

## USING IN SITU VIDEO ANALYSIS TO ASSESS JUVENILE FLATFISH BEHAVIOR ALONG THE OREGON CENTRAL COAST

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### ABSTRACT

We examined the feasibility of using a video beam trawl system to assess behavioral responses of juvenile flatfishes in relation to co-occurring habitat features, most notably dissolved oxygen (DO) concentrations. Sixteen samples were collected along a cross shelf transect in the central Oregon coast during summer 2008. We found that juvenile fish reaction duration, defined as the time in seconds from first reaction to capture, decreased with decreased DO. However, other variables such as bottom water temperature, fish size, and fish species composition by site are potentially confounding factors of the analysis. The dominant flatfish species shifted from English sole (*Parophrys vetulus*) to Pacific sanddab (*Citharichthys sordidus*) with increased depth. Escape behavior varied from “burying” in the shallows, to “hovering” at mid-depth stations, and “running” at the deepest site. Collectively, our results suggest that the video beam trawl effectively monitor behavioral metrics and community composition of nearshore flatfish assemblages.

### INTRODUCTION

In the eastern North Pacific along the central Oregon continental shelf, upwelling-driven coastal hypoxia (<1.43 ml DO l<sup>-1</sup> H<sub>2</sub>O) (Grantham et al. 2004; Chan et al. 2008) is a seasonal phenomenon, which also presents interannual variability in its intensity. Every year, northerly winds starting in late spring to early summer cause offshore Ekman transport. As the surface water moves offshore it is replaced with deep, nutrient-rich, oxygen-depleted water that has the potential to support phytoplankton blooms. The decomposition of organic matter sinking down from these phytoplankton blooms results in additional oxygen depletion (Pierce et al. 2012). Historically, hypoxia in the California Current System has not extended inshore past the 70 m isobath where important nursery grounds for economically and ecologically important fishes are located (Grantham et al. 2004).

In 2002 scientists began to detect seasonal nearshore hypoxic events on central Oregon’s continental shelf (Grantham et al. 2004). In the summer of 2002 hypoxic waters covering 820 km<sup>2</sup> were observed inshore of the 70 m isobath (Buck 2007). During this period, remotely

operated vehicle (ROV) surveys revealed areas void of live fish (dead zones) where long-lived benthic communities had previously existed, confirming decreased biodiversity (Grantham et al. 2004). In addition to scientific observation, the Oregon Department of Fish and Wildlife (ODFW) received increased reports of dead fishes washing up onshore. The hypoxic event in 2002 was not an isolated event. Four years later, in 2006, a more severe hypoxic event occurred (Chan et al. 2008). Some studies report that alterations in wind intensity and frequency due to climate change are affecting upwelling patterns (Grantham et al. 2004; Barth et al. 2007; Buck 2007) and contributing to their intensification in recent decades (Peterson et al. 2013). In 2006, anoxia (0.0 ml DO l<sup>-1</sup>) was detected inshore of the 50 m isobath with hypoxic water covering an area of approximately 3000 km<sup>2</sup> (Chan et al. 2008).

The nearshore regions affected by hypoxia include nursery grounds for commercially and ecologically important flatfish species such as the Pacific sanddab (*Citharichthys sordidus*), English sole (*Parophrys vetulus*), sand sole (*Psettichthys melanostictus*), and butter sole (*Isopsetta isolepis*) (Pattie 1969; Barss 1976; Krygier and Percy 1986; Rogers and Pikitich 1992; Buckley et al. 1999). During early life stages, flatfishes are important prey items for other species (Lassuy 1989), and if hypoxia negatively affects their behavior, there could be trophic implications for the local food web. On an ecological level, thresholds of hypoxia are taxon specific and can result in sublethal effects such as forced migration (Vaquer-Sunyer and Duarte 2008) resulting in shifts in biological communities and behavior-mediated death of individuals (Kolar and Rahel 1993; Breitburg et al. 1997). Organisms with restricted mobility, such as echinoderms, crustaceans, and early life stages of fishes, are typically some of the most heavily affected (Gray et al. 2002).

While the effects of anthropogenic hypoxia on fish communities in semi-enclosed systems such as Chesapeake Bay and the Gulf of Mexico have been well monitored (Buck 2007), similar effects in seasonal upwelling-driven nearshore hypoxic zones have not. Laboratory studies have revealed that low DO con-

TABLE 1  
 Date, station name, targeted and actual (in parenthesis) depths, number of tows, and video time (in minutes)  
 of the sampling events analyzed in this study. MB and NH station names indicate locations off of  
 Moolack Beach and along the Newport Hydrographic line (44.6517°N).

Date	Station	Depth (m)	No. of Tows	Video time (minutes)
07/26/08 & 08/11/08	MB 30	30 (30.0)	2	24.1
07/26/08, 08/11/08, & 08/26/08	MB 40	40 (42.8)	4	51.5
07/26/08, 08/25/08, 08/27/08, & 08/28/08	NH 05	60 (60.0)	4	48.7
08/11/08	NH 07	70 (73.0)	1	13.5
07/26/08, 08/11/08, 08/27/08, & 08/28/08	NH 10	80 (80.1)	5	75.4

centrations can affect marine organisms physiologically and behaviorally. For example, laboratory experiments with common sole (*Solea solea*) tracked behavioral and biochemical responses to gradual exposure to hypoxia, establishing a pattern of behaviors that correlated to shifts in metabolic response to oxygen concentration. With the onset of anaerobic metabolism, behavior shifted from reduced activity to inactivity and as tank conditions transitioned from hypoxia to anoxia fishes lifted their heads, exhibited escape responses (“panic swimming”), loss of balance, and paralysis (Dalla Via et al. 1998). Behavioral responses to low DO include alterations in activity (Dalla Via et al. 1998; Chabot and Dutil 1999; Brady and Targett 2010), reduced feeding rates (Chabot and Dutil 1999; Stierhoff et al. 2006), decreased predation efficiency (Tallqvist et al. 1999), altered predator-prey interactions (Kolar and Rahel 1993; Sandberg 1994; Breitbart et al. 1997; Long and Seitz 2008), and species specific avoidance techniques (Wannamaker and Rice 2000). Wannamaker and Rice (2000) found that juvenile spot (*Leiostomus xanthurus*), mummichog (*Fundulus heteroclitus*), pinfish (*Lagodon rhomboides*), menhaden (*Brevoortia tyrannus*), croaker (*Micropogonias undulatus*), white mullet (*Mugil curema*), and brown shrimp (*Penaeus aztecus*) could all respond to levels of 1 mg l<sup>-1</sup> DO, but the avoidance thresholds and responses varied from species to species. Some species avoided low DO by way of vertical migration while others increased surface breathing.

Ongoing improvements of environmental sensors, and in situ imaging and platforms have dramatically augmented our ability to monitor individual behavior of fish in response to environmental variability (Norcross and Mueter 1999; Kintisch 2013). Behavioral responses to external stimuli can have important consequences to the survival of an individual. By studying behavior one can reach a mechanistic understanding of the processes that affect the survival of individuals at very small spatial and temporal scales. However, there are analytical challenges in quantifying and analyzing behavioral metrics from in situ videos. Specifically, one needs to a-priori select a number of metrics that are quantifiable in video frames and that are truly representative of the behavior

under scrutiny (Laidig et al. 2013). Further, one needs to relate these behavioral metrics to species type and to changes that occur in the habitat.

