Larval Behavioral Response to Turbulence in Eastern Oyster

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Abstract


Eastern Oyster
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The Eastern oyster is a species of considerable economic importance which is harvested from naturally occurring oyster beds as well as commercially grown. Oyster beds provide a unique marine habitat, as well as contributing to healthy marine ecosystems by filtering excess nutrients from the water. Research into the effects of larval behavior on the formation of oyster beds improves understanding of the potential consequences of human interference in marine ecosystems, as well as potentially increasing the success of commercial hatchery operations. The hypothesis of this study is that turbulence is a settlement cue for oyster larvae. Oyster larvae respond to settlement cues by exhibiting a diving behavior that brings them closer to the ocean floor and potential settling locations. In order to test this hypothesis, video data of oyster larvae in a grid stirred turbulence tank were taken at several different turbulence levels. Additionally, the effect of laser light on larval behavior was explored in anticipation of the use of laser particle imaging velocimetry as a flow visualization technique. The larval dive response to turbulence has not been seen as yet due to experimental issues, however a dive response to the laser was observed. This response to the laser requires modification of the laser particle imaging velocimetry technique, however it does demonstrate that the larval dive response is detectable. Further refinements of experimental procedure and data analysis should enable quantification of the relation between turbulence level and larval diving response.

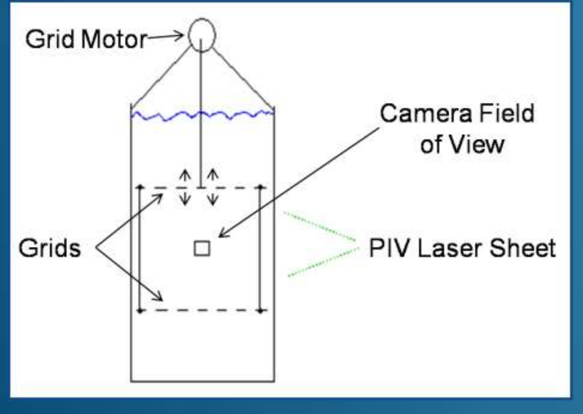
Eastern Oysters

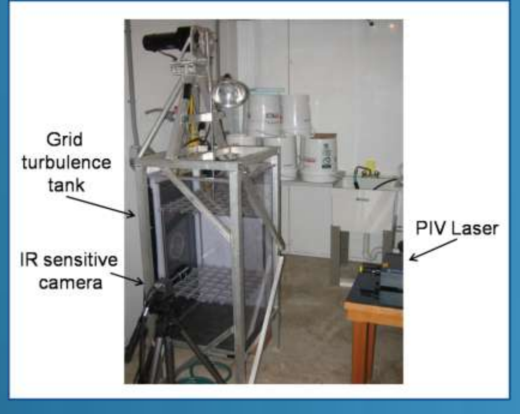
Oyster larva
Crassostrea virginica

- Eastern oysters are the primary species of oyster harvested by the oyster industry. They are collected from naturally occurring oyster beds as well as grown commercially.
- Besides their commercial importance, oyster beds provide habitats for other types of marine organisms. Oysters also support healthy marine ecosystems by filtering the water of excess nutrients, such as those resulting from fertilizer run-off, which can lead to harmful algae blooms.
- Research into the settling behavior of oyster larvae will improve understanding of the effect on oyster beds of human intervention that alters turbulence patterns in the coastal region, as well as potentially increase the productivity of commercial hatchery operations.
- Oyster larvae are normally upward swimming in still water, often moving in a helical motion. Their mean upward swimming velocity is about 0.5 mm/s, and their downward velocity when exhibiting their settling dive response is about 5 mm/s.

