

Where do the fish go in winter? A year of observing seasonal changes in Sequim Bay's nearshore fish community

July 2025

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Abstract

The use of environmental DNA (eDNA) sampling has been proposed as a complementary method to monitor fish species in marine environments. eDNA offers a non-invasive, cost-effective, and scalable way to detect aquatic species. It is appealing in environments where traditional methods are limited by access or visibility, especially in complex or sensitive habitats such as tidal channels and other marine energy sites. Before eDNA can be fully relied upon, we must verify its accuracy against established methods, like underwater photography. In this study, we collected eDNA samples and concurrently deployed a 360-degree camera near the floating dock of PNNL-Sequim in the tidal channel of Sequim Bay once a month for twelve consecutive months. During deployments, the camera remained on the seafloor for several hours overlapping slack tide and captured time-lapse photographs at ten second intervals. Counts and identifications of fish species observed in the images were used to calculate monthly Shannon diversity and Pielou evenness indices. These values were compared across the months using a Kruskal-Wallis test paired with a Conover-Iman post-hoc test. Cliff's Delta was also calculated to quantify the effect size of the monthly differences. No fish were observed from December through April, likely due to seasonal behavior changes within the local fish community. Fish returned to the shoreline in May, with the greatest diversity and evenness recorded in September. These findings reveal substantial seasonal variation in nearshore fish communities. Many monthly comparisons were found to contain statistically significant differences within the diversity and evenness, and even more were found to have large effect sizes, signifying large ecological changes throughout the seasons. The absence of fish observed during the winter months is a key outcome of this survey that will hopefully be reflected in the eDNA results still to come, which would help validate the eDNA approach.

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1.0 Introduction

Marine ecosystems are renowned for their wide variety of biodiversity. In the face of industrial activities at sea, such as energy extraction, extensive monitoring efforts are required to ensure the preservation of these important areas; however, this is made difficult by their dynamic and varied environments. Understanding these ecosystems is important for tracking biodiversity patterns, detecting ecological changes, and informing conservation strategies. Traditional fish survey methods, such as trawling and diver-based observations (Dickens et al., 2011), are often time consuming, potentially harmful to habitats (Schratzberger et al., 2002) and limited in both spatial and temporal scope. These challenges are especially pronounced in high-current or strong-wave areas designated for marine energy development, which offer promising sources of renewable energy but are not without risks for marine animals (Copping et al., 2021; Hemery et al., 2021). As researchers aim to develop more efficient and less invasive survey methods, the environmental DNA (eDNA) approach is emerging as a promising cost-effective and reliable option (Pikitch, 2018).

Through the simple collection and filtration of water samples, eDNA techniques can detect the presence of species by sequencing trace DNA fragments shed into the environment (Thomsen et al., 2012). However, in tidal areas where marine energy infrastructure would be located, constant water movement can displace eDNA before it can be captured (Kelly et al., 2018), complicating detection. Developing reliable approaches for using eDNA in these settings is critical because traditional methods may be unsafe or impractical near marine energy structures. eDNA offers a scalable, repeatable, and affordable technique that could accelerate environmental assessments during the planning, construction, operation, and decommissioning phases of marine energy projects (Fu et al., 2021)

Before eDNA can be employed as a monitoring method around tidal energy projects, it must first be compared against results generated through a traditional survey method in comparable setting. One such method is the deployment of an underwater camera, which offers a passive means of observing marine life over an extended period. Underwater 360-degree cameras are especially useful and can be deployed for several hours and reliably expected to record data on the surrounding environment (Hemery et al., 2022).

The Salish Sea, between Washington state (U.S.) and British Columbia (Canada), is renowned for the extensive marine and anadromous fish diversity that it supports (Pietsch & Orr, 2015). This region is characterized by dynamic climatic and hydrological changes that occur both daily and seasonally, like daily tidal fluctuations and seasonal temperature variations. Due to these highly dynamic environmental conditions, the fish species in this region have adopted unique strategies to survive. Examples include salmonids entering freshwater systems to spawn avoiding the harsh marine conditions (T. P. Quinn & Losee, 2022) and the shore-centered spawning behavior of many forage fish species (Frick et al., 2022). It is necessary to fully understand the fishes' seasonal dynamics over the course of the year to be able to protect sensitive populations and manage risk around human activities at sea.