The objective of this study was to assess the feasibility of using a video beam trawl system to assess the behavioral responses of juvenile flatfishes in relation to the physical and chemical properties of the water and the fish species community composition. We were particularly interested in assessing the responses of juvenile flatfishes to DO concentrations in a natural setting. To this end, we have added a video system to a beam trawl to allow for in situ behavioral observations. We hypothesized that the behavioral response of juvenile flatfishes to the approaching of the beam trawl is affected by DO concentrations and by community composition. If found to be true, our hypothesis could have implications for the survival of fishes within different ecological systems. Results from this study can thus shed light on the feasibility of applying in situ video technology in addressing the behavioral responses of organisms. Prior to this, there were a few studies to characterize the nearshore soft-sediment juvenile fish assemblages of the central Oregon coast (e.g., Pearcy 1978; Toole et al., 2011), but only for sites deeper than 50m. Given the nursery role of nearshore soft-sediment habitats (Laroche and Holton 1979), our study fills an important knowledge gap.

## MATERIALS AND METHODS

Juvenile flatfishes were sampled at five historical stations in central Oregon (table 1) off Moolack Beach (MB) and along the Newport Hydrographic (NH) line (fig. 1) during July and August of 2008. Data were collected during two 1-day cruises on the Oregon State University’s R/V *Elakha* and one 9-day cruise on the R/V *Wecoma*, for a total 16 tows. At each sample site, a conductivity, temperature, depth, and DO profiler (CTD-DO, Sea-Bird Electronics Model 25, Bellevue, WA, USA) was deployed to measure DO (ml l<sup>-1</sup>), temperature (°C), and salinity on the seafloor. The DO sensors are calibrated once to twice a year depending on availability of the gear and scheduled cruises of the R/V *Wecoma*. We sampled the flatfish community assemblage and measured their behavior using a 2 m wide beam

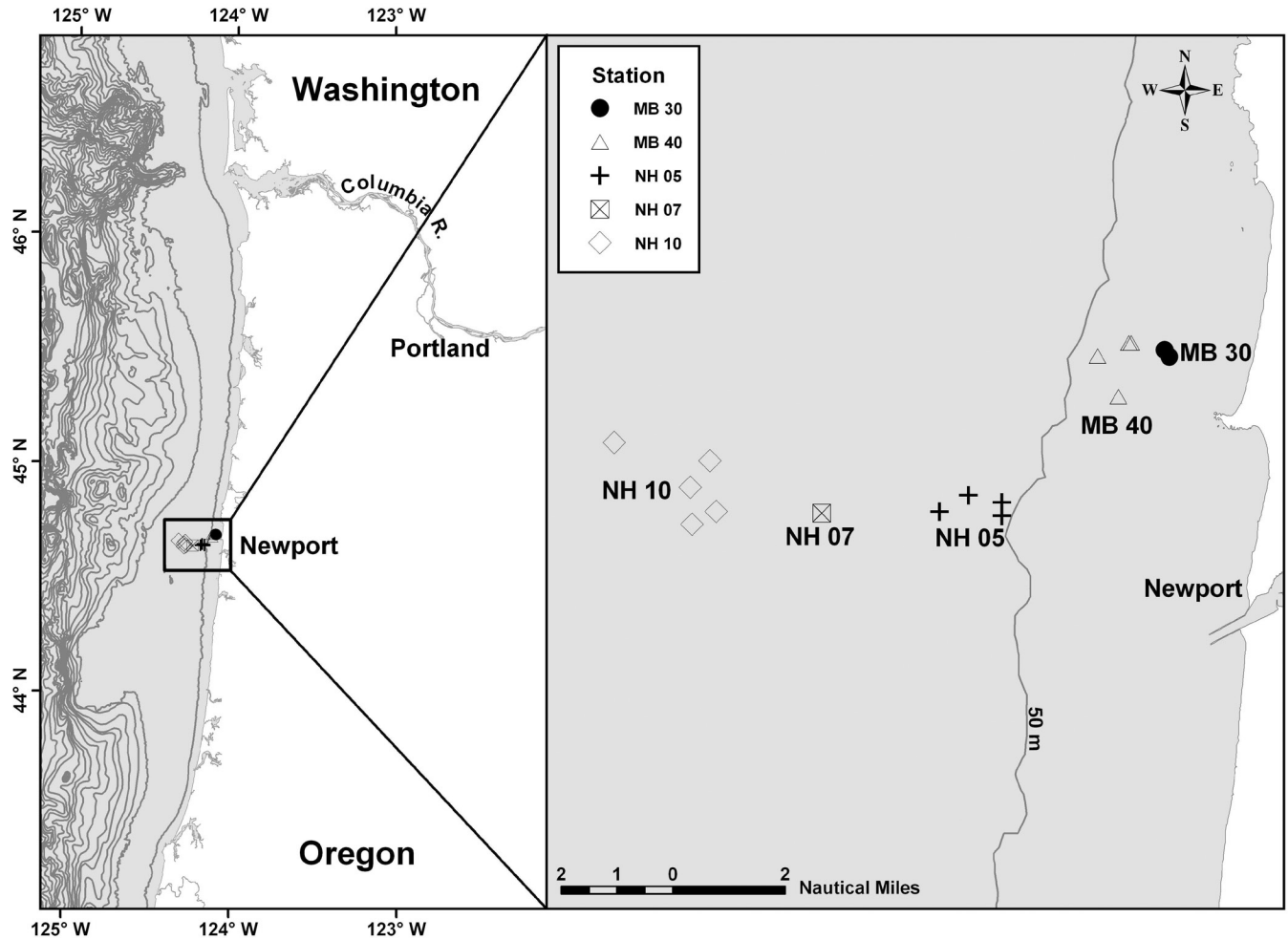


Figure 1. Locations sampled in 2008 with beam trawl and in situ video. Sampling took place along Newport Hydrographic line (NH) and at Moolack Beach (MB) stations.

trawl equipped with a 3 mm mesh liner throughout, 10-cm scaling laser, lights, tickler chain, and an NTSC high-resolution video camera mounted in a vertical downward orientation (fig. 2). Once the beam trawl made contact with the seafloor, it was towed for approximately 10 minutes at a target speed over the ground of 1 knot. The net was then brought on board and emptied. Fishes greater than 150 mm were identified to species, measured (total length [TL] in millimeters), and then released. Juvenile flatfishes less than 150 mm were frozen and subsequently thawed in the lab, identified to species, weighed (g) and measured (mm TL). Based on previous studies done in the area (Krygiar and Percy 1986), flatfishes equal to or smaller than 150 mm in length were considered juveniles (i.e., age-1 or younger). Fish length from the trawl samples was measured to the nearest mm.