Experimental Method

- Experiments were conducted in a double grid stirred turbulence tank. A diagram of the turbulence tank and a photo of the actual experimental setup appear below.
- For each trial, approximately 65,000 larvae were put into 250L of filtered sea water. Video data of the larvae were taken of a region in the center of the tank. A Particle Imaging Velocimetry (PIV) laser was aligned to fire through the camera field of view in order to investigate the potential effect of the laser light on larval behavior.
- Video data were taken of treatments at 3 different turbulence levels (still water, medium turbulence, and high turbulence). Data were taken with the PIV laser on and off at each turbulence level in order to compare larval behavior with and without the laser. The order of turbulence levels and laser light on/off treatments was randomized to prevent systematic error.



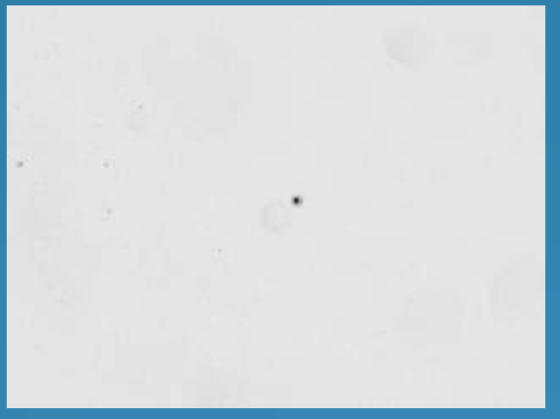


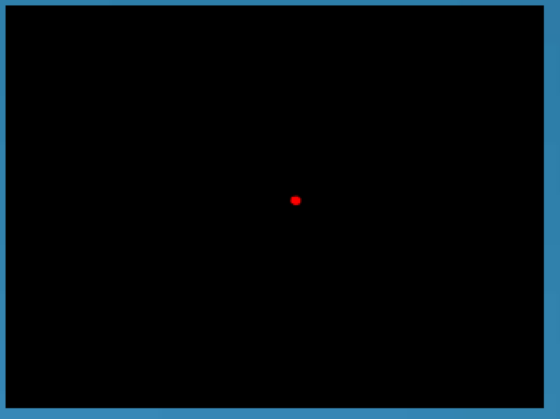
Video Data Processing

- The first step in raw data processing to extract larval behavioral data is to detect larvae positions in the video data sequence. A normal method for performing particle detection in image data is to apply a pixel intensity threshold to remove all non-particle areas of the image and then calculate the centroids of the remaining particles.
- Normal image thresholding requires a uniform image background. Since the video data taken did not have a uniform background, the image background was calculated by averaging together the frames of a given video sequence. This background, when subtracted from an individual frame, transforms the frame background to a uniform intensity. This makes thresholding to detect particles possible, and filtering the detected particles by size detects all larvae appearing in a video frame.


Example image showing one larva. Note non-uniform background (e.g. dark corners)

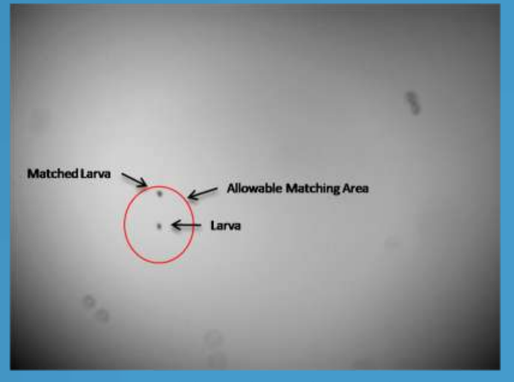

Step 1: Image background calculated by averaging video frames.


Step 2: Example image with background transformed to uniform intensity by subtraction of average image background.

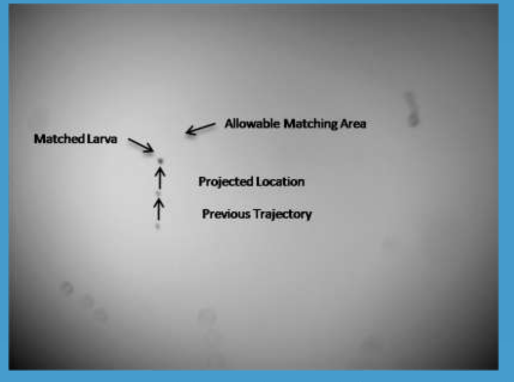

Step 3: Larva detected by image thresholding and filtering by particle size.

