Journal of Visualized Experiments

Development of new methods for quantifying fish density using underwater stereo-video tools

--Manuscript Draft--

Manuscript Number:	JoVE56635R2
Full Title:	Development of new methods for quantifying fish density using underwater stereo-video tools
Article Type:	Invited Methods Article - JoVE Produced Video
Keywords:	Fish ecology, MaxN, fisheries management, underwater visual census, groundfish, rotating drop-camera system
Manuscript Classifications:	95.51: Life Sciences (General)
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Abstract:	The use of video camera systems in ecological studies of fishes continues to gain traction as a viable, non-extractive method of measuring fish lengths and estimating fish abundances. We developed and implemented a rotating stereo-video camera tool that covers a full 360 degrees of sampling, which maximizes sampling effort compared to stationary camera tools. A variety of studies have detailed the ability of static, stereo-camera, systems to obtain highly accurate and precise measurements of fishes; our focus was on the development of methodological approaches to quantify fish density using rotating camera systems. The first approach was to develop a modification of the metric MaxN, which typically is a conservative count of the minimum number of fishes observed on a given camera survey. We redefine MaxN to be the maximum number of fish observed in any given rotation of the camera system. When precautions are taken to avoid double counting, this method for MaxN may more accurately reflect true abundance than that obtained from a fixed camera. Secondly, because stereo-video allows fishes to be mapped in three-dimensional space, precise estimates of distance-from-camera can be obtained for each fish. By using the 95% percentile of the observed distance from camera to establish species-specific areas surveyed, we account for differences in detectability among species while avoiding diluting our density estimates by using the maximum distance a species was observed. Accounting for this range of detectability is critical to accurately estimate fish abundances. This methodology will facilitate the integration of rotating stereo-video tools in both applied science and management contexts.
Author Comments:	Ryan Fields and Christian Denny contributed equally as first authorship. Does JoVE have the ability to list both authors as co-first authors? This would be preferred. If not, please keep Christian Denny as first and Ryan Fields as second.

	We have uploaded two video clips. We would like to use a portion of the survey footage in the actual JoVE video. The video of the calibration bar is intended for the supplemental materials.
Additional Information:	
Question	**Response**
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Lyndsay Troyer, Ph.D.
Science Editor
Journal of Visualized Experiments

Dear Dr. Troyer,

Please accept our submission of the manuscript, "Development of new methods for quantifying fish density using underwater stereo-video tools" for inclusion in the Journal of Visualized Experiments. There has been an increasing use of video camera systems in ecological studies of fishes across the world, especially as non-extractive methods of surveys are needed in areas closed to fishing. We developed and implemented a rotating stereo-video camera tool that covers a full 360 degrees of sampling, which maximizes sampling effort compared to stationary camera tools, and think that JoVE is a good place to let scientists know about our techniques.

Please let me know if you have any questions, and thank you for your efforts to encourage us to submit this manuscript.

Sincerely,

Richard M Starr

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TITLE:

Development of New Methods for Quantifying Fish Density using Underwater Stereo-Video Tools

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KEYWORDS:

Fish ecology, MaxN, fisheries management, underwater visual census, groundfish, rotating drop-camera system

SHORT ABSTRACT:

We describe a new method for counting fishes, and estimating relative abundance (MaxN) and fish density using rotating stereo-video camera systems. We also demonstrate how to use distance from camera (Z distance) to estimate species-specific detectability.

LONG ABSTRACT:

The use of video camera systems in ecological studies of fish continues to gain traction as a viable, non-extractive method of measuring fish lengths and estimating fish abundance. We developed and implemented a rotating stereo-video camera tool that covers a full 360 degrees of sampling, which maximizes sampling effort compared to stationary camera tools. A variety of studies have detailed the ability of static, stereo-camera systems to obtain highly accurate and precise measurements of fish; the focus here was on the development of methodological approaches to quantify fish density using rotating camera systems. The first approach was to develop a modification of the metric MaxN, which typically is a conservative count of the minimum number of fish observed on a given camera survey. We redefine MaxN to be the maximum number of fish observed in any given rotation of the camera system. When precautions are taken to avoid double counting, this method for MaxN may more accurately reflect true abundance than that obtained from a fixed camera. Secondly, because stereo-video allows fish to be mapped in three-dimensional space, precise estimates of the distance-from-camera can be obtained for each fish. By using the 95% percentile of the observed distance from camera to establish species-specific areas surveyed, we account for differences in detectability among species while avoiding diluting

density estimates by using the maximum distance a species was observed. Accounting for this range of detectability is critical to accurately estimate fish abundances. This methodology will facilitate the integration of rotating stereo-video tools in both applied science and management contexts.

INTRODUCTION:

Along the U.S. Pacific Coast, many of the species important to commercial and recreational groundfish fisheries (*e.g.*, the rockfish complex (*Sebastes* spp.) and Lingcod (*Ophiodon elongatus*)) are strongly associated with high-relief, hard-bottom habitats[1–5]. Stereo-video drop cameras are an attractive non-extractive tool to use in rocky habitats due to the relative ease and simplicity of operation. A variety of stereo-video camera systems have been developed and deployed in southern-hemisphere, shallow-water ecosystems[6–10], and recently, video drop-cameras have gained traction as a management tool for deep water rocky-reef environments along the Pacific Coast[11–13]. We sought to modify these existing stereo-camera designs by using a stereo-video camera system (hereafter referred to as "Lander") to more efficiently characterize fish populations in high-relief seafloors along the central Pacific Coast (see **Table of Materials**). The Lander used was different than existing video systems because cameras were mounted to a central rotating bar, which allowed for a full 360° of coverage of the seafloor at the drop location[14]. The Lander completed one full rotation per minute, which allowed us to rapidly characterize the abundance and community composition of an area and achieve the same level of statistical power with fewer Lander deployments. (See Starr (2016)[14] for greater detail on the specifics of the Lander configuration). Preliminary tests in the study system suggested that eight rotations of the cameras in our surveys were sufficient to characterize species abundance and richness. This determination was made by an observation of diminishing returns in species abundance and fish density over longer drops. We recommend that a pilot study including longer soak times be conducted in any new system to determine the optimal soak time for a given ecosystem/study species.

By using paired stereo cameras, both total survey area and absolute fish density can be calculated for each video survey; however, the use of rotating cameras necessitated the modification of traditional fish count metrics. Stationary video systems most often use "MaxN" as a conservative count of fishes on a deployment[6,10]. Traditional MaxN describes the maximum number of fish of a given species observed together in a single video frame, in order to avoid double counting a fish that has left and returned to frame. MaxN has therefore been an estimate of the minimum number of fish known to be present and may underestimate true fish abundance[6,10]. The MaxN metric was redefined to represent the greatest number of fish seen in each full rotation of the cameras.

The second modification to previous stereo video methods was to account for the fact that species of various sizes, color, and shapes have different maximum distances of reliable identification. For example, large species such as *O. elongatus* have a distinct elongated shape and can reliably be identified at much greater distances compared with small and cryptic species such as the Squarespot Rockfish (*Sebastes hopkinsi*). These different maximum ranges of detectability change the effective area sampled by the Lander for each species. Because the stereo cameras allow us to place every fish in three-dimensional space with a high degree of accuracy, one can determine the distance from the cameras that each fish was measured (*i.e.*, the "Z distance", named for the "z-axis" which is perpendicular to the straight line drawn between the cameras). For each species, the distance within which 95% of all individuals were observed (hereafter "95% Z distance") was considered to be the radius of the survey area, and was used to calculate the total area surveyed. In addition to species-specific characteristics, identifiability will be impacted by environmental conditions such as water turbidity. Because these factors can vary in time and space, it is important to use the 95% Z statistic only in aggregate. While it will be highly accurate for large samples, any one individual survey may vary in area surveyed.

The protocol detailed below provides guidance on how to create and use these metrics. Though the focus was to characterize deep-water rocky habitat along the Pacific Coast, the methodology described for modified MaxN count is readily applicable to any rotating drop-camera system. The number of camera rotations needed to characterize fish populations will depend on local ecosystem dynamics, but the conceptualization of the modified MaxN will remain the same. Similarly, whereas we used 3D photogrammetric software to analyze stereo video, the techniques described herein are easily applied across software platforms, as long as the precise location of fish in three-dimensional space is possible. Additionally, the approach of applying a 95% Z distance value could be considered in future studies with stereo-cameras to account for species-specific ranges of detectability and to more accurately calculate fish abundance.

PROTOCOL:

Note: Screenshots of software steps are included as **Supplementary Files.** Please note that the software steps described below are specific to the chosen software (see the **Table of Materials**). The overall approach can be extended to any stereo software platform.

1. Prepare Stereo-Camera Footage for Analysis

NOTE: Calibration using a calibration cube is recommended. A calibration cube is a three-dimensional aluminum-frame with precisely positioned reflective dots on the surface. When used in conjunction with calibration software, a calibration cube leads to greater precision and accuracy than checkerboard approaches[9].

1.1. Calibrate the Lander cameras with stereo-calibration software (**Figure 1** and **Figure 2**; see **Table of Materials** for a software recommendation).

NOTE: Calibration can be verified before use in the field by measuring targets of known sizes at varying distances (see **Supplementary Video 1**). Average measurement error for a 50-cm target at distances of 3 m (or less) should be within 2% of the known target length. Also note that a given calibration will only be valid if camera positions do not change relative to one another. It is critical to take care and avoid unintended jostling of the cameras until all sampling has been performed.

1.2. Collect field data using the calibrated Lander (**Figure 1**, **Supplementary Video 2**).

1.3. After field study is complete, create a new project folder containing both video and calibration files.

Note: In each project folder there needs to minimum of five files: the left and right ".Cam" calibration files, the left and right video files (.MP4 or.AVI format only), and the species list (.txt format).

1.4. In the stereo measurement software, start a new measurement project, and load appropriate video and calibration files.

Note: Screenshots of all software steps are available among the **Supplementary Files**.

1.4.1. Navigate to 'Measurement' > 'New measurement file'.

1.4.2. Set the picture directory by navigating to 'Picture' > 'Set picture directory', and choose the folder containing all project files.

1.4.3. Load the left camera ".Cam" file by navigating to 'Stereo' > 'Cameras' > 'Left' > 'Load camera file' and selecting the appropriate file.

1.4.4. Repeat step 1.4.3 to load the right camera ".Cam" file

Note: These files contain calibration measurements for each camera (*e.g.*, pixel size, aspect ratio, radial distortion, decentring distortion, *etc.*) and will be used to measure fish and calculate distance-from-camera (*i.e.*, Z distance).

1.4.5. Define the movie sequence for the left video file by navigating to 'Picture' > 'define movie sequence' and selecting the left camera video file.

1.4.6. Load the left video file into measurement software by selecting 'Picture' > 'load picture'.

NOTE: Be sure that the 'Lock' box is unchecked before loading video files. This allows both videos to be loaded simultaneously.

