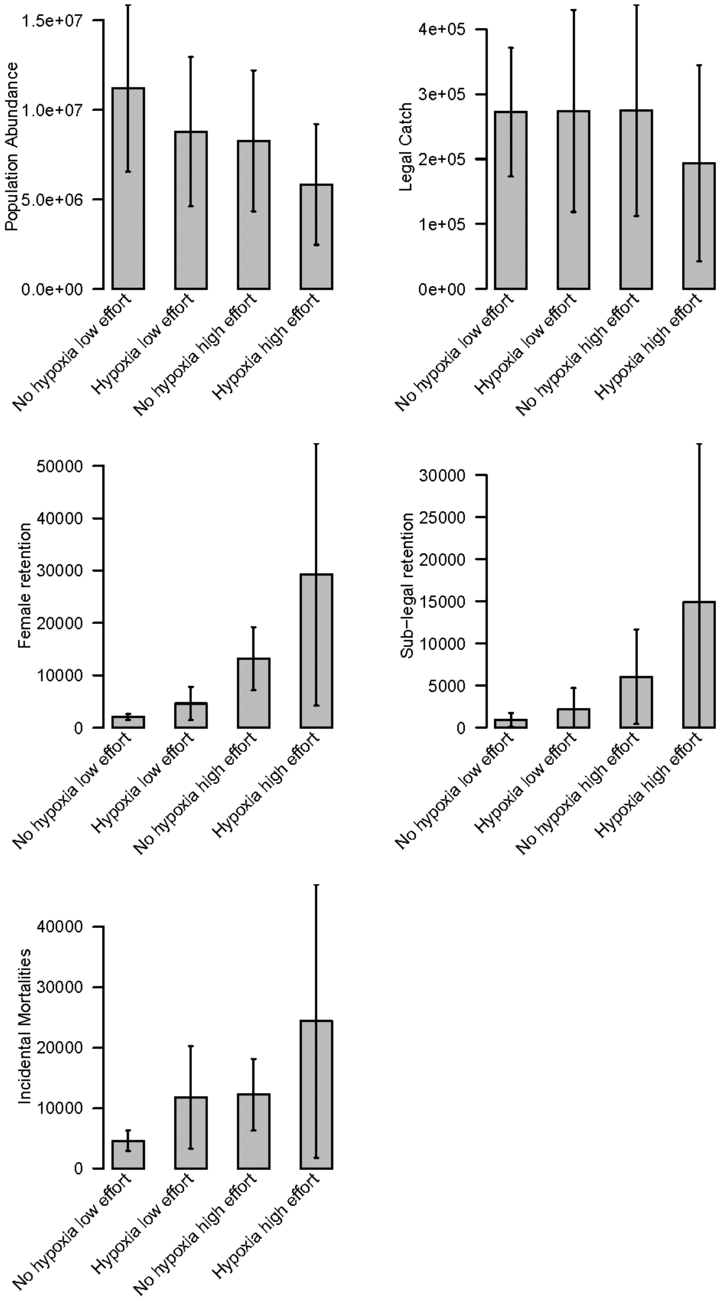

Figure 4.6 Mean and standard deviations of simulated Dungeness crab population abundance, legal (male) catch, illegal female and sub-legal retentions, and number of incidental mortalities. Values are depicted for four harvest and hypoxia scenarios: low (7,000 pots week^{-1}) and high (20,000 pots week^{-1}) effort with and without hypoxia (i.e., non-linear catchability). All results are based on 500 simulation runs ($h = 0.6$; $\sigma = 0.8$).