In situ video obtained with the video beam trawl system was analyzed frame-by-frame using Quick Time Player version 7.6.4. For each tow, we recorded fish size

(to the nearest cm using the scaling lasers), tow duration, and the duration of actual bottom contact, as observed in the video. Intervals where the beam trawl was not in contact with the seafloor were removed from the inspected footage. The number of fishes observed was standardized by the total amount of time the beam trawl was in contact with the seafloor. This gave us the number of fishes caught per minute of towing effort. Video analysis began from the point the trawl runners and tickler chain made contact with the seafloor and ended when the sampling equipment no longer was in contact with the substrate. When the equipment first made contact with the seafloor a sand plume was generated. Once the beam trawl was in constant motion the disturbed sand remained behind the tickler chain leaving the undisturbed environment in front of the sampling equipment in clear view. Excerpts of videos collected during these operations can be viewed here: <http://www.fisheriesoceanographylab.org/photos-videos/beam-trawl-videos/>.

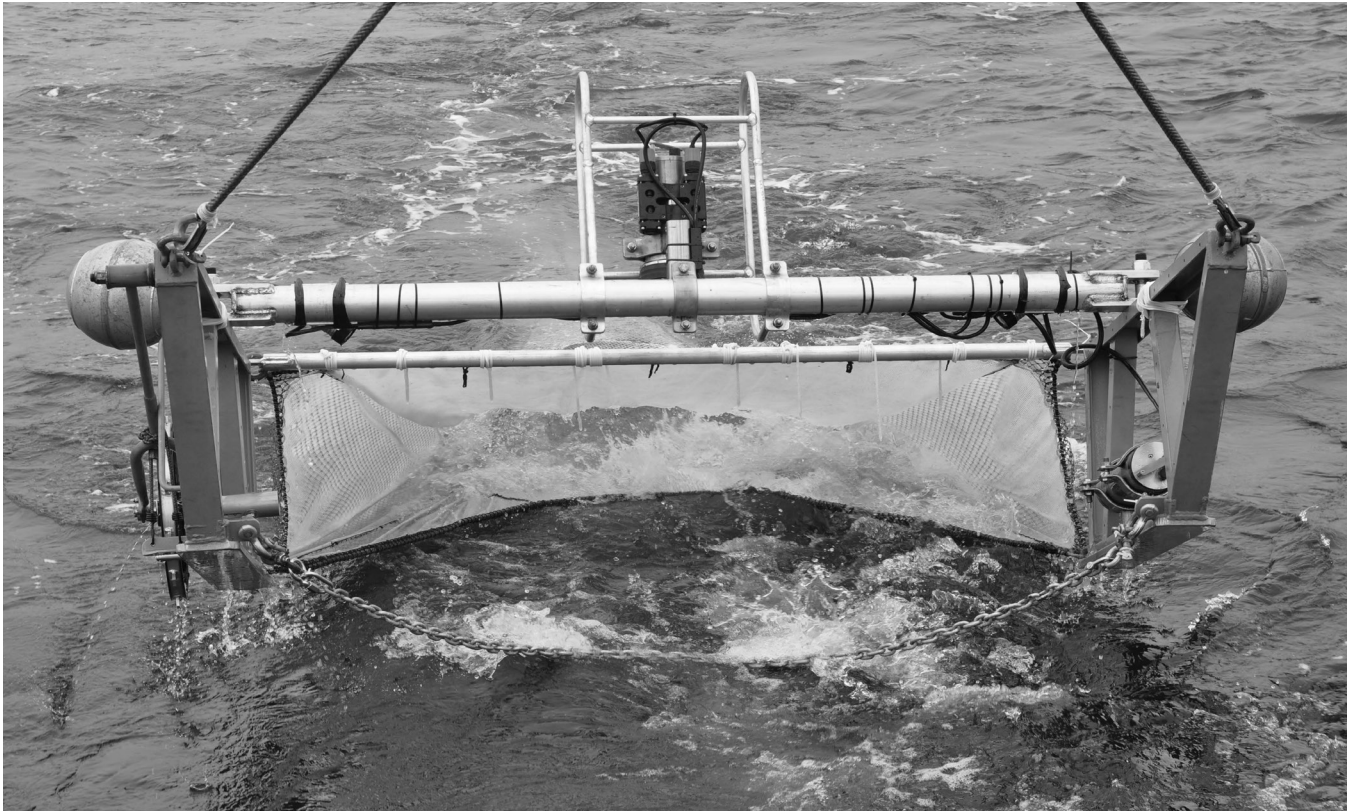


Figure 2. 2 m wide beam trawl equipped with tickler chain, 10 cm laser scaler, lights, 3 mm mesh net, and a NTSC high-resolution video camera.

During the analysis, the entire video frame was overlaid with a  $10 \times 7$  grid. The 10 cm scaling laser system generated a metric frame of reference. For each fish observed in the video frame, the following data were recorded: time first seen, location first seen (based on the overlying grid), length to the nearest cm (taken when the fish was flat over the bottom), and initially moving (1) or still (2). For fish that were not initially moving, we also recorded time of first reaction (i.e., a noticeable movement of the fish's body), distance from the tickler chain at the time it reacted, initial and post disturbance response behaviors (described below), time it fell behind the chain, time last seen, and location last seen. The time a fish fell behind the chain represents the time of capture. Fish that "out ran" the gear were classified as fish that escaped. Times were recorded in minutes and seconds. The total reaction time (total reaction time = time fish first reacted – time fish first appeared) and reaction duration (reaction duration = time fish fell behind – time fish first reacted) were calculated and recorded. Reaction duration was not recorded for fish that escaped the trawl (i.e., clearly left the video frame from the sides). In addition to the performance metrics listed above, we also quantified six initial and post disturbance response behaviors. Specifically: burying (buries in sediments), running (continuous swimming), hopping

(short bursts of swimming followed by short periods of rest), rising (rising out of sediments with no horizontal movement), hovering (hovers above sediments) and lastly, no visible reaction.

The in situ video footage was analyzed along with catch data to determine behavioral responses in relation to community composition, water DO, and other environmental conditions such as water temperature and bottom depth. Specifically, average reaction duration (time in seconds) for each tow was correlated against the respective values of bottom DO using Generalized Additive Models (GAM; Wood 2006). The average length of the fish per tow (measured from the videos) was also included in the analysis, to evaluate the effect of fish size on behavioral metrics. Initially both variables were included in the model, and removed one at a time, starting from the variables with the highest  $p$ -value. This analysis was restricted to juvenile fishes (length  $\leq 150$  mm). Analyses of variance (ANOVAs) were used to examine trends between fish size, standardized abundance (number of fish per minute of sample effort) and percent juvenile individuals observed and that escaped the tows, in relation to station. Linear regression was used to correlate DO values at each station with average fish size and with bottom temperature. All univariate analyses (ANOVA, GAM, and linear regression) were

TABLE 2  
**Average number of individual fish collected per minute at each station in the net data. The number of flatfishes per minute was calculated for net and video samples and includes individual of all sizes. The average percentage of juvenile flatfishes for net and video samples represents only flatfish that are 150 mm or less in TL.**