This study summarizes seasonal observation data obtained through the deployment of a 360-degree underwater camera in the tidal entrance channel of Sequim Bay. By identifying and analyzing fish species detected in the tidal channel over a full year, this study captures seasonal shifts that are often missed in short-term surveys. This study provides a baseline dataset of the temporal dynamics of the Sequim Bay nearshore fish community. This dataset can then be used as a baseline to which the eDNA findings can be compared, helping to assess the reliability of eDNA as a tool for monitoring fish communities in tidal stream environments.

2.0 Methods

This study combined field data collected over 12 months, data processing, and subsequent statistical analyses.

2.1 Camera Deployment and Image Processing

Twelve monthly deployments of a Boxfish 360 underwater camera were conducted in the Sequim Bay tidal channel from the floating dock at the PNNL-Sequim campus starting in July of 2024. The camera was mounted to a lander and positioned at a depth of 5 m during one day of each monthly spring tide (i.e., the monthly tides with the largest water exchange). The camera was deployed for a period of approximately four hours. The Boxfish 360 camera is composed of three synchronized individual cameras that enable a full 360-degree field of view. The camera was oriented horizontally to maximize coverage of the area surrounding the lander, especially the floating dock and nearby pier underwater structure.

The cameras were programmed to record time-lapse photography at 10 second intervals. Following the instructions in the Boxfish 360 Owner's Manual (Boxfish 360 Owner's Manual, n.d.), these images were then extracted using the Boxfish software. The photographs from each camera were reviewed individually rather than using the software to stitch them together due to the varying amounts of photographs taken by each camera. During the deployments, cameras occasionally switched randomly from the time-lapse setting to recording video footage; these video files were not reviewed nor further considered in the analyses.

2.2 Statistical Analysis

Fish were identified to the lowest possible taxonomic level using field guides for fish identification in the Salish Sea (Pietsch & Orr, 2015; (Lamb & Hanby, 2005). Unidentifiable fish were labeled as such and included as their own taxon in the analysis. Because photo counts varied throughout the deployments, subsampling was used to standardize the values across the different months. Using the total fish counts and species identifications, an average number of fish per picture for every species was calculated for each camera. Once the values for each species were calculated for all three cameras, these three values were averaged to account for the differences in the number of photos taken by each camera. Once all fish were counted and identified by a trained biologist, three random samples of 100 photos were selected from each monthly dataset for the subsampling.

Shannon diversity and Pielou evenness values were calculated individually for each monthly subsample. The three monthly values obtained for each index were then used in RStudio (Version 2025.05.1) for statistical analysis (Borcard et al., 2018). The distribution of the dataset was visually evaluated with a histogram. Because it resulted in a skewed (non-normal) distribution, non-parametric tests were selected. A Kruskal-Wallis (Davies et al., 2008) test was conducted in combination with a Conover-Iman post-hoc (Meissel & Yao, 2024) test to determine which months differed significantly from the rest. The Kruskal-Wallis test determines whether there is a significant difference between any of the combinations and the Conover-Iman test analyzes each individual monthly combination to determine which specific months differ. Cliff's Delta was also calculated (cliffdelta.shinyapps.io/calculator/) to estimate the magnitude of differences between months, providing a measure of effect size (Meissel & Yao, 2024; Davies et al., 2008).

3.0 Results

The results from the monthly camera deployments highlighted the temporal trends in observed fish community composition across the year. Over 17,000 images were analyzed and fish from nine different species were identified, counted, and included in the diversity and evenness analyses. There was a large variation in the number of recorded photos for each month due to camera malfunctions. Because of this, the month of October was not included in any analysis due to a lack of photos.

During the period from December to April, not a single fish was observed by the camera during the deployments. Fish began to be detected again in May in relatively small numbers. In July, the largest number of species was detected with a total of 9 (Fig. 1). The highest number of fish per picture was detected in June at a total of ≈ 183 fish/picture (Fig. 2).