1.4.7. Define movie sequence and load video file for the right video using the menus 'Stereo' > 'picture' > 'define movie sequence' and 'Stereo' > 'picture' > 'load picture'.

1.4.8. Load the species list by navigating to 'Measurement' > 'Attributes' > 'Edit/load species file'.

1.4.9. Enter survey ID information under 'Information Fields' > 'Edit field value' and save file to create an.EMObs project.

1.5. Sync the videos using light flash, handclap, Coordinated Universal Time (UTC) stamp, or any time specific event that occurs in both videos.

1.5.1. If using UTC time stamp, frame-step forward in the left video until the time stamp starts a new second. Else frame forward until light flash or handclap occurs.

1.5.2. Frame-step the right video forward until the time stamp matches the left video exactly. Else frame step forward until the exact moment the light flash or handclap matches the left video.

Note: It is important that videos be synchronized to the same frame. Video synchronization should be checked periodically using the video time stamp to avoid camera frame drift during analysis. A filmed hand clap could also be used at the beginning and end of the video to test that right and left videos were synced to the same frame.

1.6. Click the “Lock” button to ensure videos play together and maintain synchronization.

2. Generate Point Counts and Calculate MaxN

NOTE: Each fish is initially marked with a 2D point to the lowest possible taxonomic resolution. Fish with uncertain ID should be marked for later review.

2.1. Wait to begin counting fish until the end of a complete camera rotation to ensure that a full 360 degrees is used. Also wait until sediment has cleared (generally <1-2 min after contact with bottom).

2.1.1. As soon as the Lander starts its first rotation, right click to define a new sample period: ‘Period definitions’ > ‘Add new start period’. Enter first period name as “01” and click “OK”.

2.2. As the Lander rotates, begin marking each fish that comes into frame with a 2D point using the left camera only.

2.2.1. To add a 2D point, right click, select ‘Add point’, and choose the correct species name. Label to the lowest possible taxonomic level, selecting ‘spp.’ for unknown species and click “OK”.

2.2.2. Continue to mark each new fish according to step 2.2.1 until the conclusion of the rotation.

2.3. Repeat protocol procedures 2.1 – 2.2 for each additional Lander rotation – ensuring that a new period is defined at the start of each camera rotation.

NOTE: Species accumulation curves were used to determine that eight rotations were, on average, sufficient to characterize fish abundance in the present study. Researchers should consider conducting preliminary tests with additional camera rotations, over longer soak times, to characterize the optimum number of camera rotations within a particular ecosystem.

2.4. Calculate species-specific counts of individuals observed per camera rotation.

2.4.1. After all rotations have been enumerated, export 2D points by navigating to 'Measurement' > 'Measurement summaries' > 'Point measurements' and save 2D points as a.txt file.

2.4.2. Open the saved 2D.txt point file as a spreadsheet and create a PivotTable of species vs. rotation number to summarize counts (**Table 1**) by navigating to 'Insert' > 'PivotTable'. Select "Genus and Species" for 'Row Label', and "Period" for 'Column Label'.

2.5. Choose the MaxN for each species by selecting the camera rotation that has the greatest number of individuals of that species (**Table 1**).

2.6. For fishes identified only to genus, select a genus-level MaxN based on the rotation that had the greatest number of individuals identified to species in that particular genus.

Note: This step helps to avoid double-counting individual fish that were only identifiable to higher taxonomic groups (*e.g.*, only to genus or family). For example, in **Table 1**, 'rotation 1' contained 10 unidentified *Sebastes* spp. and 33 members of the genus *Sebastes* identified to species, whereas 'rotation 3' contained only two unidentified *Sebastes* spp. and 43 members of the genus *Sebastes* identified to species. Therefore 'rotation 3' would be used for MaxN count of unknown *Sebastes* spp. In this way, the conservative assumption is made that 8 of the unidentified *Sebastes* spp. in 'rotation 1' were identified in 'rotation 8'.

2.7. If multiple rotations have the same MaxN count for a given species, choose the first rotation with MaxN for 3D point measurements.

2.8. For each species, take 3D measurements of fish in the rotation that MaxN occurred.

2.8.1. Use the saved 2D points collected in steps 2.1 – 2.3 to navigate to the exact same fish for 3D measurement.

2.8.2. Zoom in at least 4X to better identify the tip of the fish snout and edges of caudal fins (**Figure 3**).

NOTE: It may be necessary to frame step forward or backward to find the best orientation of the fish for a 3D measurement. The 'best' orientation is one where both the snout and edges of the caudal fins are visible in both cameras.

2.8.3. Manually click on the tip of the snout, then the edge of the tail in the left camera, then repeat the selection in the same order in the right video.

2.8.4. Select correct species identification from dropdown menus as was done in 2.2.1.

2.8.5. If a 3D length measurement is not possible, for instance if the head and tail of the fish are not visible in both cameras, then mark a 3D point instead by left clicking the same position of the fish in both the left and right videos. Fill out the information fields as before and leave the comment "Exclude from length measurement".

NOTE: MaxN may occur on different rotations of the cameras for different species; however, for any given species, measurements should occur in one rotation only (**Table 1**).

2.9. After completing 3D measurements for all fishes, export data as.txt file for further analysis.

2.9.1. Navigate to 'Measurement' > 'Measurement summaries' > '3D Point and length measurements', and save.txt file to export.

3. 95% Z distance procedure for species-specific survey areas

Note: The 95% Z distance is an estimate of the average distance a species could reliably be identified in a given study while excluding cases of exceptional conditions of water clarity or lighting. This calculation takes into account the average oceanographic conditions for a given study and will need to be re-calculated for each new study.

3.1. Use simple bootstrapping to determine if the sample size is great enough to characterize the distance of reliable detection for each species.

3.1.1. For each sample size class (*e.g.*, sample size bins of 5 fish), take 1,000 random draws of the selected sample size with replacement from the sample population and calculate the mean 95% quantile of distances of these 1,000 draws, and plot the resulting asymptotic curve. See supplied code in **Supplemental Files 1** & **2**.

3.1.2. Verify that adequate samples were obtained by comparing the actual sample size with the 95% Z distance asymptote with increasing sample size.

3.2. Calculate the 95% Z distance value as the 95% quantile of distance-from-camera measured for a species across all surveys.

3.3. Calculate the effective area surveyed for each species using the 95% Z value.

Note: In the case of a rotating Lander, the 95% Z value represents the outer radius of a surveyed swath, with the inner radius determined by the physical setup of the tool and how close to the base the cameras are able to observe. As the Lander rotates, a 'donut' shaped survey area is formed (**Figure 4**).

3.3.1. Calculate area surveyed as:

$$A_{Total} = A_{Large\ circle} - A_{Small\ circle} = \pi(95\%\ Z\ distance)^2 - \pi(Z\ \text{minimum})^2$$

Note: For example, a relatively large species like Yelloweye Rockfish (*Sebastes ruberrimus*) had a 95% Z distance of 3.3 m and an effective survey area of 30.9 m^2 per Lander deployment: 34.3 m^2 (outer circle) - 3.4 m^2 (inner circle) = 30.9 m^2 (total survey area).

4. Using the calculated area surveyed (step 3.3.1), convert individual species counts (MaxN) into density estimates for each visual survey using the equation:

$$\text{D} = \left(\frac{N\ Fishes}{m^2}\right) = \frac{\text{MaxN (N Fishes)}}{\text{-Area}_{Total}\ (\text{m}^2\ )}$$

NOTE: A similar procedure could be used to calculate a volumetric density rather than an areal density; however, that process is not described here.

[Place Table 1 here]
[Place Figure 1 here]
[Place Figure 2 here]
[Place Figure 3 here]
[Place Figure 4 here]

REPRESENTATIVE RESULTS:

Between 2013 and 2014, we conducted 816 surveys with the rotating stereo-video Lander (**Figure 1**) along the central California coast and collected MaxN and 95% Z distance (**Figure 4**) data on more than 20 species. There were clear patterns in the effective detectable range of species observed, likely due to the interaction of species' size, shape and coloration (**Figure 5**). For instance, the Flag Rockfish (*Sebastes rubrivinctus*) has distinct banding on its sides allowing for confident identification at greater distances than other species of comparable size. Similarly, Canary Rockfish (*Sebastes pinniger*) are relatively large bodied, but have a pigmentation that is similar to other species, thus making it more difficult to identify at distance (**Figure 5**).

We use two species to demonstrate the calculations of both MaxN and 95% Z distance values: Pygmy Rockfish (*Sebastes wilsoni*) and Lingcod (*O. elongatus*). The former is a small-bodied fish that can be difficult to identify at distance; whereas *O. elongatus* is relatively large, has a distinct shape, and is more easily identifiable. From 2013–2014, 1,191 measurements for *S. wilsoni* and 1,222 measurements for *O. elongatus* were collected. Then, the 95% quantiles of distances at which these species were observed: the 95% Z distances were 2.65 m for *S. wilsoni* and 3.96 m for *O. elongatus* (**Figure 5**) were calculated. These 95% Z distances translate into effective survey areas of 18.6 m^2 and 46.0 m^2 for *S. wilsoni* and *O. elongatus,* respectively. A simple bootstrap analysis confirmed that sufficient sample sizes were obtained for characterization of 95% Z distance values. For both species, the estimate of 95% Z distance stabilized when greater than 50 surveys containing these species were sampled, providing strong evidence that the chosen sample sizes were more than adequate to characterize the effective Lander sample area for these species (**Figure 6**).

MaxN counts per survey were then converted into densities (number of fish/m^2). We used density estimates from the 816 surveys to test the hypothesis that Lingcod and Pygmy Rockfish would be observed primarily on high relief habitats. For both species, there were significantly greater densities over high and medium relief compared with low relief habitats (Kruskal-Wallis, p <<.001; **Figure 7**). These results were consistent with previously reported habitat associations for both species[15]. There were no differences between medium and high relief habitat for either species.

To understand how the rotating Lander compared with traditional stationary camera systems, we estimated differences in density and variability estimates between a rotating and a simulated stationary Lander. We assumed a typical stationary single-camera Lander would have a 90-degree field of view. The rotating Lander has a 60-degree field of view, and requires 5 seconds of rotation to complete a 90-degree view. Using 261 surveys, we selected fish observation data from the middle 5 seconds of Lander rotations to establish MaxN. Density estimates for the pseudo-stationary Lander were standardized by using the reduced areas of coverage (*i.e.*, approximately ¼ the area of the rotating Lander). Differences in mean density and coefficient of variation between rotating and pseudo-stationary Landers were evaluated with Welch's t-test. Mean densities obtained by the rotating camera were 18% greater than those obtained with stationary cameras (Welch's $t_{21.7}$, $p = 0.081$, **Figure 8A**). Additionally, the coefficient of variation was 1.8 times greater with the stationary camera compared to rotating cameras (Welch's $t_{15.1}$, $p < 0.001$, **Figure 8**).