	MB 30	MB 40	NH 05	NH 07	NH 10
Speckled Sanddab ( <i>Citharichthys stigmaeus</i> )	1.17	1.90	0.47	0.07	0.00
Butter Sole ( <i>Isopsetta isolepis</i> )	2.38	1.22	0.10	0.07	0.09
English Sole ( <i>Parophrys vetulus</i> )	6.04	3.48	0.43	0.00	0.20
Pacific Sanddab ( <i>Citharichthys sordidus</i> )	0.04	2.02	2.61	1.85	0.86
Curlfin Sole ( <i>Pleuronichthys decurrens</i> )	0	0.02	0.00	0.00	0.00
Sand Sole ( <i>Psetichthys melanostictus</i> )	0	0.04	0.00	0.00	0.00
Dover Sole ( <i>Microstomus pacificus</i> )	0	0.00	0.12	0.00	0.07
Petrale Sole ( <i>Eopsetta jordani</i> )	0	0.02	0.00	0.00	0.00
Slender Sole ( <i>Lyopsetta exilis</i> )	0	0.02	0.02	0.00	0.08
Flathead Sole ( <i>Hippoglossoides elassodon</i> )	0	0.00	0.00	0.00	0.01
Rex Sole ( <i>Glyptocephalus zachirus</i> )	0	0.00	0.02	0.00	0.00
Number of flatfishes minute <sup>-1</sup> (net sample)	9.63	8.72	3.79	2.00	1.31
Number of flatfishes minute <sup>-1</sup> (video sample)	14.04	14.31	12.00	6.44	4.04
Average % of juvenile flatfishes (net sample)	91.2	59.3	50.9	7.4	19.8
Average % of juvenile flatfishes (video sample)	89.9	79.1	66.3	36.8	40.9

conducted in *R*, with cut off for significance set to 0.05. GAMs were conducted using the library “mgcv” (mixed gam computation vehicle; Wood 2006).

Species assemblages from the actual catch data were analyzed using multivariate techniques in PC-ORD v5 (McCune and Mefford 1999). Specifically, we used a species composition matrix and an environmental matrix including the following variables: depth, DO, temperature, mean length, mean reaction duration, mean reaction time, and percent of each behavior type. Data transformations and their effects on the summary statistics were examined prior to the multivariate analysis by comparing row and column skewness and coefficient of variation (CV) before and after data transformation (McCune and Grace 2002). Abundance data were natural log-transformed and rare species removed from the analysis (those which only occurred once), which greatly reduced row and column skewness and coefficient of variation (CV). Nonmetric Multidimensional Scaling (NMS; Kruskal 1964) was used to run an ordination of sample stations in species space to compare community composition at the various sample stations relative to the measured environmental variables. We used the Sorensen distance measure because it is less sensitive to outliers than some other distance measures. With NMS, the best solution for the ordination is defined by a particular starting configuration and number of dimensions. The NMS procedure uses an iterative search for the best possible positions of *n* entities on *k* dimensions, which minimizes the stress of the dimensional configuration (McCune and Grace 2002). To evaluate whether NMS extracted stronger axes than would be expected by chance, PC-ORD uses a randomization (Monte Carlo) procedure. PC-ORD performs runs with randomized data by shuffling the data from the main matrix and each run uses a different ran-

dom starting configuration. A “run” consists of a series of solutions, stepping down in dimensionality from the highest number of axes to one axis. The *p*-values of this test are calculated as the proportion of randomized runs with stress less than or equal to the observed stress. The dimensionality of the ordination is then evaluated by comparing the results of the NMS runs using real data to the results obtained using the Monte Carlo simulations with randomized data. Relationships between environmental variables and ordination scores are shown as a joint plot in which the angle and length of the lines indicate the direction and strength of the relationship. In order to test for differences in species composition between depth categories, we used a multiresponse permutation procedure (MRPP) (Mielke and Berry 2001; McCune and Grace 2002; Reese and Brodeur 2006).

## RESULTS

### Description of Habitat Characteristics

During the sampled period, hypoxic conditions occurred prevalently at the deeper sampling sites of our study area, while the shallower sites were characterized by high variability of DO values. At MB 30 for example, DO ranged from 1.99 to 8.11 ml l<sup>-1</sup>, while the range of DO concentrations became smaller and less variable with increasing station depth (fig. 3). We have questioned the validity of the highest DO value of at MB 30, because it appeared unrealistically high for this location and time of the year. However, the highest (8.11 ml l<sup>-1</sup>), lowest (1.11 ml l<sup>-1</sup>) and second lowest (1.30 ml l<sup>-1</sup>) DO values of our records were all measured on the same day—August 11, 2008, suggesting that an instrument malfunction was unlikely. On that day, the area close to shore was well mixed as indicated

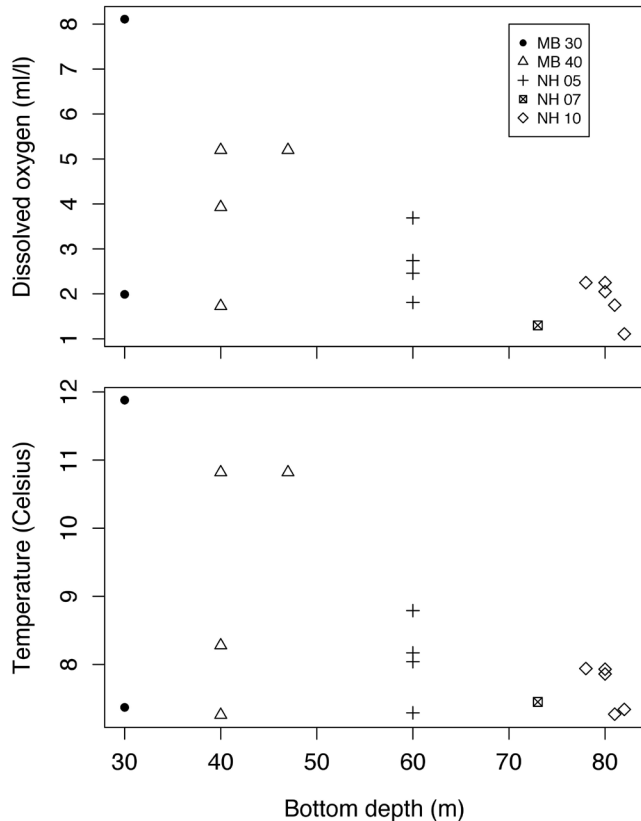


Figure 3. Dissolved oxygen concentration (top panel) and water temperature (bottom panel) measured just above the seafloor at each station (MB 30, MB 40, NH 05, NH 07 and NH 10) prior to towing the beam trawl.

by uniform temperature and salinity profiles at MB 30, while the area offshore was highly stratified. Thus, these extremes of DO values are likely the result of different hydrographic regimes of the coastal versus deeper sampled areas. At MB 30 bottom temperature ranged from 7.37 to 11.88°C, representing the largest difference in temperature of 4.51°C (fig. 3). The range and variability in temperature also decreased with depth at MB 30, MB 40, NH 05, and NH 10 (4.51°C, 3.56°C, 1.50°C, 0.67°C, respectively). Water temperature and DO were positively correlated ( $R^2 = 0.917$ ,  $p$ -value  $\ll 0.001$ ,  $n = 16$ ).

### Description of the Species Composition from the Catch Data

Flatfish species assemblages changed with the increasing of depth. At Moolack Beach (MB 30 and MB 40) the assemblage was primarily composed of English sole, speckled sanddab (*Citharichthys stigmaeus*), butter sole, and Pacific sanddab. The flatfish community composition on the deeper NH line was primarily composed of Pacific sanddab, speckled sanddab, English sole, and Dover sole (*Microstomus pacificus*) in order of decreasing abundance (table 2).