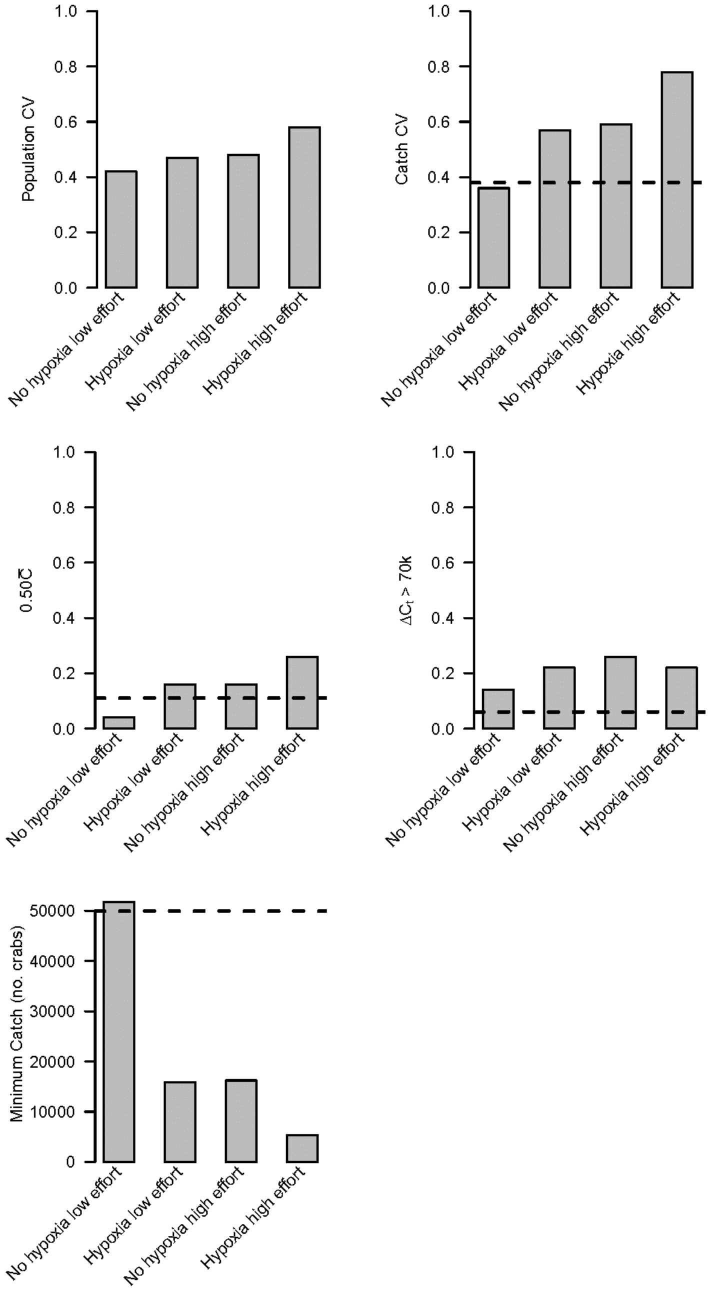

Figure 4.7 Dungeness crab metric results, including population coefficient of variation (CV), catch CV, proportion of catches less than 50% ($0.50\bar{C}$) of average yearly catch, proportion of catch declines greater than 70,000 crabs per year ($\Delta Ct > 70k$), and minimum catch. Metrics are depicted for four harvest and hypoxia scenarios: low (7,000 pots week^{-1}) and high (20,000 pots week^{-1}) effort with and without hypoxia (i.e., non-linear catchability). When applicable, horizontal *dashed lines* show the metrics calculated from the actual catch records. All results are based on 500 simulation runs ($h = 0.6$; $\sigma = 0.8$).

Synthesis

Depletion of oxygen is a growing worldwide threat to numerous marine ecosystems (Altieri and Gedan 2015; Diaz and Rosenberg 2008). The expansion and proliferation of such hypoxic [dissolved oxygen (DO) < 2 mgL^{-1}] events ultimately result in a suite of ecological consequences, both lethal and non-lethal (Diaz and Rosenberg 2008). It's well understood that hypoxia can modify the abundance and distribution of species, influencing populations and community structure, but the underlying mechanisms and magnitude of these changes is less clear – particularly for motile organisms that can avoid lethal exposure (Diaz and Rosenberg 1995; Diaz and Rosenberg 2008; Eby et al. 2005; Petersen and Pihl 1995; Pihl et al. 1991). In fact, the combination of the species physiology, severity and extent of hypoxia, and the presence of other stressors (e.g., fishing) will dictate the ecological interactions that need to be consider for the conservation and management of hypoxia prone ecosystems (Wu 2002).