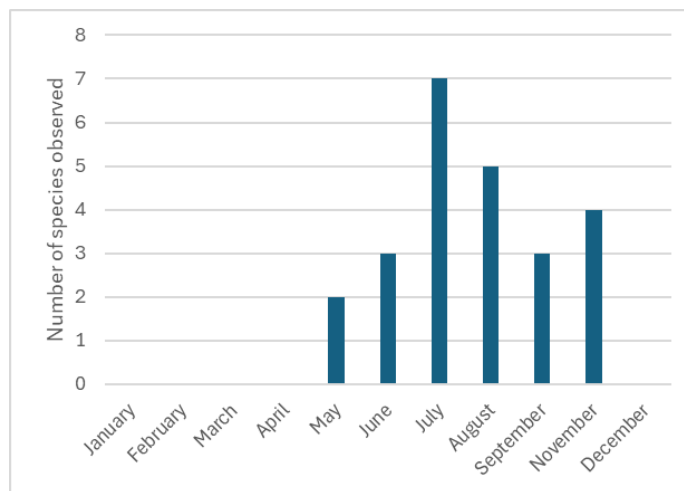


Figure 1: Number of fish species observed during each camera deployment

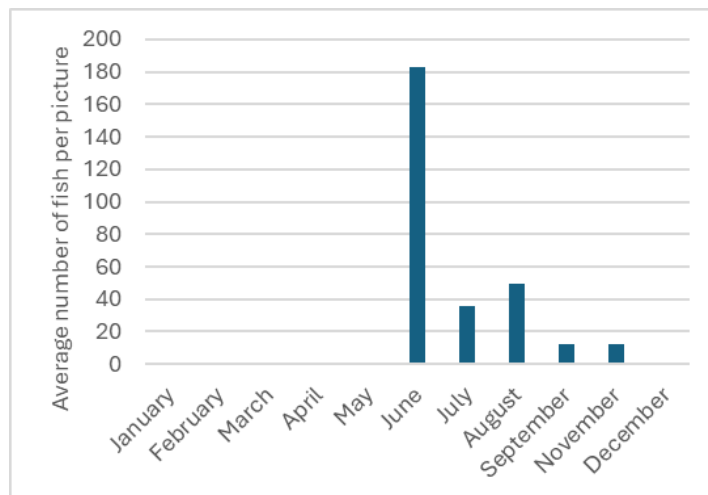


Figure 2: Average number of individual fish observed per picture during each camera deployment (May has a value of 0.841)

Looking at the dominant species for the months in which fish were detected, a clear seasonal pattern appeared when species were most prominent (Fig. 3). The first species seen in large numbers, in June, was the surf smelt (*Hypomesus pretiosus*). By July, the surf smelt have disappeared and the dominant species in July and August was the shiner perch (*Cymatogaster aggregata*). In September, there was a transition period in which the numbers of both shiner perch and Pacific sand lance (*Ammodytes personatus*) were about equal. Finally, as the fall continues, increasing numbers of Pacific sand lance were detected. Other species detected during the months were represented by low numbers of no more than 50 and often less than 10 total observations for the entire month.