[Place Figure 5 here]

[Place Figure 6 here]

[Place Figure 7 here]

[Place Figure 8 here]

FIGURE AND TABLE LEGENDS:

Figure 1: Stereo video Lander. Key hardware is numbered **(1)** 300 m umbilical, **(2)** two digital video recorders (DVR) with removable 32GB storage cards inside waterproof bottle, **(3)** two LED lights outputting 3,000 lumens at a color temperature of 5,000 K, and **(4)** two cameras with 620 TV line (TVL) resolution.

Figure 2: Calibration cube (500 mm x 500 mm x 300 mm). Example of a calibration with a 'calibration cube' shown in two different orientations: **(A)** the right side of the cube is pushed out towards cameras, and **(B)** the face of the cube is parallel to the face of the cameras. Red dots denote the reference points used in this particular calibration method and must always be identified in the numbered order.

Figure 3: 3D measurement placed on *Sebastes miniatus*. The tip of the snout and end of the tail were identified in each camera frame to allow for stereo measurement.

Figure 4: Area surveyed by the Lander tool. Effective area surveyed by the Lander tool was bounded by the minimum Z distance, and the 95% Z distance for each species. Note that this area created a 'donut' shaped survey volume around the Lander.

Figure 5: Z distances observed for select species. Red vertical bars denote the minimum Z distance (0.81 m from cameras) on the left and the 95% Z Distance value on the right. Note that this represents the average effective survey area around the Lander for each species.

Figure 6: Bootstrapped Z distance values. Bootstrapping to increase the sample size for (**A**) *S. wilsoni* and (**B**) *O. elongatus* observations. Sample sizes ranging from 3 – 300 were bootstrapped 1,000 times each to calculate the mean 95% Z distance and verify the sample sizes were adequate. Note that the y-axis values range from 2.0 – 2.6 m for *S. wilsoni* and from 2.6 – 4.0 m for *O. elongatus.*

Figure 7: Habitat differences for two select species. Average densities (± SE) of (**A**) *S. wilsoni* and (**B**) *O. elongatus* measured on low, medium, and high relief rock habitat.

Figure 8: Differences between rotating and pseudo-stationary landers. Both estimates of (**A**) mean density (fishes/m^2 ± SE) and (**B**) mean coefficient of variation (CV) ± SE for 261 surveys are presented.

Table 1: Example MaxN summary table. The selection of MaxN for each species is demonstrated with red and bold text. Note that a conservative MaxN for unidentified *Sebastes* spp. was determined by the rotation with the most *Sebastes* identified to species (rotation 3). Also, while this study used eight camera rotations, only four rotations are displayed in **Table 1** for simplicity. The process for selecting MaxN is identical regardless of the number of rotations.

Supplementary Video 1: Calibration verification. Calibration can be verified before use in the field by measuring targets of known sizes at varying distances.

Supplementary Video 2: Underwater Survey Footage.

DISCUSSION:

The traditional MaxN metric is predicated on the idea of counting a guaranteed minimum number of individuals present during a survey. If a certain number of fish are simultaneously visible in a single video frame, there cannot be any fewer present, but because fish are mobile and heterogeneously distributed, the likelihood of seeing all individuals simultaneously during a single video frame is low. It is therefore likely that traditional MaxN underestimates true fish abundance[16,17]. Additionally, it has been demonstrated that traditional MaxN may display non-linear negatively-biased relationships with increasing fish abundances[16,18]. This may be related to the phenomenon of gear saturation whereby relative abundance indices fail to detect true increases in abundance[19,20]. Conversely, the apparent stability of an index with truly declining fish abundance has been termed 'hyperstability', and may ultimately lead to the crash of fish populations[21,22]. A recent study reported that instability in MaxN could be alleviated by increasing the surveyed field of view[16]. In that study, the relationship between MaxN and true abundance became increasingly linear as the field of view approached 100% (*i.e.*, 360 degrees).

The results from the stationary camera simulation indicate congruence with these previous results, and suggest that the MaxN value may better characterize fish abundance. For example, the estimated mean coefficient of variance was reduced among density estimates derived from the rotating Lander compared with the pseudo-stationary Lander. This is likely due to the fact that fish are heterogeneously distributed, and that stationary cameras are more likely to 'miss' the fish present if the Lander faces the wrong direction. Rotating Landers maximize sampling effort by surveying the full 360 degrees around the tool, and the net effect is reductions to both sampling cost and variance, and an overall increase in the statistical power of the study. Future studies could better address this issue by directly testing a rotating Lander with a separate stationary Lander in a paired survey design. Similarly, we were unable to directly test for the relationship between MaxN and true abundance in this study, and future studies might directly test this using either simulation or controlled environments, as was done in Campbell (2015)[16].

A possible criticism of the modified MaxN approach is the possibility of double counting individuals. Because the Lander made one full rotation per minute, and the benthic species of interest in the ecosystem tend to be relatively sedentary and slow moving under most conditions, we believe the risk of double counting was low. Additionally, cases where fish would enter or leave the survey area over the course of the eight rotations were observed. Additional precautions to avoid double counting such as using the rotation with the greatest number of individuals of a given Genus to count unidentified species were taken. Other metrics have been proposed as indices of fish abundance such as Mean Count; however, these too have been shown to consistently underestimate true abundance while increasing variability among density estimates[16]. MaxN is therefore recommended as a more precise metric of fish abundance. While our modified MaxN metric does not guarantee a conservative estimate of absolute minimum number of individuals, we are overall confident that this modified MaxN approach provides better estimates of true fish abundance, and that over-counting fish is of relatively low concern.

Many side-viewing video-transect surveys use a fixed transect width to estimate density for all species. Similarly, one approach to using stereo-video Landers would be to use one maximum distance-from-camera to calculate both area surveyed and fish density. Both may lead to an underestimate of species which are only reliably identifiable to smaller distances than the fixed transect width estimates[23]. The distance to which a species is reliably identified is caused by the interaction of factors such as size, shape, coloration pattern, fish behavior, as well as environmental factors. The 95% Z distance method is particularly advantageous in that it accounts for the interaction of all these factors simultaneously. For example, *O. elongatus* was the species that we are able to identify to the greatest distance, likely as a result of its distinct, large, elongate body shape and behavioral tendency to lay on the seafloor. Rosy Rockfish (*Sebastes rosaceus*) had one of the shortest Z distances, likely because, as a member of the

Sebastomus subgenus, it has several congeners that look very similar and are difficult to distinguish at increased distances. By allowing for species-specific areas surveyed by the Lander, we may be able to more accurately estimate fish abundance. The bootstrap approach to sample size verification is simple and readily implemented in other surveys, and we believe the method of 95% Z distance could be further adapted to accommodate line transect survey design. 95% Z distance would then represent a horizontal distance of reliable detection for species observed with submersible or remotely operated vehicle (ROV) tools. In the future, researchers may investigate using distance sampling theory to model density as a function of detectability with distance[23,24].

As there is greater use of no-take reserves in fisheries management[25–27], there is an increasing need for non-extractive sampling techniques, especially in deep water habitats not accessible to diver surveys. However, it is also necessary that those techniques provide accurate, reliable data on fish length, abundance, and species composition. Video Landers are a relatively new monitoring tool that have a low cost, can be operated on relatively small vessels of opportunity, and are logistically simpler to operate than ROVs and submersibles while requiring fewer and less skilled personnel. While not discussed in these methods, stereo-camera Landers are capable of accurate length measurements with error less than 2%. Additionally, Landers can be rapidly deployed over large geographic areas, increasing statistical inference. We expect the interest in video monitoring tools to increase as research agencies look to tighten budgets and more efficiently spread sampling effort. Our modification of MaxN and 95% Z distance should be considered in future ecological studies utilizing rotating video Landers.

ACKNOWLEDGMENTS:
This work was funded by The Nature Conservancy and private donors, Resources Legacy Fund Foundation, Gordon and Betty Moore Foundation, Environmental Defense Fund, California Sea Grant Program, the NMFS National Cooperative Research Program, and a NOAA Saltonstall-Kennedy Grant #13-SWR-008. Marine Applied Research and Exploration (Dirk Rosen, Rick Botman, Andy Lauerman, and David Jefferies) developed, constructed and maintained the video Lander tool. We thank Jim Seager and SeaGIS™ software for technical support. Captain and commercial fisherman Tim Maricich and crew onboard the F/V *Donna Kathleen* provided support in deploying the Lander from 2012-2015. Thank you to all who participated in video data collection or analysis (Anne Tagini, Donna Kline, Lt. Amber Payne, Bryon Downey, Marisa Ponte, Rebecca Miller, Matt Merrifield, Walter Heady, Steve Rienecke, EJ Dick, and John Field).

DISCLOSURES:
The authors have nothing to disclose

REFERENCES:
1. Love, M. S., Yoklavich, M. M. & Thorsteinson, L. K. *The Rockfishes of the Northeast Pacific*. (University of California Press: Berkley, 2002).
2. Laidig, T. E., Watters, D. L. & Yoklavich, M. M. Demersal fish and habitat associations from visual surveys on the central California shelf. *Estuar. Coast. Shelf Sci.* **83**, (4) 629–637 (2009).

