Infrared Tomographic PIV Measurement of Aquatic Predator-Prey Interaction

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Abstract

Infrared tomographic PIV is used to measure volumetric velocity fields during aquatic predator-prey interaction. In addition, a 3-D motion tracking method is employed on the same image sets to measure the organism trajectories. Zebrafish feeding on evasive copepods are investigated. Measurement volumes of 22.5 mm × 10.5 mm × 12 mm were reconstructed from images captured on a set of four cameras framing at 500 Hz. To obtain accurate fluid velocity vectors within each volume, fish were masked using an automated visual hull method. Predator and prey were identified independently from the same image sets, and their reconstructed locations were tracked. Image sequences show that the zebrafish executes a sudden acceleration to feed on the evasive copepods. The results did not reveal any obvious difference in the volumetric flow fields between cases of successful and failed predation. However, the maximum velocity of the copepod was higher for the case of failed predation. The relative trajectory of the copepod was also different for the two cases, suggesting that a strategic combination of velocity and trajectory may determine the predation success or failure.

1. Introduction

The dynamics of aquatic predator-prey interaction differ greatly from those of terrestrial interaction. In aquatic environments, predation is significantly affected by the motion of the surrounding fluid. For instance, an evasive prey (e.g. copepod) may sense flow perturbations caused by an approaching predator (e.g. fish), which signals the prey to escape. Successful predation, however, has been attributed to the strategic approach and manipulation of the ambient fluid by the predator (Higham et al 2005). Thus, quantitative study of the fluid motion associated with predator-prey interaction, can provide insights into sensory and locomotive behavior required for predation success.

Particle image velocimetry (PIV) has been used in many studies to understand the locomotion and feeding behavior of aquatic animals. These include fish (e.g. Higham et al 2005; Epps and Techet 2007), eels (e.g. Tytell and Lauder 2004), copepods (e.g. Catton et al 2007), jellyfish (e.g. Dabiri et al 2010), and sharks (Wilga and Lauder 2004). While planar PIV has provided significant understanding on the locomotion and predation of these organisms, it provides only two- or three-component velocity vectors in a plane. By contrast, flow fields and organism motions during predation are usually three-dimensional (3-D) which suggests a need for a volumetric velocimetry technique to understand the interaction.

Recent advances in PIV have made it possible to resolve all three components of velocity in three-dimensional space. Malkiel et al (2003) applied digital holography to obtain 3-D flow fields around and the trajectory of a feeding copepod, while Murphy et al (2011) applied infrared tomographic PIV to obtain higher resolution velocity fields generated by a copepod during its motion. In another study, Flammang et al (2011) used 3-D particle tracking velocimetry (PTV) to understand the wake structure generated by a shark's tail. These studies demonstrate the recent interest in volumetric measurements when the flow is complex and three-dimensional as would
occur in cases of predator-prey interaction.

In the present work, an infrared tomographic PIV system is introduced as a means to provide volumetric velocity measurements for aquatic predator-prey interactions. The system is used to study a small freshwater fish (zebrafish) feeding on an evasive zooplankton (copepod). To ensure natural animal behavior, an infrared laser, which is invisible to fish and copepods, is used for illumination. Using the same hardware arrangement, the motion of both predator and prey can be tracked simultaneously. This provides their three-dimensional trajectory during the interaction. The purpose of this paper is to describe some features of the measurement system and demonstrate its application. The predation success and failure when the zebrafish forages on the copepod is analyzed and discussed based on the derived velocity fields and organism motions.