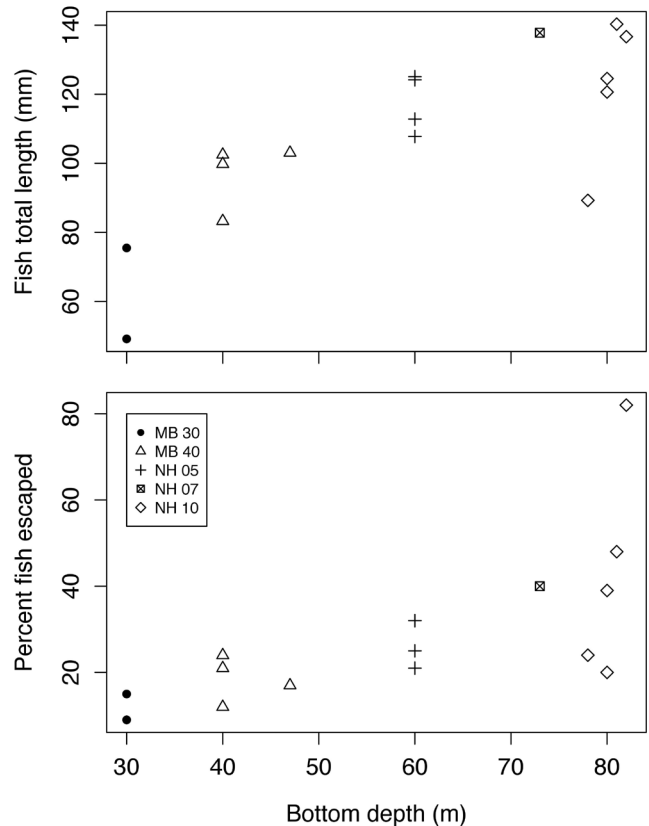


Figure 4. Fish total length (mm) and percentage of fishes escaped at each station (MB 30, MB 40, NH 05, NH 07, and NH 10). Fish total length is measured to the nearest 10 mm using scaled laser beams from video frames and it includes all fishes, not only the juvenile portion. Fishes that escaped the beam trawl were also assessed from inspection of the video frames.

### Description of Species Abundance and Size from the In Situ Video Observations

The abundance of flatfishes obtained from in situ video analysis was from 145.8 to 335.9% greater than that measured from the net data (table 2), indicating that many of the fishes that were detected in the video escaped the net. From the analysis of the video data, the average number of all flatfishes observed, standardized by effort, was highest at MB 40 (14.31 fish  $\text{min}^{-1}$ ) and decreased with depth in stations equal to and greater than 40 m. The smallest number of flatfishes was observed at NH 10 (4.04 fish  $\text{min}^{-1}$ , table 2). The only significant difference in the number of fishes was observed between MB 40 and NH 10 ( $p = 0.05$ , Tukey HSD).

The average percentage of juveniles ( $\leq 150$  mm) with respect to all flatfishes caught decreased as depth increased from 30 m to 70 m. Interestingly, the percentage of juvenile fishes increased slightly at NH 10 (80 m), indicating the probable existence of deeper juvenile settlement locations for flatfish species that are associated with deeper habitats. MB 30 had the highest average percentage (89.9%) of juvenile fishes caught ( $< 150$  mm) while NH 07 had the lowest (36.8%, table 2). The aver-

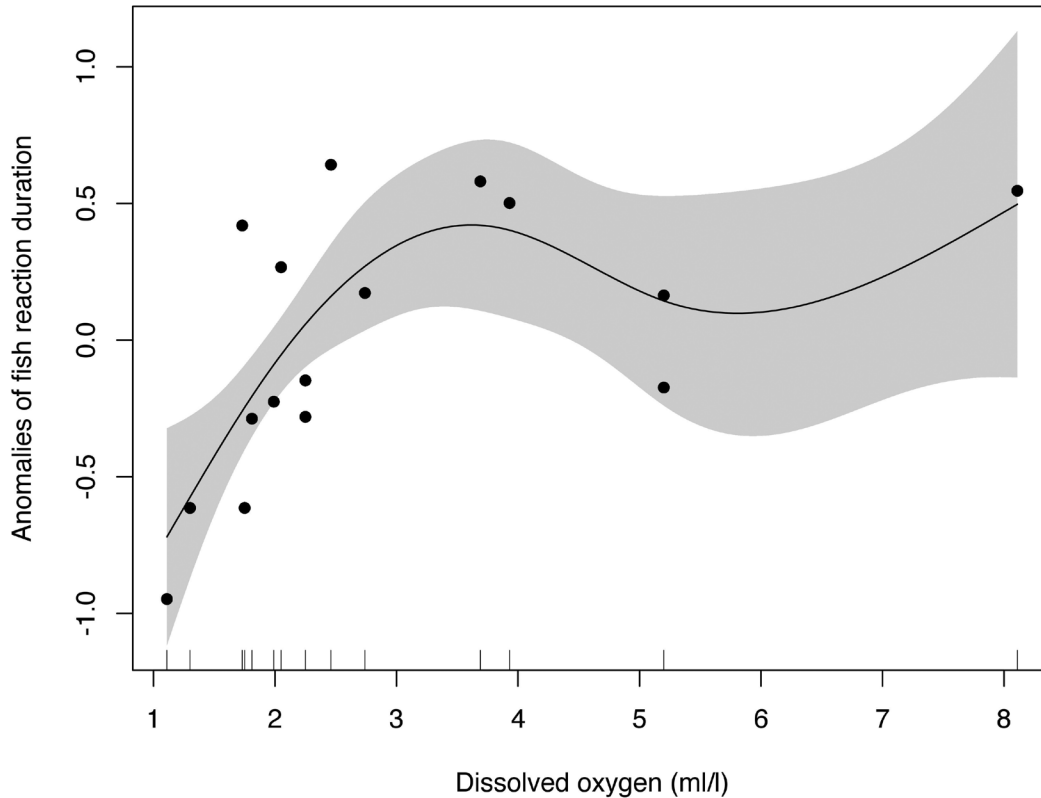


Figure 5. GAM estimated effects of dissolved oxygen (DO ml l<sup>-1</sup>) on average reaction duration (s) of juvenile flatfishes (length ≤ 150 mm) measured from the in situ video observations. The y-axis indicates the magnitude of the effect as anomalies with respect to the overall mean. Shaded regions are the 95% confidence intervals. The “rug” on the x-axis indicates the points at which observations were obtained.

age percentage of juvenile flatfishes at MB 30 was significantly different from NH 07 ( $p = 0.008$ ) and NH 10 ( $p < 0.001$ ) stations and MB 40 was significantly different than NH 07 ( $p = 0.020$ ) and NH 10 ( $p = 0.001$ ). The only significant difference between NH stations was between NH 05 and NH 10, with NH 05 having a greater percentage of juveniles ( $p = 0.020$ ). Average length (mm) of fishes had a positive trend in relation to station depth (fig. 4). The only non-significant difference in average fish size per station were found between NH 05 and MB 40 ( $p = 0.078$ ) and among all pair-wise combinations of station along the NH line.