3. Anderson, T. J. & Yoklavich, M. M. Multiscale habitat associations of deepwater demersal fishes off central California. *Fish. Bull.* **105**, (2) 168–179 (2007).
4. Yoklavich, M. M., Cailliet, G. M., Sullivan, D. E., Lea, R. N. & Love, M. S. Habitat associations deep-water rockfishes a submarine canyon : an example of a natural refuge. *Fish. Bull.* **98**, (3) 625–641 (2000).
5. PFMC *Status of the Pacific Coast Groundfish Fishery, Stock Assessment and Fishery Evaluation*. (Portland, OR, 2016).
6. Cappo, M., Harvey, E., Malcolm, H. & Speare, P. Potential of video techniques to monitor diversity, abundance and size of fish in studies of marine protected areas. In: Beumer JP, Grant A, Smith DC (eds) Aquatic protected areas—What works best and how do we know? Proc. World Congr. on Aquat Protected Areas. Australian Soc. for Fish Bio., North Beach, Western Australia February(455–464 (2003).
7. McLean, D. L., Green, M., Harvey, E. S., Williams, A., Daley, R. & Graham, K. J. Comparison of baited longlines and baited underwater cameras for assessing the composition of continental slope deepwater fish assemblages off southeast Australia. *Deep-Sea Research Part I: Oceanographic Research Papers* **98**, 10–20 (2015).
8. Parker, D., Winker, H., *et al.* Insights from baited video sampling of temperate reef fishes: How biased are angling surveys? *Fish. Res.* **179**, 191–201 (2016).
9. Boutros, N., Shortis, M. R. & Harvey, E. S. A comparison of calibration methods and system configurations of underwater stereo-video systems for applications in marine ecology. *Limnol. Oceanogr. Methodss* **13**, (5) 224–236 (2015).
10. Harvey, E. S., Cappo, M., Butler, J. J., Hall, N. & Kendrick, G. A. Bait attraction affects the performance of remote underwater video stations in assessment of demersal fish community structure. *Mar. Ecol. Prog. Ser.* **350**, 245–254 (2007).
11. Watson, J. L. & Huntington, B. E. Assessing the performance of a cost-effective video lander for estimating relative abundance and diversity of nearshore fish assemblages. *J. Exp. Mar. Bio. Ecol.* **483**, 104–111 (2016).
12. Easton, R. R., Heppell, S. S. & Hannah, R. W. Quantification of Habitat and Community Relationships among Nearshore Temperate Fishes Through Analysis of Drop Camera Video. *Mar. Coast. Fish.***7**, (1) 87–102 (2015).
13. Hannah, R. W. & Blume, M. T. O. Tests of an experimental unbaited video lander as a marine fish survey tool for high-relief deepwater rocky reefs. *J. Exp. Mar. Bio. Ecol.* **430**, 1–9 (2012).
14. Starr, R. M., Gleason, M. G., *et al.* Targeting Abundant Fish Stocks while Avoiding Overfished Species: Video and Fishing Surveys to Inform Management after Long-Term Fishery Closures. *Plos One* **11**, (12) e0168645 (2016).
15. Love, M. S. *Certainly more than you want to know about the fishes of the Pacific Coast: a postmodern experience*. (Really Big Press: Santa Barbara, CA, 2011).
16. Campbell, M. D., Pollack, A. G., Gledhill, C. T., Switzer, T. S. & DeVries, D. A. Comparison of relative abundance indices calculated from two methods of generating video count data. *Fish. Res.* **170**, 125–133 (2015).
17. Cappo, M., Speare, P. & De'ath, G. Comparison of baited remote underwater video stations (BRUVS) and prawn (shrimp) trawls for assessments of fish biodiversity in inter-reefal areas of the Great Barrier Reef Marine Park. *J. Exp. Mar. Bio. Ecol.* **302**, (2) 123–152 (2004).

18. Schobernd, Z. H., Bacheler, N. M., Conn, P. B. & Trenkel, V. Examining the utility of alternative video monitoring metrics for indexing reef fish abundance. *Can. Jour. Fish. Aquat. Sci.* **71**, (3) 464–471 (2014).
19. Hansen, M. J., Schorfhaar, R. G. & Selgeby, J. H. Gill-Net Saturation by Lake Trout in Michigan Waters of Lake Superior. *North Am. J. Fish. Manag.* **18**, (4) 847–853 (1998).
20. Dauk, P. C. & Schwarz, C. J. Catch estimation in the presence of declining catch rate due to gear saturation. *Biometrics* **57**, (1) 287–93 (2001).
21. Hilborn, R. & Walters, C. J. *Quantitative Fisheries Stock Assessment Choice, Dynamics and uncertainty.* (Springer: Dordrecht, 1992).
22. Erisman, B. E., Allen, L. G., Claisse, J. T., Pondella II, D. J., Miller, E. F. & Murray, J. H. The illusion of plenty: hyperstability masks collapses in two recreational fisheries that target fish spawning aggregations. *Can. Jour. Fish. Aquat. Sci.* **68**, 1705–1716 (2011).
23. Buckland, S. T., Anderson, D. R., Burnham, K. P. & Laake, J. L. *Distance Sampling: Estimating abundance of biological populations*. (Chapman and Hill: London, 1993).
24. Ronconi, R. A. & Burger, A. E. Estimating seabird densities from vessel transects: Distance sampling and implications for strip transects. *Aquat. Bio.* **4**, (3) 297–309 (2008).
25. Caselle, J. E., Rassweiler, A., Hamilton, S. L. & Warner, R. R. Recovery trajectories of kelp forest animals are rapid yet spatially variable across a network of temperate marine protected areas Recovery trajectories of kelp forest animals are rapid yet spatially variable across a network of temperate marine protected. *Nat. Publ. Gr.* 1–14 (2015). doi:10.1038/srep14102
26. Starr, R. M., Wendt, D. E., *et al.* Variation in Responses of Fishes across Multiple Reserves within a Network of Marine Protected Areas in Temperate Waters. *Plos One* **10**, (3) e0118502 (2015).
27. Lester, S., Halpern, B., *et al.* Biological effects within no-take marine reserves: a global synthesis. *Mar. Ecol. Prog. Ser.* **384**, 33–46 (2009).

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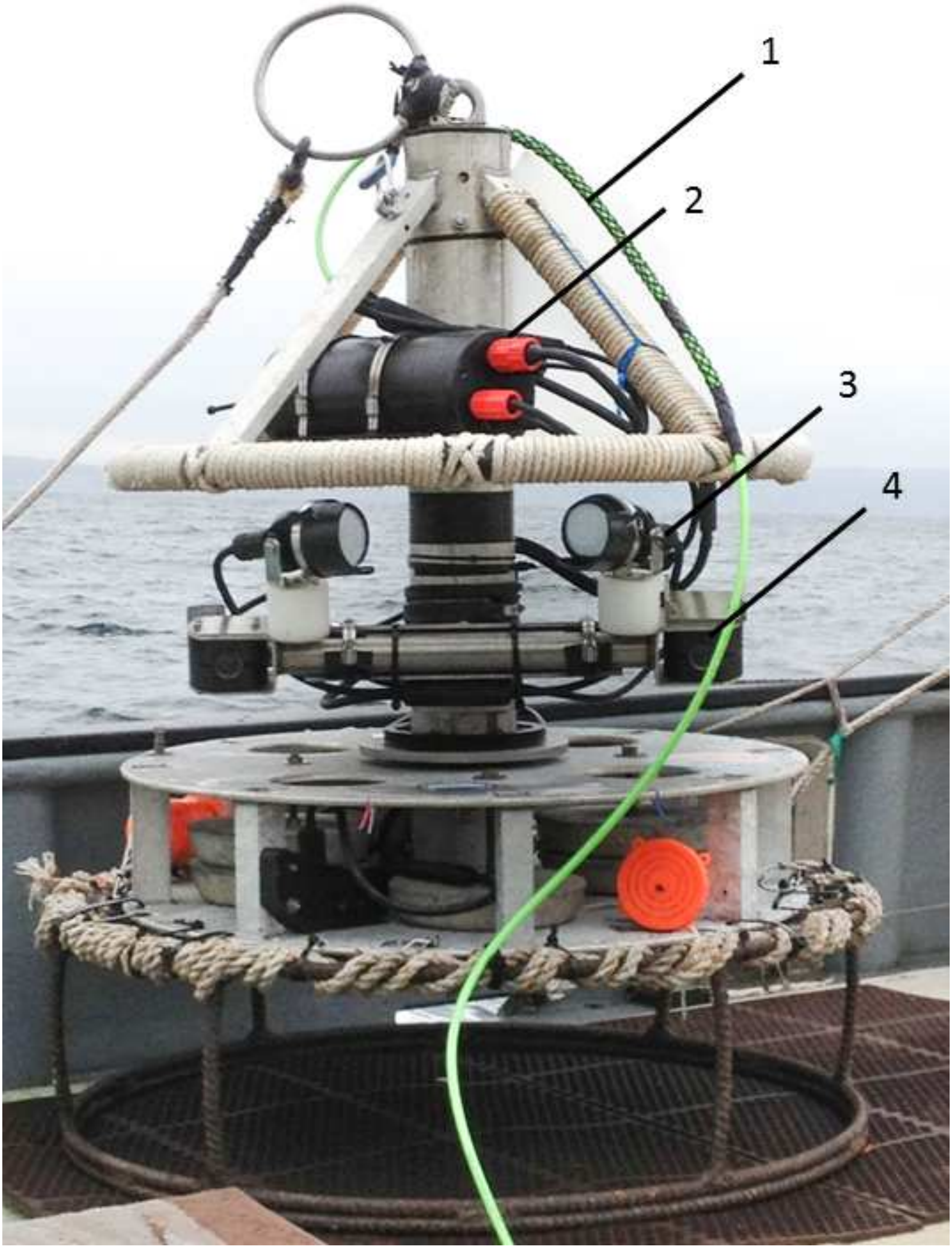

