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Evaluating User Errors and Temporal Trends in Marine Fish Communities Using 360-Degree Underwater Photography

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Benjamin J Trotter

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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, offering a non-invasive and potentially more efficient approach to marine species observations. eDNA monitoring could be especially useful in and around sites targeted for marine energy generation as these regions need regular monitoring that would be impractical with traditional techniques. Before we can fully rely upon eDNA, we must first verify its accuracy against other proven methods, such as the use of underwater photography. In this study, I deployed a 360-degree camera in the tidal channel of Sequim Bay once a month during several hours overlapping slack tide. I investigated how having multiple people identify and count fish on underwater images could affect the overall results. Using chi square tests in R, I compared my fish identifications and counts to those made by another intern on the same images recorded in August. I found significant differences in the number of species identified and the total individual counts between the two different datasets. I also tested the statistical differences in both Shannon diversity and Pielou evenness indices between the August, September, and November camera deployments using a Hutcheson t-test. Only one significant difference was found in the Shannon index comparisons, and none were found between the Pielou evenness comparisons. These findings show that if multiple identifiers are used to process underwater images, quality control checks must be made to reduce the potential for error. This also points toward the possibility to leverage more advanced image analysis processes, such as automated image analysis software. The findings from this study also show that the dynamics of marine fish communities can vary over a few months; however, further analysis is needed to determine the extent of the seasonal changes in Sequim Bay.

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1.0 Introduction

Marine ecosystems are among the most biodiverse environments on Earth, yet their complexity presents significant challenges for effective monitoring. Understanding these ecosystems is essential for tracking biodiversity patterns, detecting ecological shifts, and informing conservation strategies. Traditional survey methods, such as trawling and scuba 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 areas designated for marine energy development, which offers promising sources of clean energy but are not without risks for marine animals (Hemery et al., 2021) (Copping et al., 2021). As researchers seek more efficient and less invasive survey methods, the environmental DNA (eDNA) approach has emerged as a promising option.

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. 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 in a tidal stream, it must first be compared against results generated through a traditional surveying process. 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). Despite the advantages of underwater cameras, the process of identifying fish species from footages (photos and videos) presents unique problems such as limited visibility and difficulties in identifying discernable characteristics between similar fish species. These issues can be further exacerbated when combined with variations in observer data.

This study explores both the potential and the limitations of using 360-degree underwater photography to support marine biodiversity monitoring in a tidal channel. It also constitutes a preliminary study of the temporal variations of fish species present within the Sequim Bay tidal channel, in parallel to eDNA sampling. By analyzing photographic observations with statistical analyses, this project contributes to improving marine monitoring approaches by increasing the reliability of eDNA sampling. These results will provide a foundation for more accurate, efficient and scalable methods for characterizing marine ecosystems in tidal environments.

2.0 Methods

This study combined monthly field data collection, data processing, and subsequent statistical analyses.

2.1 Camera Deployment

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. The camera was mounted to a lander and positioned at a depth of five meters 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 allow it to have a full 360-degree field of view (Boxfish 360 Owner's Manual, n.d.). 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 splice them together due to the varying amounts of photographs taken by each camera. During the deployments, cameras occasionally switched from the time-lapse setting to recording video footage; these video files were not reviewed nor further considered in the analyses.

2.2 Fish Identification, Counts, and Analysis

Fish species were counted and identified to the lowest possible taxonomic level by myself and another intern associated with the project (respectively Identifier 1 and Identifier 2). Several field guides of marine fishes from the Salish Sea (Pietsch & Orr, 2015) or the Pacific Northwest (Lamb & Hanby, 2005) were used to assist with species identifications**.** Unrecognizable fish individuals were included in the data and labeled as "unidentified". Multiple categories were made to group together unknown fish that had similar shapes and behaviors. Because sculpins (family: Cottidae) are notoriously difficult to identify on images alone, they were included as a single taxon or occasionally placed into two groups based on size.

For the month of August, the two identifiers manually counted and classified the fish species that were photographed by each camera. In each picture, the individual fish species were counted in groups of ten to reduce the amount of time needed to count them. From the two sets of identifications, 100 photos were randomly selected from each of the three camera datasets to be compared for quality control. Chi-square tests

for both the total fish counts and the number of species recorded were conducted in R studio to test for differences between the observers' results.