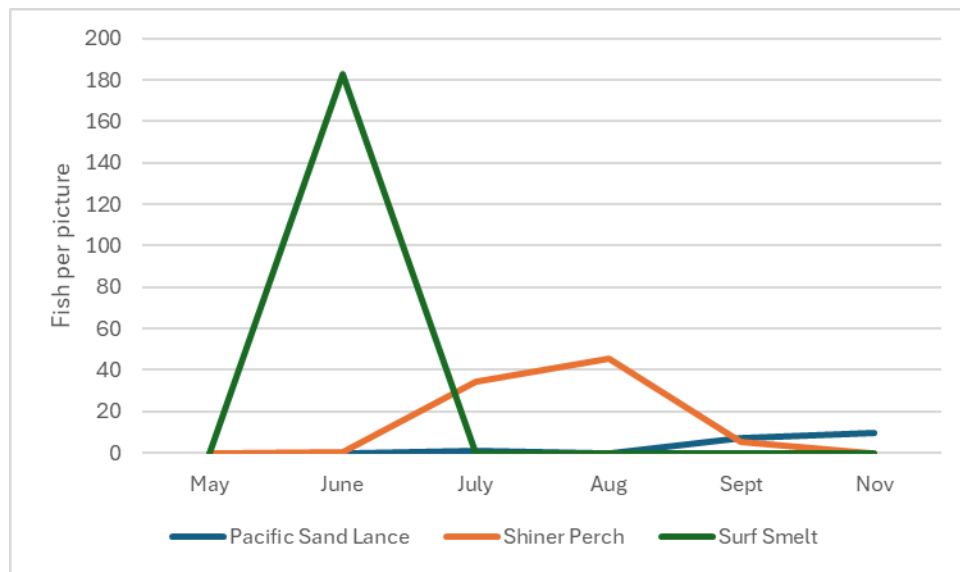


Figure 3: Seasonal changes in the fish per picture count for the three dominant fish species: Pacific sand lance (*Ammodytes personatus*), shiner perch (*Cymatogaster aggregata*), and surf smelt (*Hypomesus pretiosus*)

The analysis of both the Shannon diversity (Tab. 1) and Pielou evenness (Tab. 2) indices revealed stark differences throughout the year. Indices could not be calculated for the winter months due to the absence of fish observations. The highest recorded diversity and evenness indices were found in the month of September, with values of 0.6710 and 0.6110 respectively. The lowest diversity and evenness indices were seen in August, with values of 0.0048 and 0.0069 respectively.

Table 1: P-values (italicized) for the post-hoc Conover-Iman test to analyze the differences between the monthly Shannon diversity indices (diagonal), with statistical significance ($p < 0.05$) denoted by *

Shannon	May	June	July	August	September	November
May	0.0065					
June	<i>0.1678</i>	0.0170				
July	<i>0.0568</i>	<i>0.4316</i>	0.0887			
August	<i>0.3171</i>	<i>0.2591</i>	<i>0.1088</i>	0.0048		
September	<i>0.0002</i>	<i>0.0048*</i>	<i>0.0189*</i>	<i>0.0004*</i>	0.6710	
November	<i>0.0019</i>	<i>0.0504</i>	<i>0.1399</i>	<i>0.0039*</i>	<i>0.2530</i>	0.3263

Table 2: P-values (*italicized*) for the post-hoc Conover-Iman test to analyze the differences between the monthly Pielou evenness indices (diagonal), with statistical significance ($p < 0.05$) denoted by *

Pielou	May	June	July	August	September	November
May	0.0094					
June	<i>0.4463</i>	0.0155				
July	<i>0.1277</i>	<i>0.4137</i>	0.1103			
August	<i>0.4444</i>	<i>0.2758</i>	<i>0.1449</i>	0.0069		
September	<i>0.0012*</i>	<i>0.0089*</i>	<i>0.0556</i>	<i>0.0014*</i>	0.6110	
November	<i>0.0106*</i>	<i>0.0850</i>	<i>0.4271</i>	<i>0.0126*</i>	<i>0.4474</i>	0.3263

The analysis of effect size using Cliff's Delta provided a broader look at the seasonal differences in the nearshore fish community (Tab. 3 & 4). Cliff's Delta effect sizes range from 1 to -1 and the further the value is away from 0, the greater the effect size is. There were more monthly comparisons with a large effect size (i.e., Cliff's Delta) than there were with a statistically significant difference (i.e., Conover-Iman test). In addition, the months of September and November stood out in terms of both diversity and evenness. These results may provide a more practical understanding of the changes that occur throughout the year in Sequim Bay.