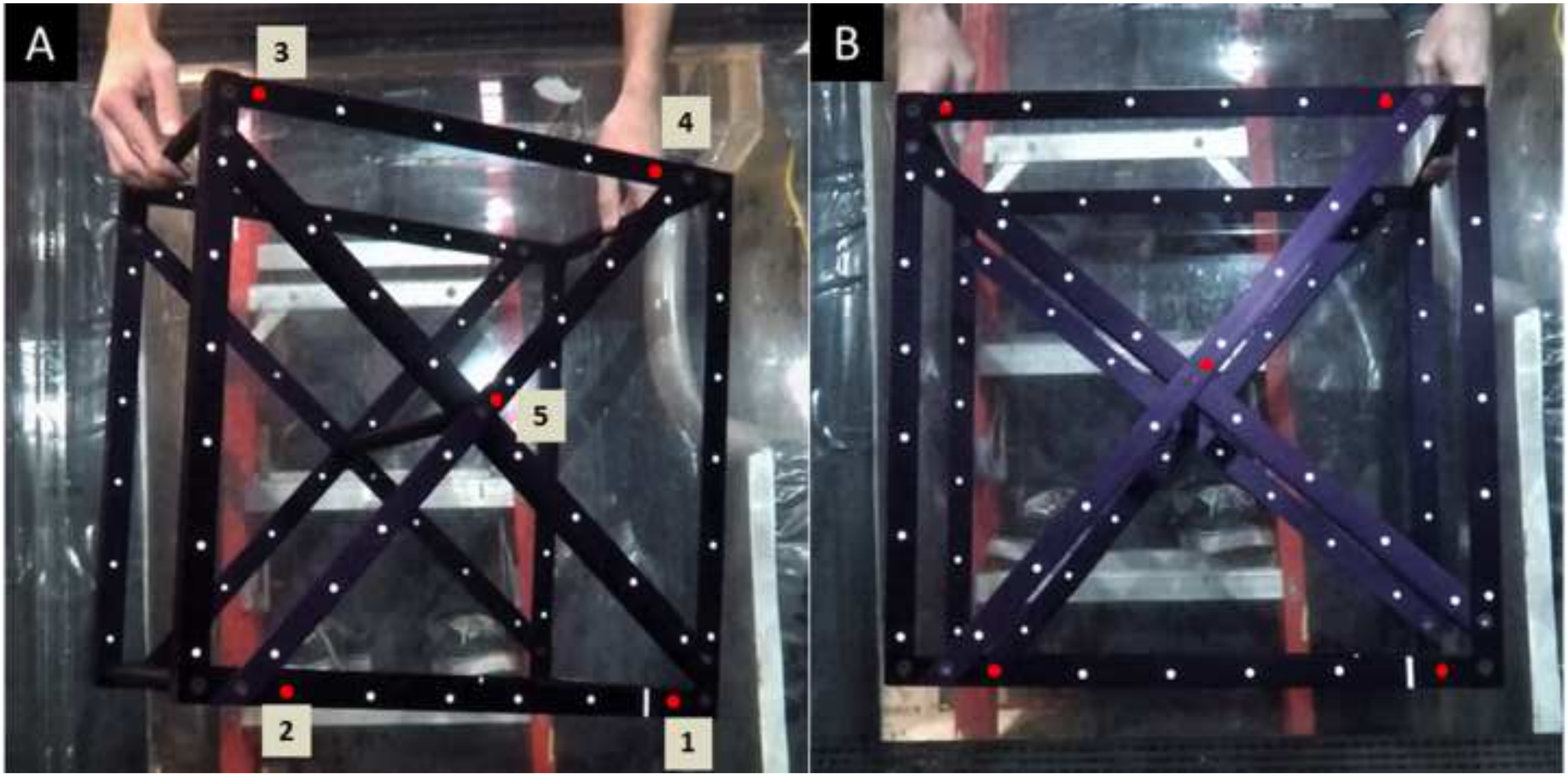
A
3
4
5
2
1
B

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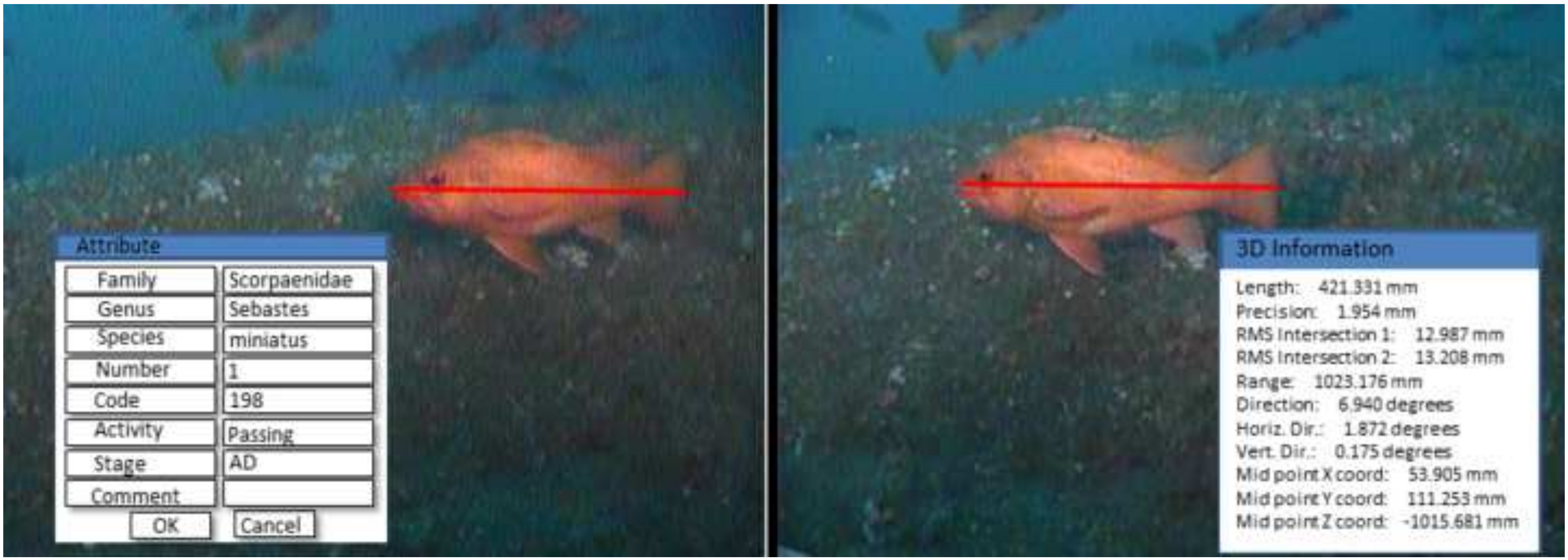

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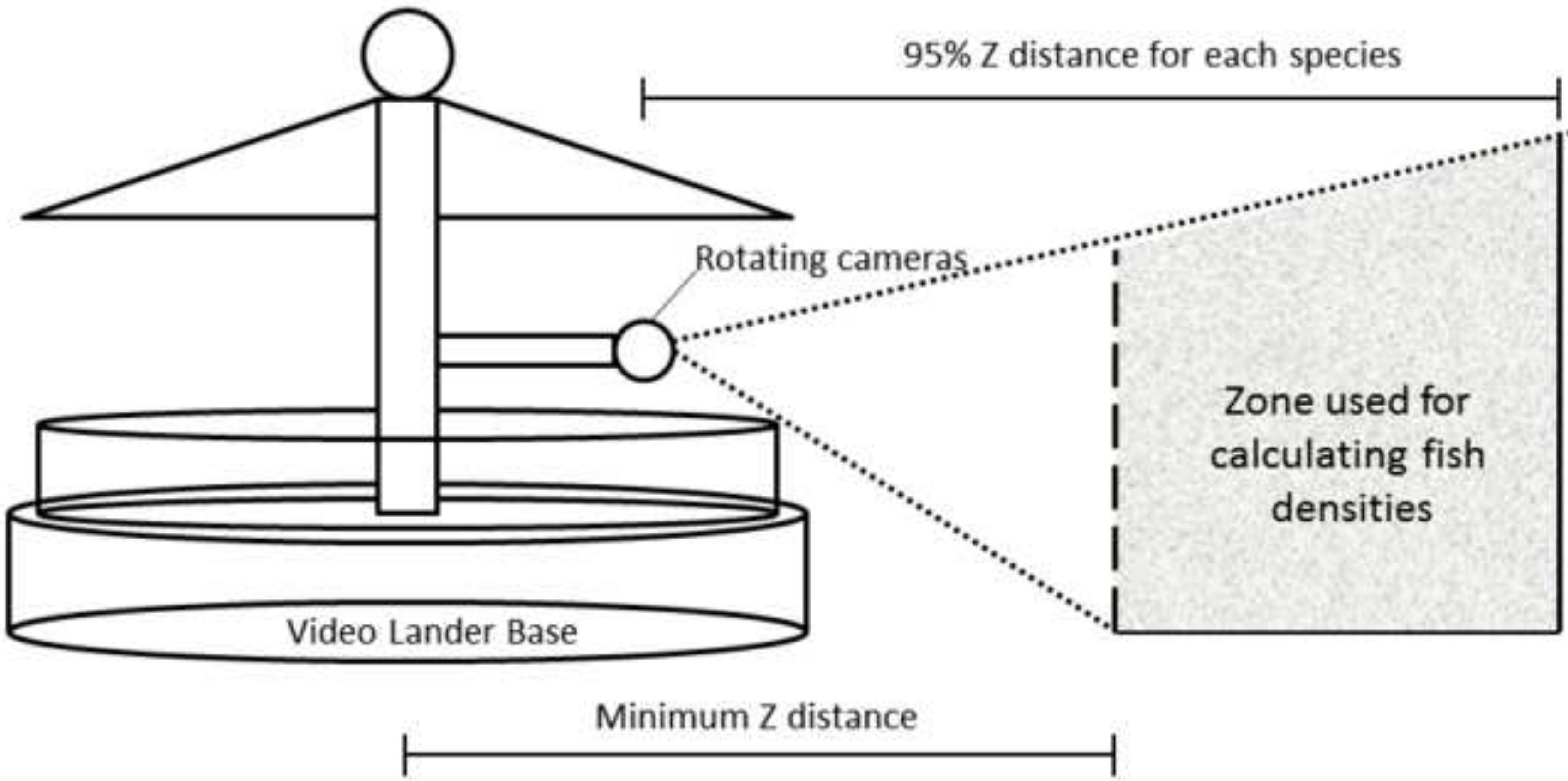

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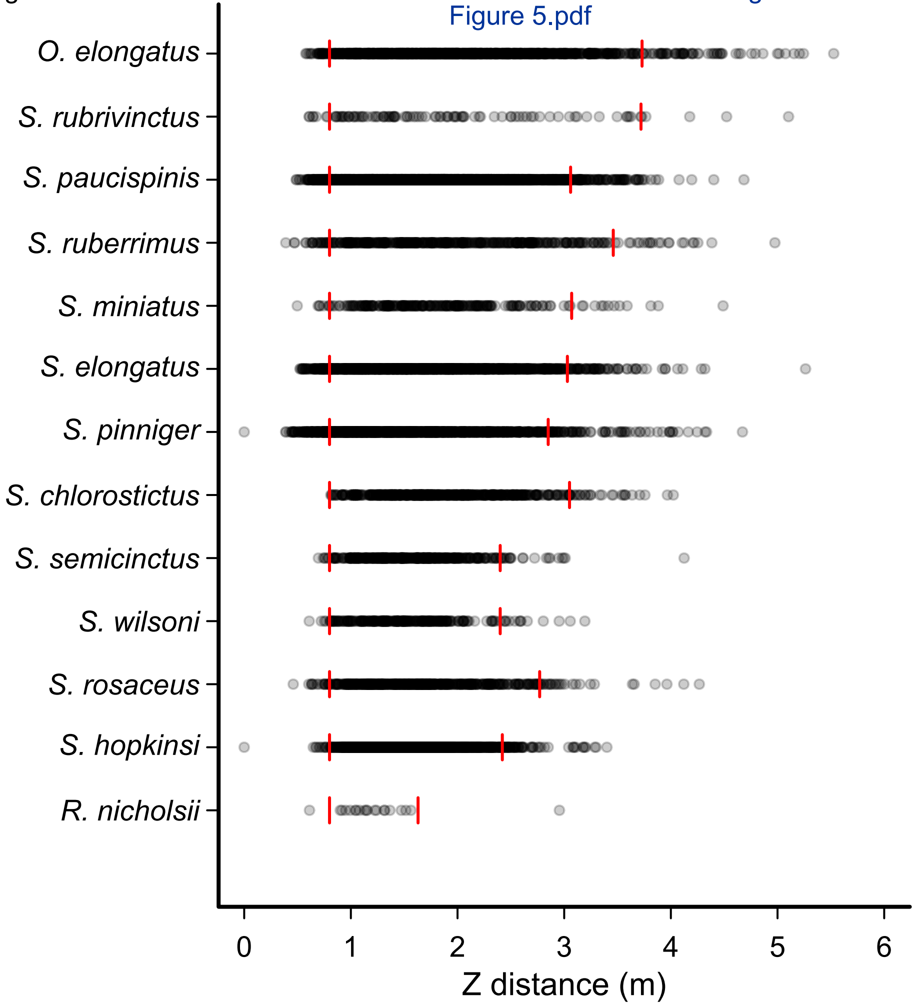
O. elongatus
S. rubrivinctus
S. paucispinis
S. ruberrimus
S. miniatus
S. elongatus
S. pinniger
S. chlorostictus
S. semicinctus
S. wilsoni
S. rosaceus
S. hopkinsi
R. nicholsii
0
1
2
3
4
5
6
Z distance (m)