#### Description of Juvenile Fish Performance and Behavioral Responses from In Situ Video Observations

The escape frequency increased with increased station depth (fig. 4), however, there were not statistically significant differences of escape frequencies among stations (ANOVA,  $p$ -value = 0.141). We compared the size of escaped and captured fishes at each station (two-way ANOVA) and found that the former were significantly larger (13.97 mm,  $p = 0.047$ ) than the latter. Reaction duration for all fishes and reaction time are a mea-

sure of performance. The GAM analysis indicates that the average reaction duration at each station decreased with decreasing DO concentrations ( $p = 0.018$ , fig. 5). A stronger (i.e.,  $p$ -value < 0.05) and positive relationship is found after removing the highest (8.11 ml l<sup>-1</sup>) and second highest (5.20 ml l<sup>-1</sup>) DO observations from the analysis. Average fish size per station did not have a significant effect and was removed from the model at the first iteration. The final fitted model only contained the DO term and explained 52.7% of the variance on a total of 16 stations sampled. However, there was a trend for negative correlation between station DO and average fish size ( $p = 0.065$ ,  $R^2 = 16.7\%$ ), therefore, due to their colinearity, it is not straightforward to disentangle effects of these two variables on fish reaction duration. No significant effects were observed between reaction time and DO concentration even after taking into account the distance from the chain when the fish was first seen. Running and hopping were the two most observed behavior types at all stations (combined 78.6%–90.4%, table 3).

Running was the dominant behavior at station MB 40 (50.9%), NH 05 (53.6%), NH 07 (70.6%), and NH 10 (54.2%). Hopping was the dominant behavior at

TABLE 3  
 Percentage of behavior type (burying, running, hopping, rising, hovering, and no reaction)  
 at stations MB 30, MB 40, NH 05, NH 07, and NH 10.

Station	Burying	Running (R)	Hopping (H)	R+H	Rising	Hovering	No reaction
MB 30	10.5%	37.0%	41.6%	78.6%	5.1%	0.4%	5.4%
MB 40	4.4%	50.9%	37.5%	88.4%	5.6%	0.2%	1.4%
NH 05	1.2%	53.6%	31.7%	85.3%	7.5%	1.6%	4.4%
NH 07	0.0%	70.6%	17.6%	88.2%	11.8%	0.0%	0.0%
NH 10	0.0%	54.2%	36.1%	90.4%	8.4%	0.0%	1.2%

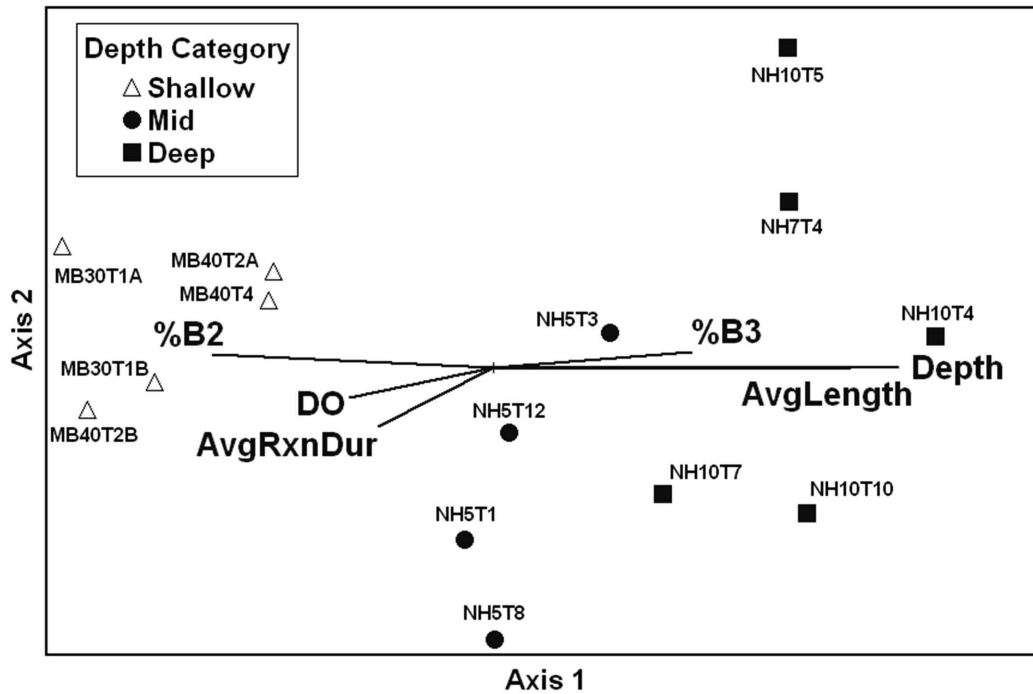


Figure 6. Multivariate analysis comparing the species composition of the catch data to environmental conditions (stress = 9.10). Behavioral values were added to the environmental matrix (B2 is burying and B3 is running). Stations were named using station names (MB 30, MB 40, NH 05, NH 07, and NH 10) and tow (T) number. DO: dissolved oxygen (ml l<sup>-1</sup>).

MB 30 (41.6%). Burying occurred only at stations MB 30, MB 40, and NH 05 (table 3). Hovering was the least common behavior and only observed at mid depth (NH 05).

The non-metric multidimensional scaling (NMS) ordination of the catches data revealed three distinct species assemblages separated by depth (fig. 6). Most of the variance in the community composition was captured by two dimensions containing 78.8% and 11.6%, respectively, of the information in the analytical data set (cumulative for the two-dimensional solution = 90.5%; stress = 9.10). The three community groups suggested by the ordination analysis were tested for similarity between the depth categories as indicated in Figure 6 with a multi-response permutation procedure (MRPP). Results from the MRPP analysis found high within-group agreement ( $A = 0.36, p < 0.001$ ). In community ecology, values for  $A$  are commonly below 0.1, so an  $A > 0.3$  is determined to be high (McCune and Grace 2002).

These results indicate that community composition varied between the three depth categories. English sole, speckled sanddab, and butter sole dominated numerically at the shallower Moolack Beach stations. Along the NH stations, Pacific sanddab dominated numerically, however, flatfish diversity was generally higher at the shallower NH 05 station than further offshore at the NH 07 and NH 10 stations (table 2).

Of the variables measured several showed strong levels of correlation to axis 1, depth ( $r^2 = 0.929$ ), average fish length ( $r^2 = 0.818$ ), DO concentration ( $r^2 = 0.329$ ), and average reaction duration ( $r^2 = 0.264$ ) (fig. 6). Thus, community composition was found to vary with geographic location of the sampled stations and the environmental characteristics occurring within these areas. In addition, average fish length increases with increasing depth whereas DO concentration decreases. The deepest stations (NH 07 and NH 10) have larger fishes and lower DO concentrations.



A relationship between the community composition found at the shallowest depths and burying was observed ( $r^2 = 0.645$ ) (B2 in fig. 6). This behavior was primarily noted at stations off Moolack Beach (table 3). At the deepest stations (NH 07 and NH 10), a relationship was found between the flatfish community composition and running ( $r^2 = 0.455$ ) (B3 in fig. 6) indicating that species in this community tended to run when disturbed rather than bury as the species did in shallower locations at Moolack Beach (table 3; fig. 6).