In Puget Sound, Washington, U.S.A, a hypoxic fjord estuarine basin known as Hood Canal experiences seasonal bouts of hypoxia, that appear to becoming more chronic in nature (Newton et al. 2007; Newton 1995). This dissertation investigated several facets of the non-lethal ecological impacts of hypoxia on the fish and motile macroinvertebrates in Hood Canal. Prior to this research, there was limited understanding of the role of hypoxia in species' seasonal movement patterns and exposure, nearshore community structure, and harvest. We contributed important foundational information regarding the scale of distributional shifts of some of the most abundant, bottom-oriented species in Hood Canal: Dungeness crab (*Metacarcinus magister*) and English sole (*Parophrys vetulus*). We used the information garnered from the behavioral study to explore the possible consequences of non-lethal hypoxia and other sources of demographic influence on the management strategy of Dungeness crab using simulation modelling. In addition, we provided multiple lines of evidence of low DO conditions – levels above the traditional 2 mgL^{-1} – influencing the composition of the nearshore community relative to physiological tolerance. Lastly, we also provided more insight into hypoxia exposure and interactions of pelagic species by exploring a possible genetic biomarker, hypoxia-inducible factor-1α.

More localized responses of benthic-oriented species, particularly Dungeness crab, to hypoxia suggested a potential increased vulnerability to nearshore fishing pressure, which we explored using a Management Strategy Evaluation. The relative scale of organismal movements has import implications for the magnitude and type of secondary ecological effects (Eby 2004). Indeed, we found little evidence of large, regional shifts of Dungeness crab and English sole into more northern, oxygenated areas of Hood Canal. Instead, the majority of tagged individuals remained relatively localized (5-15 km) and the Dungeness crab fitted with pressure-sensing tags showed significant reductions in depth, coinciding with the vertically expanding hypoxia. Assuming the localized shoaling patterns of the crabs increases their catchability during summer months, when state recreational and commercial tribal crab fishing occurs, we found the 3-S management strategy performance eroded. Using an age-structured population model for Hood Canal Dungeness crab, we found accounting for variable hypoxia scenarios and changes in important demographic parameters, including reduced reproductive capacity and additional sources of mortality (i.e., illegal and incidental capture mortality) decreased legal catch and population abundance, increased the variability and minimum catches, but reduced the extremes in the variability. Outcomes were further exacerbated by increases in effort, which represented the possible repercussion of allowing the recreational, open-access fishery to expand without limit.

The traditional definition of hypoxia (< 2 mgL^{-1}) does not capture the full suite of non-lethal consequences of low DO (Vaquer-Sunyer and Duarte 2008), particularly when considering the nearshore (≤ 30m) environment – a habitat that does not always experience severe hypoxic conditions (Breitburg 1990; Breitburg 1992c). Comparing nearshore, subtidal mobile species composition between a southern, lower DO region and a northern reference region of Hood Canal revealed significantly different structures that aligned with the hypoxia tolerance of the most abundant species. Moreover, division of the species into DO-tolerance groups (i.e., 'sensitive' and 'tolerant') revealed distinct thresholds – both above 2 mg L^{-1} – and responses in presence relative to the level of oxygen; tolerant species had an overall lower threshold and nearly 3-fold increase in probability of presence at the minimum DO level (1.8 mg L^{-1}), while the sensitive species were almost entirely absent under the same conditions. Given the patterns explored in this chapter, the continual lower DO conditions in southern Hood Canal could result in a more persistent, perhaps more sensitive, low DO community state.

Bathymetric features greatly restrict the behavioral options for benthic species when faced with hypoxia, while interactions of highly mobile marine pelagic species with hypoxia are more complex and not well understood (Ekau et al. 2010; Zhang et al. 2009a), supporting the need for a multitude of approaches to explore such interactions. We developed a hypoxia biomarker for Pacific herring (*Clupea pallasii*) using hepatic mRNA expression of hypoxia-inducible factor-1α (HIF-1α) to measure and detect biologically significant levels of acute (i.e., hours) hypoxia exposure. Pacific herring showed significant increases in HIF-1α when exposure to ca. 2-8hrs of traditional hypoxic conditions (2-3 mgL^{-1}); this level also resulted in mortalities, suggesting they were at or near the lethal limit. In addition, mRNA levels returned to equivalent control levels after ca. 24hrs of normoxia – a trend that appears to hold across multiple species

(Kodama et al. 2012b; Rahman and Thomas 2007; Rimoldi et al. 2012; Terova et al. 2008). Using the expression patterns of HIF-1α determined in the laboratory, we found that significant expression of HIF-1α tracked broader scales of time and space. Most likely due to the high mobile nature of the species, HIF-1α did not coincide with the south to north hypoxia gradient in Hood Canal. However, levels of HIF-1α mRNA were significantly elevated during the more severe hypoxic year (2013). Increases in HIF-1α have been linked to negative, long-term impacts on reproductive function in other teleost fishes (Cheung et al. 2014; Thomas and Rahman 2009; Thomas and Rahman 2012); an important non-lethal consequence of hypoxia that warrants consideration if hypoxic conditions continue to deteriorate in Hood Canal.