Figure 6

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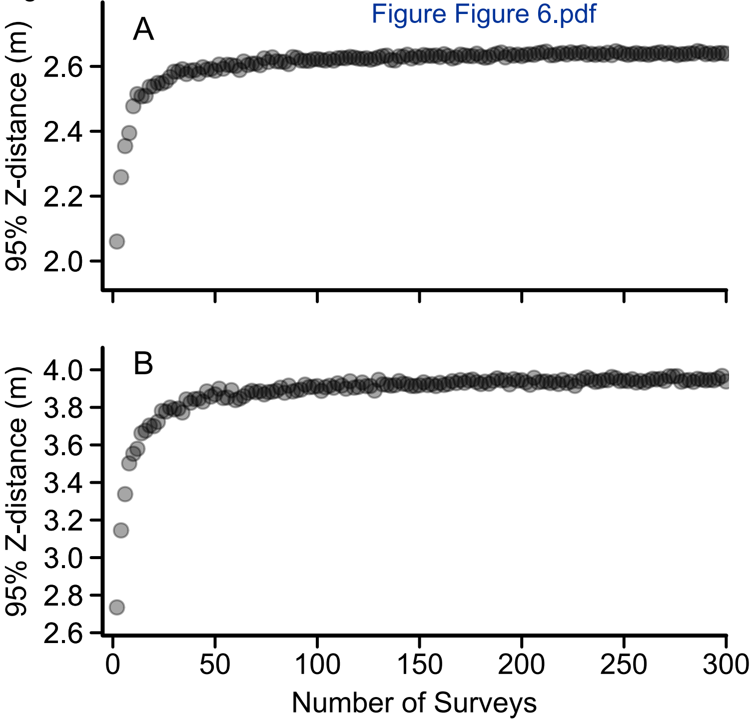
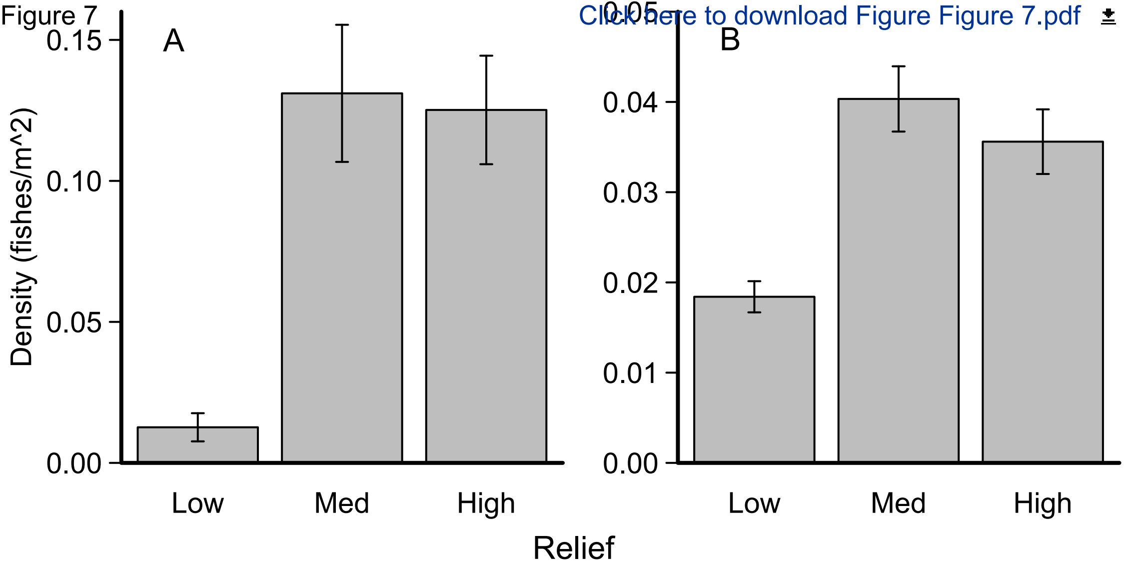


Figure 7



A
B
Density (fishes/m^2)
0.15
0.10
0.05
0.00
0.05
0.04
0.03
0.02
0.01
0.00
Low
Med
High
Low
Med
High
Relief

Figure 8

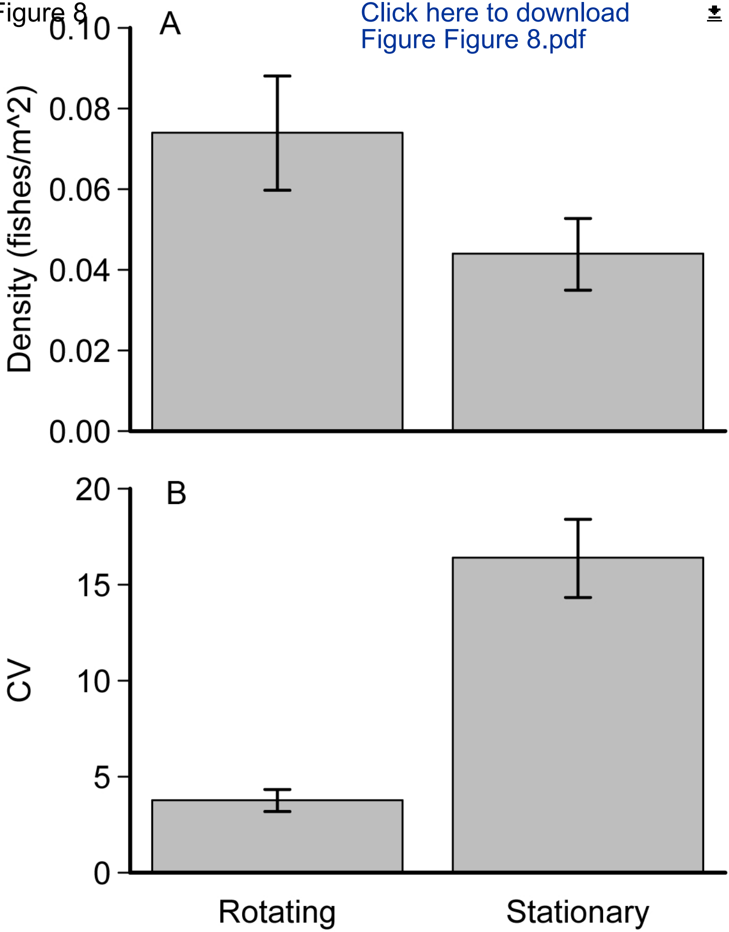

A
Density (fishes/m^2)
0.10
0.08
0.06
0.04
0.02
0.00
B
CV
20
15
10
5
0
Rotating
Stationary

Table 1 Click here to download Table Table 1.xlsx

Species		Rotation 1	Rotation 2	Rotation 3	Rotation 4	MaxN
Eptatretus						
	stoutii		**1**		1	**1**
Ophiodon						
	elongatus		1	**2**	2	**2**
Sebastes						
	flavidus	2	2	2	**4**	**4**
	miniatus	28	28	**37**	32	**37**
	rosaceus		**2**	2	1	**2**
	ruberrimus	2	2	2	1	**3**
	rubrivinctus	**1**	1		1	**1**
	spp.	10	8	**2**	1	**2**
Grand Total		43	45	47	43	**52**

Name of Material/ Equipment	Company	Catalog Number
calibration cube	SeaGIS	http://www.seagis.com.au/hardware.html
CAL calibration software	SeaGIS	http://www.seagis.com.au/bundle.html
EventMeasure stereo measurement software	SeaGIS	http://www.seagis.com.au/event.html
Statistical software	R Core Team 2017 (v. 3.4.0)	
Spreadsheet Software	Microsoft Excel	
2 waterproof cameras	Deep Sea Power and Light	
2 depth rated, waterproof lights	Deep Sea Power and Light : 3000 lumen LED with 5000k color temperature	
DVR recorder	Stack LTD DVR	
standard PC		
rotating Lander platform	Marine Applied Research and Engineering (MARE)	

Comments/Description

1000x1000x500 mm is the preferred dimensions. Other methods of calibration are available.

Bootstrapping code can be found: https://github.com/rfields2017/JoVE-Bootstrap-Function

HD quality preferred

Windows 10 preferred OS

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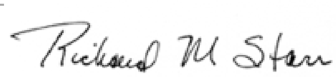
Signature:	
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July 24th 2017

Dear JoVE Reviewers,

Thank you for your detailed comments and revisions. The authors of "*Development of new methods for quantifying fish density using underwater stereo-video tools*" have revised the manuscript based on the recommendations of the reviewers. Attached is a copy of the manuscript with track changes, as well as a clean version of the manuscript. We have also attached a folder with supplemental materials. Supplemental screenshots are labeled according to the step numbers in the manuscript. Supplemental R-code and data has been uploaded and referenced on GitHub to help readers understand and perform the bootstrapping procedure. We have included a summary below our actions to reviewer's suggested edits.

In brief, the focus of this manuscript is the description of a MaxN technique for rotating drop camera tools as well as the use of a 95% Z distance metric to define the mean distance of reliable fish measurements. As such, we significantly reduced language in the manuscript that compared our tool directly with other existing survey tools. Similarly, calibration of cameras for 3D photogrametry is well documented in other studies and is not the focus of this present paper. We therefore reduced the description of camera calibration, and instead focus on MaxN and 95% Z Distance.

Changes recommended by the JoVE Scientific Review Editor:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.
We read through the manuscript to check for grammatical and spelling errors.

• Please include at least six keywords/phrases.
Six Keywords and phrases are now included.

• **Protocol Language:** Please ensure that ALL text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.
1) Example NOT in imperative tense: "Some fishes will only be identifiable to higher taxonomic groups"
The protocol was checked for imperative tense at each step.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more details to the following protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Major Edit: The focus of our paper is the description of MaxN for rotating cameras as well as the use of 95% Z-distance values for calculating the mean distance a fish could reliably be measured. We therefore made the choice to limit protocol discussion of camera calibration and instead focus on the intended methods. Calibration and measurements can readily be performed across a variety of software platforms. We made the recommendation of using a 'calibration cube' with references to the table of materials. Again, the choice of calibration method will not affect the MaxN statistic calculated in this paper.

It should be noted that we used specific software for these steps and that complete replication may require referencing both the CAL software manual (80 pages) as well as the EventMeasure manual (110 pages). Neither proprietary manual can adequately be abbreviated in this manuscript. We assume that individuals implementing our protocol will have some basic understanding of their selected software. Nonetheless, we have added specific menu commands for each step. Screenshots of these software steps will be available in the supplemental materials.