2.3 Biodiversity and Evenness Analysis

Fish counts and species were also recorded for the months of September and November by a single identifier. The Boxfish-360 failed to record any photos in October, hence the omission of that month. 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 (Fig. 1). The number of pictures used to obtain the average fish-per-picture value only included the photographs taken after the lander settled on the seafloor and any suspended material had cleared from the field of view. Using the *vegan* package in R studio, Shannon-Wiener diversity indices (hereafter Shannon indices) and Pielou evenness indices were calculated for each month using the average fish-per-picture value to assess ecological trends within the Sequim Bay channel (Borcard et al., 2018). These values were then compared in a pair-wise fashion using a Hutcheson t-test in excel (Scheer et al., n.d.).

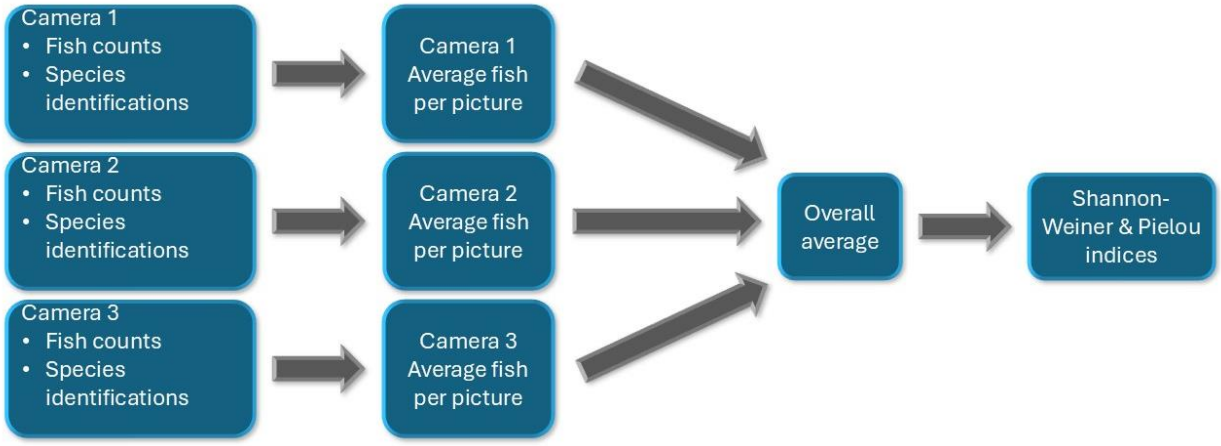


Figure 1: Flow chart describing the process for calculating the Shannon diversity and Pielou evenness indices for each monthly deployment for the Boxfish 360.

3.0 Results

The results of this study highlight both the influence of observer variability, and the temporal trends observed in fish community composition across the sampled months.

3.1 August Quality Control

The August camera deployment resulted in the three cameras recording 176, 237, and 714 images, for a total of 1,127 photographs. There were significant differences between the fish counts recorded by the two identifiers for each of the three cameras (Tab. 1). However, the number of species that were detected by the two identifiers did not differ, except for Camera 2 that showed a significant difference in the number of species (Tab. 2). Identifier 1 systematically counted less fish than the other identifier, with the average number of fish per picture for each camera in August being 34.5, 76.7, and 73.2 for Identifier 1, and 44.8, 90.3, and 83 for Identifier 2 (Fig. 2).

Table 1: The chi-squared and p-values for the quality control analysis of the differences in the total numbers of fish counted in August by the two identifiers (significance denoted by *).

	Camera 1	**Camera 2**	**Camera 3**
Chi-Squared Value	518.9	226.3	241.2
P-Value	2.2e-16*	1.1e-13*	1.1e-7*

Table 2: The chi-squared values and p-values for the quality control analysis of the differences in the total numbers of species identified in August by the two identifiers (significance denoted by *).

	Camera 1	**Camera 2**	**Camera 3**
Chi-Squared Value	1.05	6.28	1.11e-29
P-Value	0.79	0.04*	1