Larval Tracking

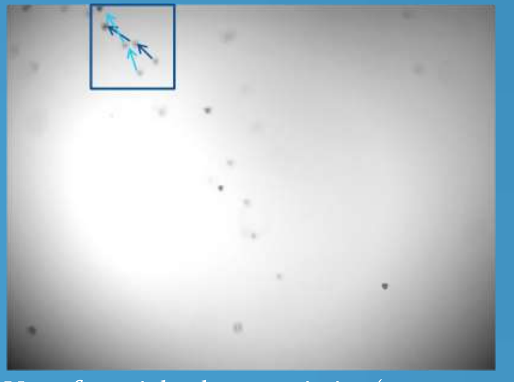
- Video data processing creates a list of larval positions in video frames. By comparing larval positions in subsequent frames, it is possible to match the positions of a particular larva together over a sequence of frames, thus detecting the larval movement track.
- A nearest neighbor matching algorithm was used (illustrated graphically below). The nearest neighbor algorithm searches for the larval positions closest together between subsequent images. If these positions fall within an allowable movement range, the positions are assumed to represent the location of the same individual larva in both images, leading to the creation of a larval track.
- To improve matching accuracy, after the beginning of a larval track the next predicted larval position was calculated, and the allowable movement range (i.e. where the algorithm looks for a position match) was shifted towards the predicted position. Also, in ambiguous cases such as when two larval paths cross close to each other, comparison of average particle intensity was used to ensure correct larval tracking.

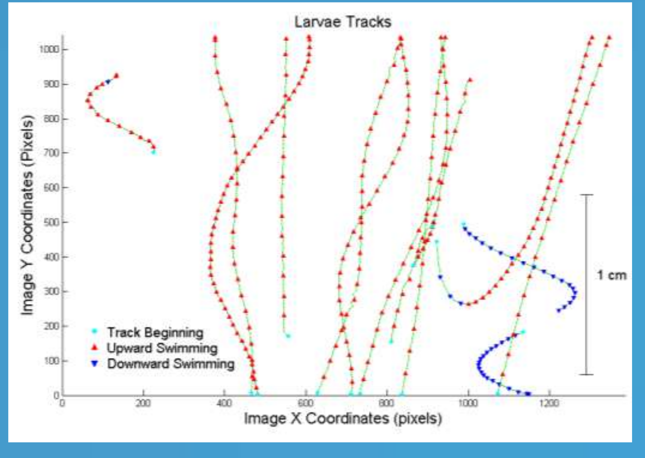


Nearest neighbor larval match between video frames.



Nearest neighbor adjusted for predicted larval location

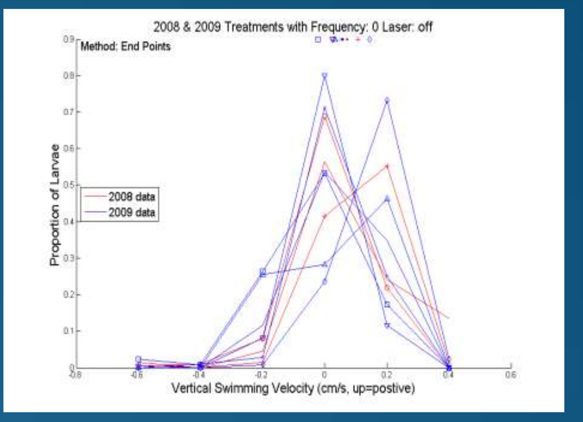

Use of particle characteristics (average particle intensity) in ambiguous cases.