1) 1.1: Is it unclear what “cube” is and what it does here (please add a short note after this step to mention this?).
A description of the Calibration Cube was provided as well as a reference to the table of materials.

Please expand the calibration steps. Please mention what button is clicked on in the software to do this, or which menu items need to be selected.
2) 1.1.1: What are the 5 cube orientations?
As per the note above, we have eliminated the description of our specific calibration procedure. The use of 3D photogrammetry was not the focus of our paper and has been adequately described in previous research. Menu items and buttons were explicitly referenced.

3) 1.2,1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.2.7, 1.3,1.3.1, 1.3.2, 1.3.3., 1.3.4,1.5 ,1.6, 1.6.1, 1.6.2., 1.6.3., 1.7, and all other software-based steps: Please mention what button is clicked on in the software to do this, or which menu items need to be selected. Please also provide us screenshots to show each software step, these can be added a supplementary files.

4) 2.6: Unclear what is done here.

We have changed the text to try and clarify the step. The accompanying note/example in the protocol is intended to help readers understand the procedure. This step is admittedly tricky to understand, but is important and deserves the long comment and nuanced explanation. For the reviewer: Some fish are only identifiable to the genus - level, and we wanted a count metric that would conservatively avoid over counting fishes. We therefore decided to only count “unknown species’ during rotations where the majority of fishes had in fact been identified to species. This made it more likely that the ‘unknown species’ marked as 2D points had not been previously identified.

We believe that this step will be easier to understand with a visual aid.

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.
Care was taken to ensure that all protocol numbering matched the required formatting.

• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE's instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

Highlighted text meets the 3 page maximum requirement.

o **Underwater step: Can these be filmed in mock? Our videographers cannot film underwater.**
As per the notes above, we have reduced the text describing calibration since this was not the focus of the paper. If a segment of calibration were to be included in the video, then this step could easily be filmed in mock. Another possibility is for us to supply video we took ourselves while calibrating underwater. We also thought that some of our GoPro footage of actual fish surveys could be included, both to show the cameras rotating around and also to make the overall video more interesting.

o The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
o The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
o Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
o Notes cannot be filmed and should be excluded from highlighting.
Effort was made to conform to highlight formatting outlined above

o Please bear in mind that software steps without a graphical user interface/calculations (e.g. section 3) cannot be filmed.
We have modified section 3 according to suggestions outlined above. We have included a screenshot in the supplemental material showing this step performed with a GUI.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

We believe that our discussion follows the outlined format well.

• **Figures:**:
1) Fig 3: The text in the inset windows cannot be read.
Figure 3 has been updated to be legible

• **Figure/Table Legends:**
1) Fig2: Please mention cube dimensions.
Cube dimensions have been added to Fig 2 legend. Note that calibration is no longer a focus of this paper, and so the figure legend describes 'an example calibration'.
2) Fig 4: Please define the red markers.
No red lines were found in Fig 4; however, 'Red' was added to the description of vertical bars in Figure 5

• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are SeaGIS, Inc, LTD™, Deep Sea Power and Light (DSPL), etc.
1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

We removed all commercial sounding language from the text of the manuscript, and instead only reference company names in the materials table.

• Please define all abbreviations at first use.
Abbreviations were defined at first use.
• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.
Unit abbreviations conform to these requirements.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Our figures and tables are original.

Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:

I have reviewed the paper titled "Development of new methods for quantifying fish densities using underwater stereo-video tools". The manuscript describes a modification to the traditional MaxN approach to enumerating fish abundances for a rotating stereo-video lander. The primary objective of this study was to determine species-specific detectability to estimate densities using a rotating stereo-video camera system. This research is well thought out and clearly presented. I believe that this manuscript represents an important contribution on survey methods for quantifying densities from stereo-video systems. However, there are several clarifications that with regards to the process for determining that an appropriate calibration was created that need to be added to the protocols. Addressing these suggestions will make the manuscript more re-producible. Therefore, I recommend that the manuscript be published after undergoing minor revisions to address the points listed in this review. Comments by section are presented below.

*Title:
I believe that the title accurately describes the scope of the work.
*Keywords:
I would suggest adding in the following: Underwater visual census, groundfish, rotating drop-camera system, and removing stereo video (as it is already in the title).
Underwater visual census, groundfish, rotating drop-camera system were added to the keywords and Stereo Video was removed
*Short Abstract:
The structure and content of the short abstract is suitable.
*Long Abstract:

The long abstract sufficiently describes the background and justification for the application of this modified protocol for future research and is appropriate for this methods article.
*Suggested edits are as follows:
Lines (88-90): Suggest adding in more information about the preliminary studies - what were the analyses used to determine that eight rotations was ideal for this system. Since the author recommends that this metric be determined for a given study site it would help increase reproducibility if more information in conducting these preliminary analyses were added.
A generic description of the determination of the 8 minute soak time was provided. Other reviewers cautioned against inclusion of specific results in the introduction so specific numbers were avoided.

*Protocol:
The steps listed in the procedure overall are clearly explained. All the critical steps are highlighted.

However, the protocol should be clearer in specifying that terms, cited file extensions, and software functions are SeaGis dependent and may not be synonymous with other software applications.
While we wholeheartedly agree that specific software used should be cited within the text, JoVE requires the exclusion of any commercial language from the manuscript. SeaGIS is mentioned in the materials table. The specific steps outlined are only possible in SeaGIS; however the overall approach to the MaxN approach we have outlined is transferable to any stereo software platform.

Also, the syncing steps would be useful to include earlier in the procedure.
Syncing the video is not possible before this point in the procedure.

A possible addition to this protocol maybe a sentence on checking for camera frame drift to ensure that the cameras remained synced.
A sentence was added to our note to recommend checking for camera frame drift during analysis

*Suggested edits are as follows:
Lines (144-145, 149,154): You should clarify if these file extensions are specific to the SeaGIS software and may not be synonymous to all other systems.
See comment above: JoVE will not allow this.

Lines (151-152): Specify the camera specific parameter estimates contained in this file that are needed for calibration.
Example parameters are now included in the text. See supplemental material for a screengrab of all parameters included in the calibration file

Line 154: Specify the cube specific parameter estimates contained in this file for those not using SeaGIS software.

This step in the protocol has been removed; however the cube parameter file contains the precisely calibrated distances and dimensions between calibration cube points.

Line 158: Provide information on how to sync videos. Suggest including information on the process for syncing cameras (i.e. light flash, clapper board, etc.) between 1.1 and 1.1.1 and state how often this process should be conducted during field sampling to identify if there is camera drift.
We have recommended the use of a time UTC time stamp to sync videos and to periodically check that synchronization has been maintained. We also mention other techniques used to calibrate.

Line (160-162): Specify if there is an order needed to when selecting the reference points.
*Overall, in the calibrations section the author needs to specify the acceptable and not acceptable limits for metrics during this calibration. For example, what are the metrics that would make a calibration not meet standards for moving forward (i.e. # of exclusions, relative positioning, etc.).
We have narrowed the scope of our protocol section to focus on the measurements of fishes and the calculation of MaxN and 95% Z-distance values. See previous comments for further details. We did however note that measurement error after calibration should be within 2% of an object's known length. The choice of software and calibration technique can be determined by the researcher and will not affect the strategy employed in the calculations of MaxN and 95% z-distance.

Lines (214-15, 219, 221-222): Specify that these processes are SeaGIS specific and that other software packages may not have these specifications and tabs.
See above comment: JoVE will not allow this

Lines (224-225): This is the first time the syncing process is mentioned see comment above about including this earlier in the protocol.

As addressed above, syncing is now addressed in more detail the first time it is required in the protocol.

*Representative Results:
The results from this study seem reasonable and are useful to readers operating stationary stereo-video drop cameras.

*Suggested edits are as follows:
Lines (379-383): Consider adding a reference to support whether these results are consistent with the known habitat associations of these species.
Reference added citing known habitat associations for both example species

*Discussion:
The discussion details the use and applicability of this approach by highlighting the strengths, limitations, and caveats of both the stationary and rotating lander.

*Suggested edits are as follows:
Line 590: This citation is not cited in the paper.
Citations were updated to excluded references not used

Reviewer #2:
Manuscript Summary:
Nice work. I think this is an interesting paper that warrants publication. I checked "major revision" only because I think you need to think hard about the way in which the work is contextualized. Papers that compare approaches are fraught with peril, because there are so many potential explanations for why one approach might be better under one set of circumstances, but not others. For that reason, I recommend that rather than juxtaposing this approach (lander with stereo video) as the solution to problems inherent in other tools/approaches (ROVs/subs using lasers), I suggest that you describe the universe of challenges in collecting data from deep water, perhaps describing how some other tools address some of those challenges, and then introduce your approach as a means to addressing selected challenges without directly juxtaposing it to other tools. I think that could make a really useful contribution. I'm happy to talk further about this if it would be helpful. Good luck!

Lines 54-55 Should read "...these known habitat associations..."
Line 56 " ...that are better suited to sampling low-relief sedimentary environments" This provides a better juxtaposition to the prior sentence.

Lines 59 -65 Since you assert above that relief and substrate is the primary issue, I think it will be important to better flesh out that particular issue with respect to these traditional gears, particularly trawls. For instance, it is not simply that trawls may underestimate counts, rather trawl surveys will intentionally avoid the types of high relief habitat you are discussing due to the way the gear is deployed and fished. Explain that. This is an important point that sets the stage for your approaches' utility. Next I am not convinced by your critiques of acoustic surveys and hook and line methods. While both may have the limitations you describe, they can be dealt with by someone who understands how to use the tools. They are not necessarily problems unique to those tools. If your goal is to cast landers in a better light than these other tools, I would make a different case..

Lines 68-71 Similar to my comment above, while it is true that most ROV surveys to-date have relied on paired sizing lasers rather than stereo video, it is not true that this limitation is inherent to ROVs or subs (which this statement implies). Indeed prior to your tool, the same can be said of landers (e.g., ODFW's). If you want to flesh out differences between transect-based visual tools here to set the stage for a discussion of your lander, that could be useful. For instance, for a given day at sea, an ROV/Sub can survey x amount of seafloor, distributed over a total area of y, given the logistics of getting the vehicle in and out of the water and down to the seafloor. How do lander stats compare? Smaller area surveyed, but over a wider area? This would be an interesting way to compare while avoiding the economic comparison I mention below.

Also, I recommend the use of human-occupied submersibles (HOVs) rather than manned submersibles.

Lines 72-73 Consider removing value judgments about the "expense" of particular tools. Expense is relative. The widely variable types of ROVs and associated vessels make this type of statement difficult to support without a more dedicated evaluation of cost associated with the use of any particular tool.

