SUPPLEMENT TO METHODS

Volumetric imaging of fish locomotion

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We used a new but experimentally validated technique to analyze the three-dimensional structure of the wake of swimming fishes, which allows an instantaneous snapshot of the entire wake volume, thus obviating the need to reconstruct 3D wake flow patterns from 2D slices. For reference please see the article cited below and the citations therein, and the supplemental figure on the next page. Also please note that Dr. Dan Troolin, an author on this paper, was one of the engineers who developed the volumetric visualization system used to acquire these data.

To briefly summarize, fish were placed into a recirculating flow tank and swam within the volume imaged by the volumetric imaging camera system (Figure S1 below). This camera system has three independent lenses and CCD arrays (2,048 by 2,048) that were calibrated by traversing a known target across the transverse (z) plane of the flow tank, in the volume and downstream of where the fish swam. Groups of image pairs (one pair per camera – 3 pairs total) were captured at 7.25 Hz with a time of 3.5 ms in between each image pair, at 12 bit resolution. The volume imaged was 14*14*10 cm, and for each image pair approximately 70,000 particles were identified in all three images, and from these approximately 35,000 triplets representing three views of the same particle were identified and tracked between laser pulses. These particles were gridded to give a final volumetric matrix of 57*57*33 vectors (= 107,217 total vectors within the volume). As a control, particle images were captured while the flow tank was running with no fish inside. Under control conditions, mean vorticity magnitude was 0.74 ± 0.11 s^{-1} (mean ± s.e.; X vorticity = -0.10 ± 0.09 s^{-1}, Y vorticity = -0.20 ± 0.19 s^{-1}, Z vorticity = -0.037 ± 0.079 s^{-1})."

The uncertainty of the V3V measurement derives from two primary sources, uncertainty in the time separation between laser pulses and uncertainty in measuring the 3-dimensional spatial location of the particles. The firing of the laser pulses is controlled by the timing electronics of the system, for which the timing uncertainty is 1 ns, resulting in a negligible effect

on velocity. The uncertainty in the measured particle positions has been determined using a planar grid of dots with known spacing placed at various locations within the measurement volume. Measurement of the dot positions showed an uncertainty of 20 microns in the x- and y-directions, and 80 microns in the z-direction. The time between laser pulses is set such that the particle displacement between two successive image frames is approximately 2 mm in the area of interest. Thus, the inherent uncertainty in the velocity measurement is approximately 1% in x- and y-directions, and 4% in the z-direction.

The current construction of the camera probe (figure S1b) is a limiting factor in the volume that may be imaged (approximately 15*15*15 cm). Therefore, the V3V technique could be applied to any aquatic organism for which this volume of interest would be sufficient. Future iterations of this system, if developed to allow for modifications of the camera probe, scaling of the calibration target, and rate of frame capture, would greatly increase the adaptability of this tool for other biological systems of interest.

Troolin, D. and Longmire, E. (2010). Volumetric velocity measurements of vortex rings from inclined exits. Exp. Fluids 48, 409-420.

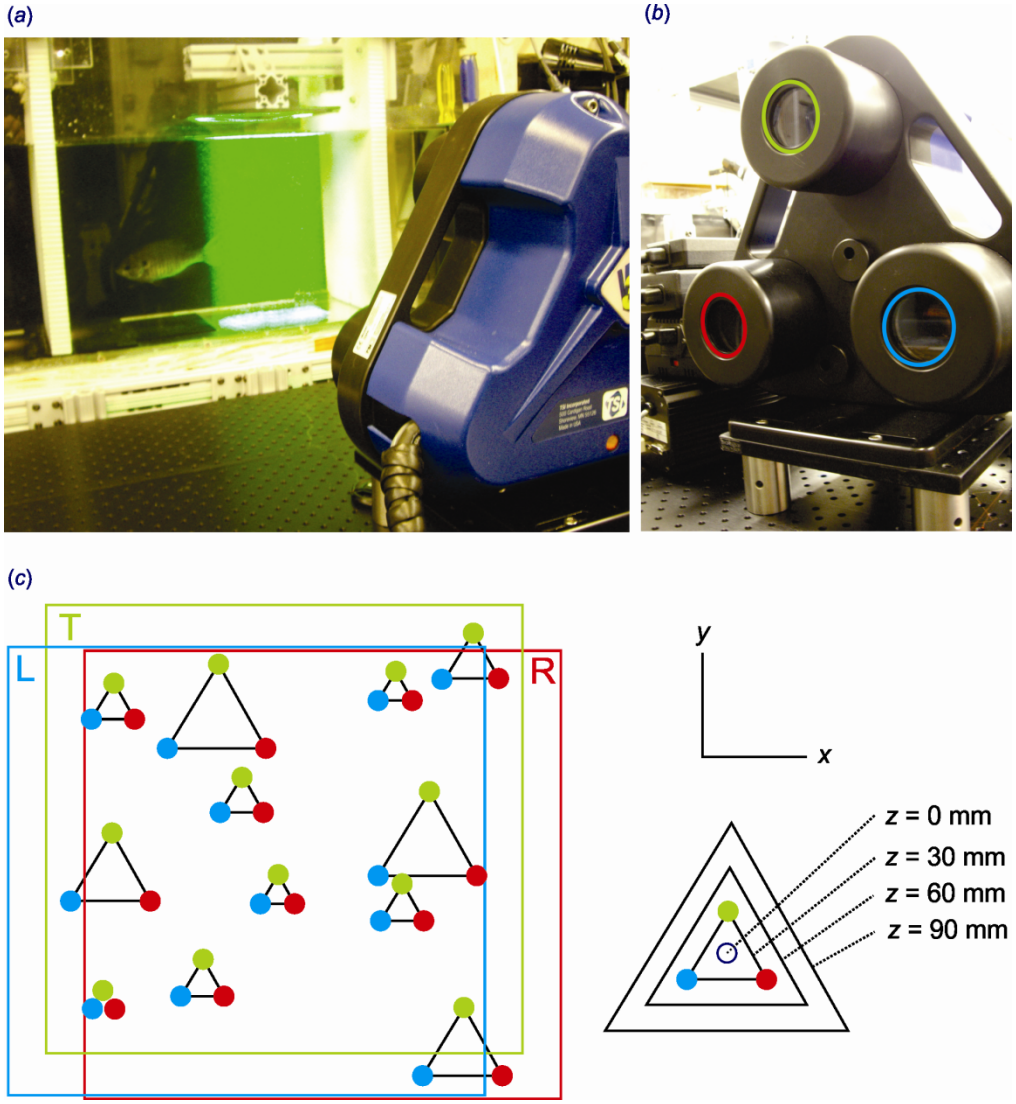

Figure S1. (*a*) Camera probe relative to bluegill swimming in front of laser volume in flow tank. (*b*) Front view of the camera probe. (*c*) Illustration of particle triangulation technique; modified from Troolin and Longmire (2010).