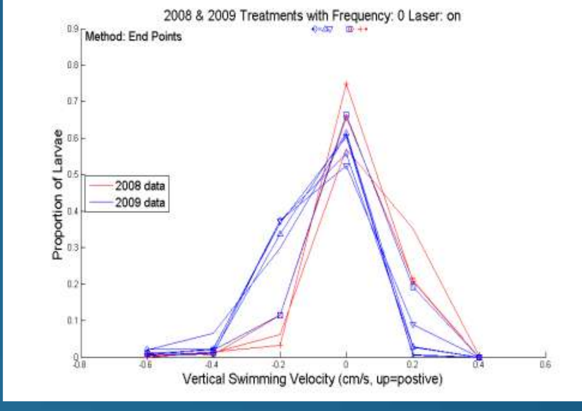
- After tracking is completed, several hundred larval tracks per trial are available for statistical analysis of larval behavior.

Results

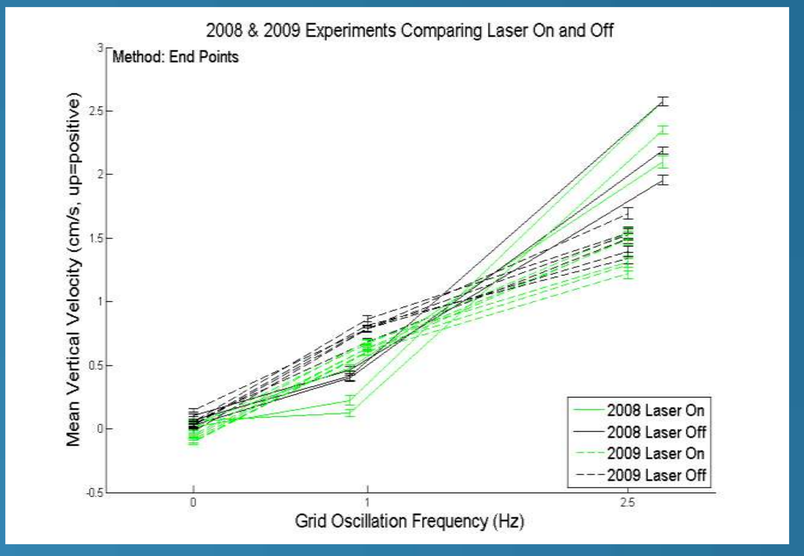
- Several experimental issues inhibited the detection of a larval dive response to turbulence. Chiefly, an unmeasured mean vertical flow in the tank prevented calculation of the larval component of vertical swimming velocity in turbulence.
- A clear larval response to the PIV laser light was detected in this year's experiments. The use of a higher powered pulsed laser led to a larval dive response that was not evident in previous experiments with a lower powered continuous laser.
- Although a dive response to turbulence could not be observed, the dive response to the laser verified that the experimental method was capable of detecting a larval dive response.



Larval swimming speeds were similar in still water with the laser off between years.



Larval swimming speeds in still water with the lower powered laser in 2008 are the same as with the laser off, but in 2009 with the more powerful laser many larvae dive.



- This figure shows a summary of larval swimming behaviors in 2008 and 2009 with the laser on versus the laser off. The increasing vertical slope as grid oscillation frequency (i.e. turbulence) increases is indicative of the mean vertical flow in the tank.
- The 2008 laser on and off data show little effect of the laser on larval vertical swimming speed.
- The 2009 laser on and off data show a marked effect of the laser light on swimming behavior. For all turbulence levels, the laser on swimming speeds underlie the laser off swimming speeds.

Conclusions

- The larval dive response to the PIV laser was well established, and demonstrated the capability of the experimental method to detect a larval dive response. In order to retain the capability to perform PIV without affecting larval behavior, a different wavelength laser or a lower power level may be necessary.
- The mean flow generated in the tank makes it difficult to accurately measure larval contribution to their velocity versus the velocity due to the mean flow. In order to resolve this, the tank may be improved to reduce the mean flow problem, and/or the mean flow may be measured in order to calculate and account for its contribution to larval velocity.
- Although the larval dive response to the PIV laser was confidently detected, the difference in mean larval swimming speeds with the laser on and off was on the order of 1 mm/s. In order to confidently detect a larval dive response to turbulence, therefore, measurements of larval swimming velocity and mean flow must be accurate to 1 mm/s or better.

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