## DISCUSSION

From the analysis of the in situ video, we have quantified a number of fish performance and behavioral metrics that correlated with water hydrographic properties and species community composition. Because behavior is likely the first trait affected by adverse environmental conditions, we were particularly interested in relating the in situ behavioral response of fishes to DO. Indeed, results from previous laboratory studies indicate that fishes exposed to hypoxia encounter changes in behavior (i.e., panic swimming, reduced spontaneous activity, and paralysis) (Dalla Via et al. 1998; Brady and Targett 2010), in turn affecting predator-prey interactions (Tallqvist et al. 1999), recovery time from exposure to diel-cycling hypoxia (Brady and Targett 2010), and reductions in growth and feeding rates (Stierhoff et al. 2006). However, prior to our work there were no other studies that have looked at the effect of hypoxia on juvenile flatfish species in the nearshore region of the US Pacific coast.

We found that reaction duration, representing the length of time fishes responded to an approaching of the sampling gear, decreased with decreased DO concentration. The effect was particularly strong up to bottom DO concentrations of 3 ml l<sup>-1</sup> and then leveled off. Decreased reaction duration with decreased DO indicates that fishes responded sluggishly at lower levels of DO. The beam trawl is dragged across the seafloor at approximately 0.5–1 knots. This means that fishes that respond sluggishly fall behind the chain quickly and are captured. On the other hand, fishes that respond more energetically have the ability to stay in front of the chain for a longer period of time or outrun the chain and escape. There are at least three potentially interacting reasons that contribute to this result. First, as suggested above, lower DO may cause fishes to reduce the length of their sustained swimming following disturbance. Second, lower reaction duration may be driven by changes in species composition and fish size. We found a clear shift of species assemblages and an increase of fish size with depth where DO levels are lower. However, it is somewhat counterintuitive that increased fish size causes a reduction of reaction duration at low DO

levels, because one would expect that larger fishes are able to perform longer and faster swimming bouts. In our analysis we also found that fish size did not significantly contribute to reaction duration, but fish size and DO are both correlated to depth, so their effect on reaction duration is hard to disentangle. Third, other environmental covariates, in addition to DO, may affect the fish reaction duration. For example, we found that bottom DO was strongly and positively correlated with bottom temperature ( $R^2 = 0.917$ ,  $p$ -value  $\ll 0.001$ )—a result that limits our ability to discern between a DO and a temperature effect on fish behavior.

We found no significant relationship between DO and reaction time (time fish reacted minus time fish was first seen). This neither supports nor rejects the idea that DO affects the ability of juvenile flatfishes to respond to a disturbance. During this study, reaction time ranged from 0–3 seconds and the time the fish was first seen and time the fish first reacted were recorded in one-second increments. This increment may be too limiting, and in future applications it would be more appropriate to use time increments smaller than a second. Other factors to consider are the orientation of fishes to the chain, the direction of the initial response to the disturbance, substrate composition, and whether to redefine the circumstance of initial response time. By redefining the stimuli of an initial response time researchers could take into account circumstances such as fish response to sand plumes generated by sampling equipment and reactions to disturbances by organisms that had previously reacted.

We found differences in juvenile flatfish in situ behavior over the stations sampled. Running and hopping were the two most common behavior types and when combined made up between 78.6% to 90.4% of the observed behaviors at all stations, with increasing percentages toward deeper sites. Because the percentages of fishes that escaped the trawl also increased going offshore, it is clear that running and hopping are effective disturbance avoidance behaviors. Burying on the other hand was only observed at MB 30, MB 40, and NH 05. The multivariate analysis indicated that burying had a strong relationship to the species composition at the shallower stations MB 30 and MB 40. The prevalence of burying at shallow stations may also be related to substrate type, which in this area is dominated by fine sand at depths shallower than 40 m and increases to medium sand for deeper stations. The flatfish communities of the shallower stations were largely composed of small English sole, butter sole, and speckled sanddab in order of dominance.

Besides variations of species composition, we showed that fish size also changed with depth, with the percentage of juvenile fishes being higher at the shallower stations. This trend would suggest a possible nursery role of

inshore locations and an ontogenetic shift toward deeper habitats. While this certainly applies to some of the species we sampled, like English sole (Laroche and Holton 1979; Kryger and Pearcy 1986), it is difficult to conclude that larger individuals of all species move offshore, given that we could not discriminate among species with our video samples. For example, butter sole and speckled sanddab are also abundant at the shallower stations (table 2), but their size does not change in relation to depth (Johnson 2012). It is possible that for these species, shallower habitats (i.e., <30 m) than those sampled here (i.e., >30 m) may serve as nursery habitats. Although not statistically significant from other NH stations, we found that the percentage of juvenile fish decreased slightly at NH 10 compared to NH 07. It is possible that deeper locations may serve as nursery habitats for flatfish species that are deeper in their adult distribution range, such as Pacific sanddab, petrale sole, and Dover sole.

## CONCLUSION

Juvenile flatfishes are an important component of the ecosystem and as they grow into adults and move into deeper waters they become part of the economically important flatfish fishery. The ability for juveniles to escape predation and capture food is key to their survival. If behavioral performance is affected by low DO or temperature values, there can be ecological consequences on the survival of fishes. These effects can be species specific. Benthic species that have low thresholds to DO and experience sublethal effects such as reduced activity (Chabot and Dutil 1999), loss of balance, panic swimming, inactivity, or paralysis (Dalla Via et al. 1998) are consequently vulnerable to predation by species with lower DO thresholds in hypoxic waters (Long and Seitz 2008).

The objective of this study was to examine the feasibility of using an in situ video system and sampling gear to assess behavioral responses of juvenile flatfishes in relation to the physical and chemical properties of the water and the species community composition. We were particularly interested in assessing the responses of juvenile flatfishes to DO concentrations. When looking at the flatfish behavioral metrics we found a positive trend between DO and reaction duration. This result suggests that the ability of juvenile flatfishes to escape predation could be affected by DO concentrations, but this result may be confounded by the covariation of water temperature and species assemblages. Abundance of flatfishes obtained from in situ video analysis was 143% to 322% greater than that measured from the net data (table 2) and has expanded our ability to quantify individual performance and behavioral metrics of juvenile flatfishes. In situ video has proven to be an effective tool to complement trawl survey data (Norcross and Mueter 1999).

The video data allows us to observe greater numbers of organisms and their behavioral responses while the net data provides us with the community composition. An ideal development would be that of identifying species from the video data. However, with the camera system used in this study it was not possible without a considerable margin of error. Newer technologies and higher definition cameras may provide better results. In addition to improved equipment, more data on substrate physical and chemical characteristics could also prove useful in understanding behavioral and abundance metrics of the nearshore flatfishes in relation to hypoxic events. Finally, findings from the analysis of catch data contribute to our current understanding (Pearcy 1978; Toole et al. 2011) of the nearshore juvenile fish assemblages in the Oregon central coast. We have identified clear changes of species assemblages along a depth gradient. Of interest was the fact that while there was a clear trend to larger fishes from inshore to offshore stations, the average size of fishes observed at the deepest station (NH 10) was not the largest. It is possible that deeper locations along the shelf edge may serve as nursery habitats for slope flatfishes like Dover and petrale sole. Our sampling design was developed to monitor coastal habitats, thus additional studies over greater temporal and spatial extent are needed to corroborate this hypothesis and to further characterize the juvenile fish assemblages.