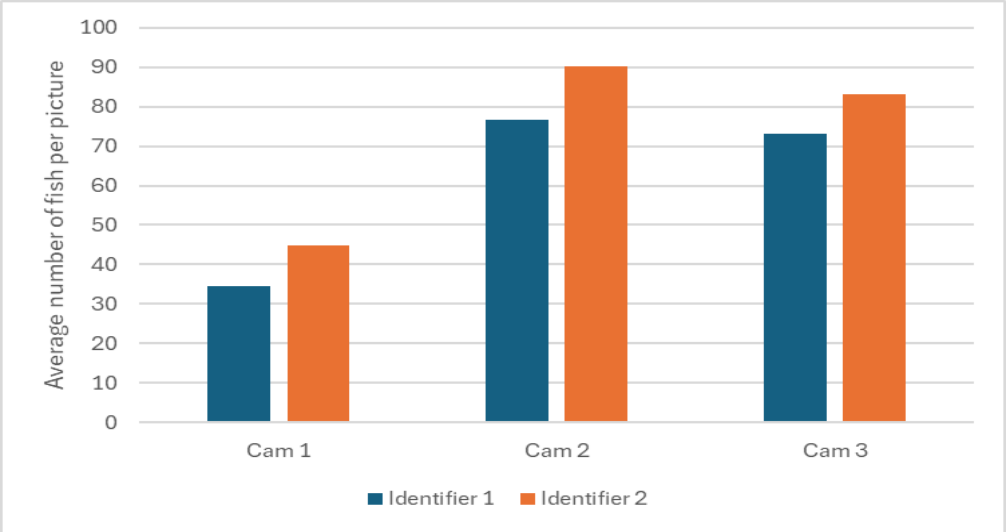
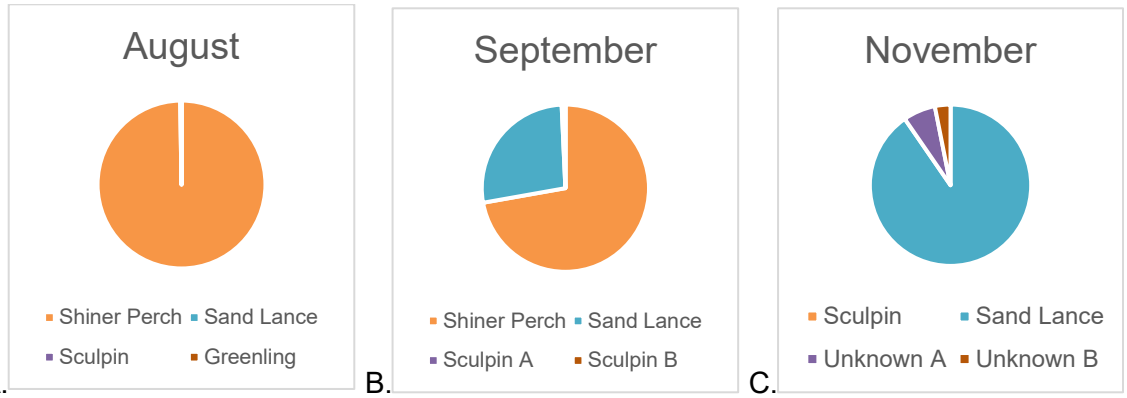


Figure 2: Average number of fish counted per picture by the two different identifiers for the August camera deployment.

3.2 Comparison of August, September and November Datasets

The most abundant fish species in each of the months shifted from shiner perch (*Cymatogaster aggregata*) in August and September to Pacific sand lance (*Ammodytes personatus*) in November. There were no recordings of shiner perch on the November dataset. The other species, such as the unidentified sculpins (family: Cottidae), kelp greenling (*Hexagrammos decagrammus*), and unknown fish, were seen in much lower numbers throughout those months (Fig. 3 A, B, and C).



A. B. C.

Figure 3: Pie-charts showing the proportion of each fish species counted in August (A), September (B), and November (C).

The fish counts and species identifications for the months of August, September, and November resulted in Shannon indices of 0.0198, 0.6245, and 0.3807 respectively (Tab. 3). August-September was the only statistically significant pairwise difference (Tab. 3). The Pielou evenness index calculations for the three months resulted in values of 0.0048, 0.2219, and 0.1481 respectively. None of these values were significantly different from each other (Tab. 4).

Table 3: Shannon diversity index values (diagonal) for August, September, and November; Hutcheson t-test p-values (italicized) are shown on the lower half, with statistical significance denoted by *.

	August	**September**	**November**
August	0.0198	-	-
September	*0.0017**	0.6245	-
November	*0.1914*	*0.428*	0.3807

Table 4: Pielou evenness values (diagonal) for August, September, and November; Hutcheson t-test p-values (italicized) are shown on the lower half, with statistical significance denoted by *.