Lines 75-76 same comment as above. This critique is too broad. Does "relative ease" refer to smaller vessels and fewer surface support staff? Either explain that, or don't make that claim. I can put a small ROV on a small boat, using student support, and the cost would be minimal. Of course, conditions would be a huge issue, and perhaps your lander would be preferable for certain sea states or certain water depths, but you don't talk about that. Also, what about the cost of stereo video processing software? ROV/sub video can be reviewed using a free VLC media player. What about the time required to calibrate the camera systems with a cube? I've got a small ROV that I can turn on and deploy in two minutes, no time intensive calibration needed.

Line 82 As you can probably surmise by now, I'm not sure that "more efficiently" actually applies.

Major Edit: In reference to the above comments from Reviewer #2, the entire introduction has been refocused on the MaxN and 95% characteristics. We agree with the reviewer's concerns that 'tool comparison' studies are difficult and often wrought with problems. We therefore significantly reduced language that compared our tool with existing tools. We agree with the reviewers overall comment that those comparisons were overly broad and not necessary for the primary aims of the manuscript. Our philosophy is that this paper best explains a technique to researchers who have *a priori* made the decision to use a rotating camera system.

Line 102 This paragraph describes issues associated with species ID. You need to explain that along with species morphology, color, and behavior, the environmental conditions (light level, suspended particulate) are critical determinants of our ability to ID species. Like an ROV or Sub, the lander will be similarly impacted by these conditions.
A sentence addressing these concerns has been added.

Lines 127 onward. The following protocol is obviously based on SeaGIS' technology. I would precede this discussion with a short paragraph indicating that a variety of stereo video tools exist out there in the world, possibly name a few, and then explain that for the purposes of this paper you are going to limit the discussion to SeaGIS.
See above comments: JoVE will not allow commercial language in Manuscript text. SeaGIS is referenced in the materials table.

Lines 448 onward. I think that there are three related, but distinct, dimensions to this paper: landers vs other platforms, stereo video vs traditional sizing lasers, and MaxN versus other estimates. The problem is that the three dimensions are not uniformly addressed throughout the paper. For instance, your introduction (subject to my comments above) really sets the stage for a comparison between landers and other visual tools/traditional tools, without really talking about MaxN at all. But the first paragraph of your discussion, asserts the importance of MaxN as assumedly your most important finding.
The introduction has been modified to de-emphasize comparisons with other tools and instead focus on MaxN and 95% Z distance calculations

Reviewer #3:

Manuscript Summary:

The manuscript describes the use of a rotating stereo video lander for estimating fish densities and discusses statistical modifications of established methods for estimations of MaxN and maximum distance estimations for fish detection.

-The challenges of estimating fish densities for mobile species that are distributed quite variably in time and space are many, and the development of this rotating stereo video lander system is a great step forward. But this manuscript does not do it justice in it present form. The modification of MaxN and estimations of maximum detectable distances seem appropriate and were

necessary developments for incorporating the results of this tool to other scientists and managers. Clearly, this instrument covers a larger area than static video systems as shown by the comparisons of MaxN between rotating video and non-rotating video subsampled from the video. Each tool produces estimates of MaxN that are unique to each and are only useful in a relative context. For the rotating system (which by the way should be named for ease of use in manuscripts) to be accepted and useful it must be used solely in all areas of interest, otherwise estimates derived from its use are not scalable to other static video systems. While static systems underestimate fish densities relative to the rotating system, the rotating system also underestimates fish densities but less than static systems as the author's claim. I think it is important to develop a method to intercalibrate the rotating system with static systems (especially if the rotating system becomes more prevalent) to prevent historical baseline estimates developed using static systems from becoming incomparable. This should be attempted using multiple methods besides video as mentioned in the Discussion.
We fully agree that to properly do ‘tool comparison’, all tools need to be inter-calibrated. Because that was not the focus of this paper, we have reduced language of tool comparison as per the response to Reviewer #2’s comments.

Finally, I am concerned about pooling distance estimates among different locales given the highly variable nature of water clarity and camera light penetration among settings. In my mind, this was not dealt with adequately in the manuscript or the study methods, yet it is presented as an important modification element.

We have added text to a note to try and clarify this issue. We agree that each particular survey will have differences in water clarity and camera light penetration. The 95% Z distance represents the average water clarity conditions across the entire study. We believe that it is statistically valid to pool results this way; however, this procedure would need to be recalculated if a different study were performed (say a different survey year).

-The Introduction is well written and introduces the topics of importance, however it includes results starting at line 85 which are not appropriate in the introduction. These should be limited to the results section.
We have added a few more sentences to clarify the introduction section, but it was unclear what ‘results’ were included. We have eliminated specific quantitative ‘result’ language from the introduction.

-The Protocol section is vague in describing general concepts and how these were applied (e.g., bootstrapping details are vague and inadequate).

We have added additional details to many protocol steps as was requested by other reviewers. It is unclear what level of detail is needed for a widely used (and software generic) statistical approach such as bootstrapping. In subsection 3.1.1.1, we describe taking '1000 random draws of the data with replacement' for each tested sample size and calculating a statistic (95% Z distance). We have also included code online at: https://github.com/rfields2017/JoVE-Bootstrap-Function

-Line 394: "Welches" should be "Welch's" - the use of Welch's t-test assumes independence of samples and the way that the "pseudo-stationary" estimates were derived by subsampling video sections of the rotating system means that they are not independent. I understand why this was done given the difficulty and logistics of the work and think this is a minor flaw given the magnitude of the differences.

We agree with the reviewer that assumptions for Welch's t-test were not fully met, and also agree that this is a "minor flaw given the magnitude of the differences". We therefore left the result unaltered.

July 31, 2017

Dear JoVE Reviewers,

Thank you for the additional comments and revisions. The authors of "*Development of new methods for quantifying fish density using underwater stereo-video tools*" have revised the manuscript based on the recommendations of the reviewers. Attached is a copy of the manuscript with track changes.

We have largely accepted the recommendations of the JoVE editors below, and have added additional text where necessary to clarify protocol steps. We have included supplementary video as well as survey footage that we would like to use, either integrated into the protocol or as part of the introduction.

• **Protocol:** Anything you would like shown in the video will need to be described in the text.
1) 1.1: Unclear what you wish to show here. Can you cite a reference for calibration? We have unhighlighted this step and also edited step 1 as shown in the text.

We agree that this step would be difficult to film and have not highlighted this section. The note above step 1 includes a reference to using calibration cubes. We also reference the table of materials where CAL software by SeaGIS is listed as the preferred calibration software to use.

We have also uploaded supplemental video that shows the calibrated target of a known length being measured underwater. We suggest using footage in between the times 00:05 – 00:45 of the uploaded video.

2) 1.2: What do you wish to show here? If you have footage of the cameras rotating underwater conducting fish surveys, these will need to be reviewed before proceeding. Please also note that the protocol text will be used to produce the script for the video and content for the voice-over, so ideally the visuals in the footage you provide should match the content in the steps. Please use this link to upload any uncompressed video files and let us know which video matches which step- > http://www.jove.com/files_upload.php?src=17273723

We suggest using approximately 1 minute of survey video footage to narrate background information for this technique. We suggest using a full rotation of the video in between times 00:14 -1:19. We believe it would be interesting and valuable for viewers to both see a video survey as well as get background details on why we are using the described methods. An alternative is to splice some survey footage into the introduction portion of the video. We have provided the following text, adapted from the abstract, as a guide for the narration of this video.

1. The use of video camera systems in ecological studies of fishes continues to gain traction as a viable, non-extractive method of measuring fish lengths and estimating fish abundances. We developed and implemented a rotating stereo-video camera tool, which maximizes sampling effort compared to stationary camera tools.
2. Our focus was on the development of methodological approaches to quantify fish density using rotating camera systems. We first developed a modification of the metric MaxN, which typically is a conservative count of the minimum number of fishes observed on a given camera survey. We redefine MaxN to be the maximum number of fish observed in any given rotation of the camera system. When precautions are taken to avoid double counting, this method for MaxN may reduce between-sample variability and more accurately reflect true abundance than that obtained from a fixed camera.

3. Because stereo-video allows fishes to be mapped in three-dimensional space, precise estimates of distance-from-camera can be obtained for each fish. By using the 95% percentile of the observed distance from camera to establish species-specific areas surveyed, we avoid either underestimating species which can only be identified near the cameras or unnecessarily excluding individuals of species identifiable to greater distances; both of which can be caused by using a single distance for all species. Accounting for this range of detectability is critical to accurately estimate fish abundances.

3) Do you wish to include all the supplementary software screenshots with the final publication (if accepted), or are these intended for use by JoVE staff only to guide scripting and filming?

The last editor requested that screenshots be provided for all software steps. We suggest that all the supplementary screenshots be included as supplemental materials for readers to view.

4) 2.8.1: Which 2D points? If this does not have any specific action to be filmed, please unhighlight this.

We added the step number that the 2D points were generated. The software allows the user to navigate to the exact same fish that was counted in order to measure it.

5) 2.8.5: How do you mark a 3D point?

We have added a brief description of the details needed to make a 3D point

6) Section 3 being computation work using scripts cannot be filmed but the screenshot provided can be shown. However, we adjusted the highlight a bit.

We believe the edited highlights are appropriate and would like the screenshot of the computational work in step 3.1 (bootstrap) to be shown.

• **References:** Please abbreviate all journal titles.

We abbreviated all journal titles

Supplemental_Figure_1

Click here to access/download
Supplemental File
S_1.3_New Project Folder.PNG

Supplemental_Figure_2

Click here to access/download
Supplemental File
S_1.4.2_Set Picture Directory.png

Supplemental_Figure_3

Click here to access/download
Supplemental File
S_1.4.3b_Cam file parameters.PNG

Supplemental_Figure_4

Click here to access/download
Supplemental File
S_1.4.3_Load Cam Files.png

Supplemental_Figure_5

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Supplemental File
S_1.4.5_Define Movie Sequence.tif

Supplemental_Figure_6

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Supplemental File
S_1.4.6_Load Left Camera.png

Supplemental_Figure_7

Supplemental_Figure_8

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Supplemental File
S_1.4.7_Define right video sequence.png

Supplemental_Figure_9

Supplemental_Figure_10

Click here to access/download
Supplemental File
S_1.4.9_Edit Field Values.tif

Supplemental_Figure_11

Supplemental_Figure_12

Supplemental_Figure_14

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S_2.4.1_2D Point measurements.png

Supplemental_Figure_15

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S_2.4.2_Pivot Table.tif

Supplemental_Figure_16

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Supplemental File
S_2.9.1_3D point measurements.png

Supplemental_Figure_17

Click here to access/download
Supplemental File
S_3.1_Bootstrap of 95% Zdistance.png

Click here to access/download
Supplemental File
Suggested text for narration to accompany video footage.docx

Supplementary File 1- Bootstrap Function_R Code as .txt

Click here to access/download
Supplemental Code
Bootstrap_Function_R.txt

Supplementary File 2- Example data for Bootstrap Function