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