Currently, most conservation and management strategies of natural resources in Hood Canal revolve around severe hypoxic levels (< 2 mgL^{-1}) that can lead to fish-kill events. However, non-lethal impacts of hypoxia are more common and ecologically significant for organisms that can move. Distributional shifts and changes in community composition can alter the interactions in the ecosystem, including the magnitude of fishing impacts and sensitivity to other environmental pressures (e.g., temperature). Moreover, sublethal exposure can induce biological costly consequences on reproduction and growth. The non-lethal threat of hypoxia is thus context dependent, including, but not limited to, scale, species' physiological limitations, and other external perturbations. As a result, understanding and managing for the impacts of hypoxia in aquatic ecosystems cannot simply focus on one species or process, but rather requires a more holistic approach to account for the range of biotic and abiotic factors and their associated uncertainties. Moving forward, hypoxia research will need to consider ecosystem tipping points (Conley et al. 2009), fisher interactions with these hypoxic environments (Selberg et al. 2001), and the potential synergistic effects of hypoxia with other environmental stressors, including temperature and ocean acidification (Altieri and Gedan 2015).

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Vita

Halley Elizabeth Froehlich earned a Doctor of Philosophy degree in Aquatic and Fishery Sciences at the University of Washington in 2015. In her graduate research, Halley used field-based and quantitative approaches to investigate ecological questions pertaining to the non-lethal impacts of hypoxia on species in an estuarine ecosystem. Broadly, Halley is interested in the ecological responses of marine populations and communities to anthropogenic-related disturbances over multiple scales.

Ms. Froehlich received several awards over her graduate career. Halley was a National Science Foundation Graduate Research Fellow (2012-2015). She also received the Northwest Scientific Associate Student Research Grant (2014). Halley ultimately graduated Magna Cum Laude from the University of Washington.

Born in New Mexico, Halley spent the latter-portion of her life in Northern California where she received a Bachelor of Science degree in Animal Biology from the University of California, Davis in 2010. During her time in Davis, Halley worked in several collaborative laborites as a technician studying fish physiology and conservation. The several years of experience in fish ecological research inspired Halley to pursue a higher degree of education in the field, with an eye towards the marine realm.

Outside her dissertation, Halley also collaborated on projects studying the human side of scientific research. Working with Dr. Julia K. Parrish and others, she assessed the untapped potential of citizen science and found tremendous opportunities for research and increased public awareness of conservation issues. Halley sees great benefit in community involvement through citizen based science for policy, management, and research of coastal ecosystems.

Beyond research, Ms. Froehlich highly values scientific communication and teaching. Although Halley was a teaching assistant (2013) and guest lecturer (2012-2014) at SAFS, she wanted more experience as a primary instructor. As a result, she sought out another Ph.D. graduate student to help develop, implement, and teach a new, graduate student driven course in SAFS, entitled 'FISH 511: Advanced Marine Ecology'. Halley successfully co-instructed the course in fall 2015, which was structured as a seminar to teach classic marine ecological theories and compare the foundational information with contemporary research through lecture, readings and in-class discussions. Importantly, the course was designed to help prepare Ph.D. students for their general and qualifying exams.

Peer-Reviewed Publications (as of June 2015)

Froehlich, H.E., S.M. Hennessey, T.E. Essington, A.H. Beaudreau, P.S. Levin. 2015. Spatial and temporal variation in nearshore macrofaunal community structure in a seasonally hypoxic estuary. *Mar. Ecol. Prog. Ser.* 520:67-83. 10.3354/meps11105

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Essington, T.E., P. E. Moriarty, **H. E. Froehlich**, E. E. Hodgson, L. E. Koehn, K. L. Oken, M. C. Siple, C. C. Stawitz. 2015. Fishing amplifies forage fish population collapses. *PNAS*

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