	August	**September**	**November**
August	0.0048	-	-
September	*0.2045*	0.2219	-
November	*0.5935*	*0.8094*	0.1481

4.0 Discussion

As demonstrated by the quality control results, analyzing visual data can be a subjective process and may lead to large variations in the numbers of fish counted and species identified. For this kind of study to achieve a high degree of accuracy, there must be a greater level of standardization in how the photographs are evaluated and the counts and identifications are made to avoid disparities between identifiers. The difference in fish counts in this study was due to one identifier consistently counting around 10-20 individuals less than the other identifier in larger schools of fish (Fig. 1). Having more identifiers review the images would allow the calculation of an averaged count of fish for each picture. However, this solution would be expensive and time consuming to properly implement. A more realistic to way standardize the identification of marine fish on underwater photographies would be to develop and train a deep learning model to recognize and identify marine fish species in various conditions (Villon et al., 2018).

The Shannon diversity and Pielou evenness indices are commonly used in ecology to quantitatively represent biodiversity in study areas. They are normally calculated using raw counts of the different species that are observed. Multiple statistical tests, such as t-tests and ANOVA (analysis of variance), are commonly used to compare the results from these indices. However, in the case of this study, the counts had to be corrected to account for each camera having a different number of usable photos. Because the diversity and evenness values were calculated from a single deployment, average values for the indices could not be calculated. Performing one of the more common statistical tests was then impossible because they analyze the difference between the average of a dataset. The Hutcheson t-test is a modified t-test specifically designed to compare Shannon values without replicated data, and has been previously used to also compare Pielou values (Scheer et al., n.d.). To compare the index values, this test uses the observations to calculate the variance. Although a useful solution to the problem of a limited dataset, this test is less sensitive to differences in the Shannon and Pielou values being compared because the generated variances are much larger than those commonly used in the other statistical tests. To avoid this problem in the future, it would be best to generate multiple diversity and evenness values for each month, through either more camera deployments or by drawing multiple independent sets of images from the dataset of a single deployment.

The maximum number of fish species identified during this study is seven; however, preliminary eDNA results from the project showed that 62 fish species where present in Sequim Bay tidal channel between July and October (Hemery et al., n.d.). The underwater camera approach may be limited to some fish species as certain behaviors can make some fish less likely to be recorded by the camera or the identifier. Species that are less curious or nocturnal may be underrepresented in daytime underwater camera surveys. Bottom-dwelling fish, like sculpins, can be difficult to detect due to their seafloor-like coloration and the fact that they do not exhibit schooling behavior. In comparison, shiner perch (*Cymatogaster aggregata*) and Pacific sand lance (*Ammodytes personatus*) are much easier to find and count on the camera because they are often high in the water column and travel in large schools. In addition, shiner

perch and sand lance tend to aggregate around artificial structures in the water, like the PNNL-Sequim floating dock and pier. This could have resulted in the large representation of shiner perch in August and September and Pacific sand lance in November (Fig. 3 A, B, C). Both species seem to be the dominant fish species throughout the three months. Their presence during these months is further supported by previous findings stating that the breeding season for shiner perch is in the early Summer and the early Fall for Pacific sand lance (Robards et al., 2000) (Wiebe, 1968). In addition, the area around the PNNL-Sequim campus is known as spawning ground for Pacific sand lance, herring, and surf smelt(Dionne et al., 2019).

The results and limitations in this study point toward the necessity of developing new techniques to better understand the fish diversity and dynamics in tidal areas. Studies using underwater photography can serve as a baseline and comparison for new data collected with eDNA techniques. eDNA surveys will help eliminate the biases caused by the differing fish behaviors and the issues in the subjectiveness of fish count data produced by various identifiers. This will be especially helpful in the context of the continuing growth of marine energy development. As these projects increase, the need to expand the environmental monitoring around the deployment sites will expand as well. The more efficient the sampling process is, the quicker and cheaper it will be to ensure marine energy projects do not cause irreparable harm to their local marine ecosystems.

To further the understanding of the seasonal changes in the Sequim Bay tidal channel, the Boxfish 360 camera will continue to be deployed monthly. The resulting fish identification data will be processed and analyzed to allow for a more sensitive statistical analysis of the temporal trends. This will achieve higher accuracy and clarity in the future results. Overall, this will allow for the creation of a well-established baseline assessment with which future findings can be compared.

5.0 Acknowledgement

I am immensely grateful to my mentor, Dr. Lenaïg Hemery, and my other teammates Kristin Jones, Ian Coughran, and Luciana Pereira for their guidance and help on this project. This work was supported by the U.S. Department of Energy, Office of Science, Office of Workforce Development for Teachers and Scientists (WDTS) under the Science Undergraduate Laboratory Internship Program.

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Pacific Northwest National Laboratory

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