Table 3: Cliff's Delta values comparing the Shannon diversity of the months listed in the left column with the months listed in the top row. Values from 0 to 1 mean that the months in the left column have a greater diversity than the months in the top row. Values from 0 to -1 mean that the months in the left column have a lower diversity than the months in the top row

Shannon	May	June	July	August	September
June	0.78				
July	0.78	0.56			
August	-0.33	-0.78	-0.78		
September	1	1	1	1	
November	1	1	1	1	-1

Table 4: Cliff's Delta values comparing the Pielou evenness of the months listed in the left column with the months listed in the top row. Values from 0 to 1 mean that the months in the left column have a greater evenness than the months in the top row. Values from 0 to -1 mean that the months in the left column have a lower diversity than the months in the top row

Pielou	May	June	July	August	September
June	0.33				
July	0.78	0.56			
August	-0.33	-0.78	-0.78		
September	1	1	1	1	
November	1	1	0.78	1	-1

4.0 Discussion

This study reveals stark differences in the seasonal trends within the Sequim Bay nearshore fish community. There are changes within both the number of fish and individual species that are present. As winter conditions settle upon the Pacific Northwest and Sequim Bay, fish observations stop from December to the end of April at PNNL-Sequim floating dock. The fish begin to return in small numbers starting in May and persist in varying numbers until the end of the fall. Many of the seasonal changes are characterized as statistically significant and even more are considered to have a large effect size, meaning there is at least some ecological significance to the observed differences between the months.

The analysis of the diversity and evenness using both statistical significance and effect size provides a clear picture of the changes that occur throughout the year in the Sequim Bay tidal channel. The p-values generated by the Conover-Iman post-hoc test take a more conservative approach to the interpretation of the results than the Cliff's Delta test in that it requires a more pronounced difference to be considered statistically significant. The Cliff's Delta test generates a quantitative look at how to interpret the changes. Although the Cliff's Delta values may leave some ambiguity over which results are more deserving of attention, it provides a look into the level of 'ecological' significance present within the different monthly comparisons. Using statistical significance and effect size leads to variation within the interpretation of the results; however, both methods reveal the striking changes that occur throughout the year within the nearshore fish community.

The complete absence of fish detections from December through April is likely due to seasonal migrations or behavioral changes due to winter conditions. Other studies within the Puget Sound area have come to similar conclusions and provide a variety of reasons for the wintertime disappearances. Pacific sand lance have been found to hibernate in the nearshore sand and gravel during the winter (T. Quinn, 1998). Although the spawning behavior of both surf smelt and shiner perch is well-studied (Quinn et al., 2012; Gordon, 1963), little is known about their whereabouts outside of the spawning season. It is likely that both the surf smelt and shiner perch migrate to deeper water to avoid harsh wintertime conditions along the shoreline such as low temperatures and more dynamic water movements like crashing storm-driven waves. In a similar camera-based study conducted in the Sequim Bay tidal channel during the winter of 2023-2024, forage fish, including shiner perch and surf smelt, were observed in the deeper portions of the channel (Cotter et al., under review).

Similarities to other studies can also be found within the observed fish behavior during the spawning seasons. Shiner perch are known to spawn at various times across their range. Within the Salish Sea region, they have been observed to begin moving into shallower nearshore waters in early June and remain there for the duration of the summer until their numbers begin to decrease as the fall begins (Wiebe, 1968; Gordon, 1963). Surf smelt can be seen spawning at all times of the year within the Salish Sea region; specifically, within the Strait of Juan de Fuca, they are known to spawn from mid-May to October (WDFW, 2013). In Sequim Bay, there is a documented surf smelt spawning site approximately 2 km south of the PNNL-Sequim floating dock where the camera was deployed (Stick et al., 2012). Pacific sand lance are known to spawn from November to February (Matta & Baker, 2020). It is likely that during the later portion of the spawning period, the Pacific sand lance in Sequim Bay have buried themselves in the sand near their spawning grounds (Dionne & Krueger, 2020; (Gotthardt et al., 2005).

As with all ecological survey methods, this camera-based study encountered multiple difficulties that may have altered some of the findings. One major issue was the deployments only once a

month, which limited the power of the statistical analyses and required subsampling within each monthly dataset to create random triplicates. Because some species were represented by low numbers of observations, they were occasionally not included in the subsamples created for each month. This led to some of the months having artificially lower diversity and evenness values than what was observed. In addition, distinguishing cryptic species of fish on underwater imagery can be particularly challenging, and several fish observations were classified as “unknown species”. It is possible that some of these observations of “unknown species” corresponded to more common species that were already included in the dataset for that month, leading to artificially higher diversity and evenness scores. This most likely happened in November, a month with two unknown species that were observed many times. The unknown species in November appeared in smaller schools of around 10-20 individuals that never came close enough to the camera to discern unique features to be able to identify them.