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## LITERATURE CITED

- Barth, J. A., B. A. Menge, J. Lubchenco, F. Chan, J. M. Bane, A. R. Kirincich, M. A. McManus, K. J. Nielson, S. D. Pierce, and L. Washburn, 2007. Delayed upwelling alters coastal ocean ecosystems in the northern California current. *PNAS* 104:3719–3724.
- Barss, W. H. 1976. The English Sole. Oregon Dep. Fish Wildl. Inf. Rep. No. 76-1:7.
- Brady, D. C., and T. E. Targett. 2010. Characterizing the escape response of juvenile summer flounder *Paralichthys dentatus* to diel-cycling hypoxia. *J. Fish Biol.* 77:137–152.

- Breitburg, D. L., T. Loher, C. A. Pacey, and A. Gerstein. 1997. Varying effects of low dissolved oxygen on trophic interactions in an estuarine food web. *Ecol. Monogr.* 67:489–507.
- Buck, E. H. 2007. Marine dead zones: understanding the problem. Congressional Research Service Report for Congress. Order code 98–869.
- Buckley, T. W., G. E. Tyler, D. M. Smith, and P. A. Livingston. 1999. Food habits of some commercially important groundfish off the coasts of California, Oregon, Washington, and British Columbia, 173 p. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-102.
- Chabot, D., and J. D. Dutil. 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. *J. Fish Biol.* 55:472–491.
- Chan, F., J. A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W. T. Peterson, and B. A. Menge. 2008. Emergence of anoxia in the California Current Large Marine Ecosystem. *Science*. 319:920.
- Dalla Via, J., G. Van den Thillart, O. Cattani, and P. Cortesi. 1998. Behavioural responses and biochemical correlates in *Solea solea* to gradual hypoxic exposure. *Can. J. Zool.* 76:2108–2113.
- Grantham, B. A., F. Chan, K. J. Nielsen, D. S. Fox, J. A. Barth, A. Huyer, J. Lubchenco, and B. A. Menge. 2004. Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. *Nature*. 429:749–754.
- Gray, J. S., R. S. S. Wu, and Y. Y. OR. 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. *Mar. Ecol. Progr. Ser.* 238:249–279.
- Johnson, A. 2012. An investigation of the distribution and abundance of ichthyoplankton and juvenile benthic fishes in relation to nearshore hypoxia within the Northern California Current System. MS thesis, Oregon State University, Pp. 92.
- Kintisch, E. 2013. A sea change for U.S. oceanography. *Science* 339:1138–1143.
- Kolar, C. S., and F. J. Rahel. 1993. Interactions of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. *Oecologia*. 95:210–219.
- Kruskal, J. B. 1964. Multidimensional scaling by optimizing Goodness of fit to a Nonmetric Hypothesis. *Psychometrika* 29, 1–27.
- Krygier, E. E., and W. G. Pearcy. 1986. The role of estuarine and offshore nursery areas for young English sole, *Parophrys vetulus* Girard, of Oregon. *Fish. Bull. U.S.* 84:119–132.
- Laidig, T. E., L. M. Kringsman, and M. M. Yoklavich, 2013. Reactions of fishes to two underwater survey tools, a manned submersible and a remotely operated vehicle. *Fish. Bull.* 111:54–67.
- Larocque, W., and R. L. Holton. 1979. Occurrence of age-0 English sole, *Parophrys vetulus*, along the Oregon coast: an open coast nursery area? *Northwest science*, 53(2):94–96.
- Lassuy, D. R. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest) English sole, 17 p. U.S. Fish Wildl. Serv. Biol. Rep. 82 (11.101). U.S. Army Corps of Engineers, TR EL-82-4.
- Long, W. C., and R. D. Seitz. 2008. Trophic interactions under stress: hypoxia enhances foraging in an estuarine food web. *Mar. Ecol. Progr. Ser.* 362:59–68.
- McCune, B., and J. B. Grace. 2002. Analysis of Ecological Communities, 300 p. MjM Software Design, Gleneden Beach, OR.
- McCune, B., and M. J. Mefford. 1999. PC-ORD, Multivariate analysis of ecological data, users guide, 237 p. MjM Software Design, Gleneden Beach, OR.
- Mielke, Jr., P. W., and K. J. Berry. 2001. Permutation Methods: A Distance Function Approach, 344 p. Springer Series in Statistics, Berlin.
- Norcross, B. L., and F. J. Mueter. 1999. The use of an ROV in the study of juvenile flatfish. *Fish. Res.* 39:241–251.
- Pattie, B. H. 1969. Dispersal of English sole, *Parophrys vetulus* tagged off the Washington coast in 1956. *Pac. Mar. Fish. Comm. Bull.* 7:11–14.
- Pearcy, W. G. 1978. Distribution and abundance of small flatfishes and other demersal fishes in a region of diverse sediments and bathymetry off Oregon. *Fishery Bulletin* 76: 629–640.
- Peterson, J. O., C. A. Morgan, W. T. Peterson, and E. Di Lorenzo. 2013. Seasonal and interannual variation in the extent of hypoxia in the Northern California Current from 1982–2012. *Limnol. Oceanogr.* 58(6): 2279–2292.
- Pierce S. D., J. A. Barth, R. K. Shearman and A. Y. Erofeev. 2012. Declining oxygen in the Northeast Pacific. *J. Phys. Ocean.* 42:495–501.
- Reese, D. C., and R. D. Brodeur. 2006. Identifying and characterizing biological hotspots in the northern California Current. *Deep-Sea Res. II* 53:291–314.
- Rogers, J. B., and E. K. Pikitch. 1992. Numerical definition of groundfish assemblages caught off the coasts of Oregon and Washington using commercial fishing strategies. *Can. J. Fish. Aquat. Sci.* 49:2648–2656.
- Sandberg, E. 1994. Does short-term oxygen depletion affect predator-prey relationships in zoobenthos? Experiments with the isopod *Saduria entomon*. *Mar. Ecol. Progr. Series.* 103:73–80.
- Stierhoff, K. L., T. E. Targett, and K. Miller. 2006. Ecophysiological responses of juvenile summer and winter flounder: experimental and modeling analyses of effects on estuarine nursery quality. *Mar. Ecol. Progr. Ser.* 325:255–266.
- Tallqvist, M., E. Sandberg-Kilpi, and E. Bonsdorff. 1999. Juvenile flounder, *Platichthys flesus* (L.), under hypoxia: effects on tolerance, ventilation rate and predation efficiency. *J. Exp. Mar. Biol. Ecol.* 242: 75–93.
- Toole, C. L., R. D. Brodeur, C. J., Donohoe, and D.F. Markle (2011) Seasonal and interannual variability in the community structure of small demersal fishes off the Oregon Central coast. *Mar. Ecol. Progr. Ser.* 428: 201–217.
- Vaquier-Sunyer, R., and C. M. Duarte. 2008. Thresholds of hypoxia for marine biodiversity. *PNAS.* 105:15452–15457.
- Wannamaker, C. M., and J. A. Rice. 2000. Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. *J. Exp. Mar. Biol. Ecol.* 249:45–163.
- Wood, S. N. 2006. Generalized Additive Models: An Introduction with R, 401 p. Chapman and Hall/CRC.