This study provides a year-long visual record of seasonal variation in the nearshore fish community of Sequim Bay tidal channel, creating a baseline dataset for future ecological assessments. The absence of fish during winter months near the floating dock and the heightened diversity and evenness seen in the early fall show clear seasonal patterns, likely due to behavioral and environmental changes. These findings emphasize the need to consider temporal dynamics when interpreting fish survey data. If similar shifts are reflected in the eDNA data collected from the same site in parallel to the underwater imagery, it will help validate the molecular method’s reliability in tidal stream environments. This work supports the integration of camera surveys and eDNA to improve the monitoring of marine fish in the vicinity of renewable energy development.

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Appendix A – RStudio Script

Detailed RStudio script describing the diversity and evenness calculations and their subsequent statistical analyses

```
#packages

library(vegan)

library(picante)

library(knitr)

library(conover.test)

#sample Shannon-diversity calculation

july1_species_counts <- c(36.68, 0.01, 0.01)

sdi_jul1 <- diversity(july1_species_counts, index="shannon")

#this calculation was repeated once for each sample for all months

#sample pielou-evenness calculation using the values from the July1 sample

> pei_jul1 <- sdi_jul1/log(3)

#this calculation was repeated once for each sample for all months

#sample Kruskal-Wallis test and subsequent Conover-Iman post-hoc test

#diversity

> x <- c(0, 0.0195, 0, 0.0257, 0.0207, 0.00473, 0.00502, 0.1665, 0.0947, 0.0102, 0.00211,
0.00204, 0.69, 0.659, 0.664, 0.435, 0.228, 0.412) # response variable

g <- factor(c("may", "may", "may", "june", "june", "june", "july", "july", "july", "aug", "aug", "aug",
"sept", "sept", "sept", "nov", "nov", "nov")) # group variable

kruskal.test(x, g)

conover.test(x, g, method = "holm")

#evenness
```

```
a <- c(0, 0.0282, 0, 0.0234, 0.0189, 0.00431, 0.00457, 0.24, 0.0862, 0.0147, 0.00305, 0.00294,  
0.628, 0.6, 0.605, 0.396, 0.208, 0.375)
```

```
kruskal.test(a, g)
```

```
conover.test(a, g, method = "holm")
```

Appendix B – Selected Deployment Photographs

Included below is a selection of photographs taken during the June 2025 Boxfish-360 lander deployment. The photos show the lander design, the setup and deployment procedures. They were taken at PNNL-Sequim by Shanon Dell on the pier and floating dock.



Figure 4: Team members ensuring the Boxfish-360 camera is securely attached to the weighted lander and the camera settings are adjusted to the correct format.



Figure 5: The Boxfish-360 camera mounted securely on the lander before deployment. GoPro's were mounted to the lander as well to collect timelapse photographs in case of Boxfish-360 malfunction.



Figure 6: Team members on the PNNL-Sequim floating dock lifting the lander and attached Boxfish-360 camera to lower it into the Sequim Bay tidal channel.

Appendix C – Selected Boxfish-360 Timelapse Photographs

Included below is a selection of photos taken by the Boxfish-360 camera during three of the deployments. The pictures show the three dominant fish species that were seen during the summer and fall. They also show some of the different camera angles and features of the tidal channel seafloor.

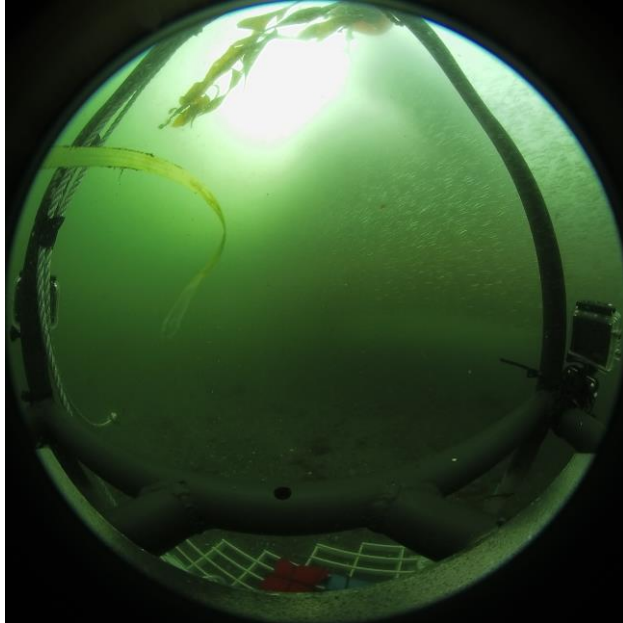


Figure 7: An image taken in June showing a large school of ≈ 250 surf smelt; some kelp pieces were wrapped on the left and top of the lander frame.

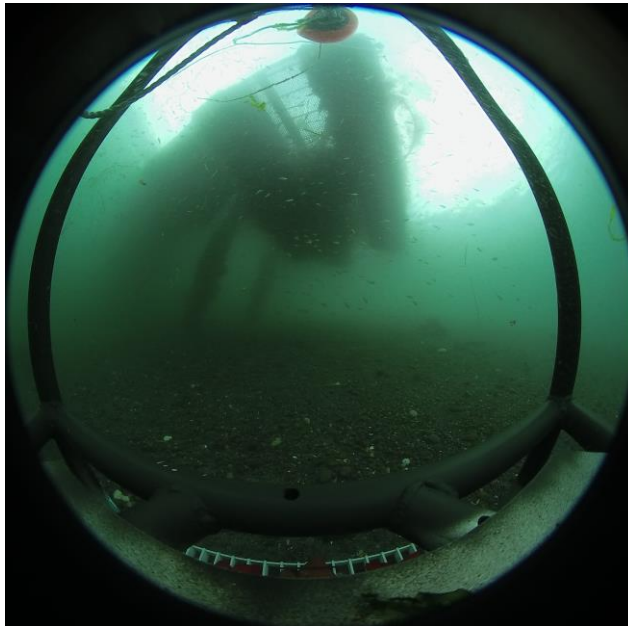


Figure 8: An image taken in August showing a scattered school of shiner perch. The PNNL-Sequim floating dock and pier pilings can be seen in the background, as well as a toggle float at the top of the image.

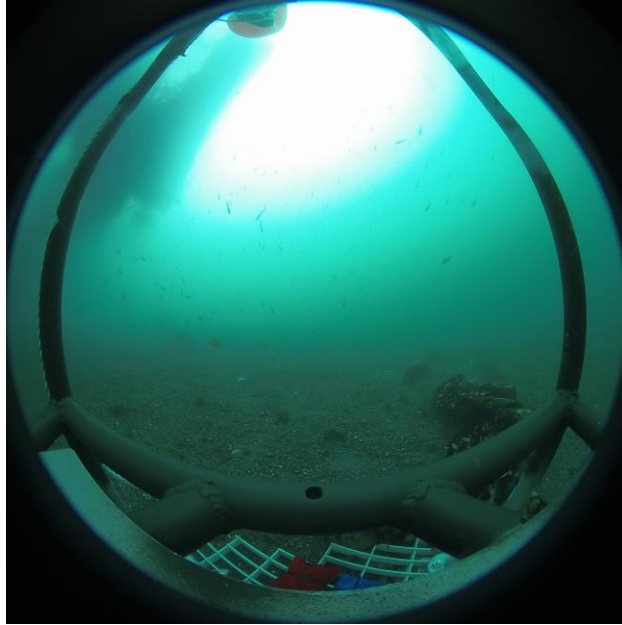


Figure 9: An image taken in September showing a scattered school of shiner perch and sand lance. Part of the PNNL-Sequim floating dock can be seen on the left, as well as a toggle float at the top of the image.

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