2. Experiment Description

Experimental Setup
The measurements were carried out in a transparent tank measuring 300 mm (L) × 150 mm (W) × 205 mm (H), filled with water to depth of 120 mm (Fig. 1). The water was seeded with 55 µm polyamide (filled with 11% titanium dioxide) tracer particles of density 1.23 g/cm³. Polyamide particles were used instead of silver-coated glass spheres (e.g. Epps and Techet 2007; Higham et al 2005) since silver is known to be toxic to zooplankton (Hook and Fisher 2001). The measurement volume was illuminated with an Oxford Firefly infrared laser (wavelength: 808 nm) with 6 mJ/pulse and a pulse duration of 20 µs. The beam was expanded into a sheet with thickness 13 mm. Four high-speed cameras were mounted and aimed at the measurement volume. Front surface mirrors were installed at the bottom of the tank in an inclined manner to reflect the laser sheet back towards the measurement volume. The near infrared laser was used as it is known to be invisible to the fish (Lythgoe and Partridge 1989), preventing any unnatural behavior, but within the spectral sensitivity range of the CMOS cameras. Near infrared illumination has been applied in some studies, including high-speed visualization (Clarke et al 2009) and PIV (Epps and Techet 2007; Murphy et al 2011). Four high-speed cameras (Phantom v210) from Vision Research Inc., each with a 12-bit monochrome CMOS sensor and 1280 × 800 pixel resolution were used for image acquisition. The camera frame rate was set to 500 Hz, and synchronized with the laser modulation frequency. Each camera was fitted with a Scheimpflug adapter, and angled such that the image focus remained the same throughout. Four camera lenses (Nikon Micro-Nikkor) of focal length 200 mm were set with aperture of f/11.

All four cameras were mounted on the same side of the laser sheet, with the cameras tilted at an angle of approximately 30°. The distance between the cameras and the measurement volume was approximately 600 mm (Fig. 1b). In all subsequent discussion, the x and y-axes are defined parallel to the laser sheet, and the z-axis is defined positive towards the cameras with z = 0 furthest from the cameras. Image acquisition from the cameras was synchronized by supplying the frame synchronization signal from one master camera to the other three cameras and the laser.
Fig. 1 Schematic diagram of the experimental setup. (a) x-y view, (b) x-z view.
Predator and Prey Species
Zebrafish (Danio Rerio; size: ~20 mm) were used as predators, while freshwater copepods (Diaptomus species; size: ~1 mm) were used as evasive prey. Twelve zebrafish, purchased from a local pet store, were housed in a 38-liter aquarium at 25°C. The fish were provided with 12 hours of light per day and fed daily with mixed diet of flaked food and brine shrimp. Copepods were collected from Lake of the Isles, Minneapolis, MN USA (44°57'6.42"N 93°18'27.28"W) using 0.25 m diameter plankton net (mesh size: 150 µm). They were kept in 20-liter aerated containers and used within 24 hours of collection.

A zebrafish was transferred from the aquarium to the measurement tank and allowed to acclimate for 15 minutes before laser illumination and data acquisition. The laser illumination and image acquisition were then initiated, whereby the images were acquired continuously in a 12-second buffered loop. Evasive copepods were then placed judiciously near the illuminated volume in order to ensure a good chance of the predator-prey interaction within this volume. A post-trigger was applied when the fish was observed executing its predation within the real-time video. Video sequences were examined, and only those showing predator-prey interaction were saved in the computer for later tomographic PIV processing and organism motion tracking. For a given fish, fewer than 10 recordings were obtained in order to prevent satiation. After being subjected to an experimental series, the fish were placed into a second, identical 38-liter aquarium to avoid being used twice.

Tomographic PIV
All of the tomographic PIV analysis with visual hull implementation was carried out using DaVis 8.0 software from LaVision. The steps were as follows:

i) Image preprocessing. Normally 'subtract sliding minimum' and peak normalization were applied. This eliminated the background noise and reduced the intensity of the fish image by subtracting minimum intensity values within the window.

ii) Volume calibration and self-calibration. The volume was calibrated using a plate of 64 mm x 38 mm with a grid of dots spaced every 1 mm along its surface. The plate was traversed in the z-direction to seven positions, spanning the volume depth of 18 mm and recorded by each camera. A third order polynomial mapping function in x and y was obtained by the volume calibration (Elsinga et al. 2006), which was used to triangulate seeding particles in the physical measurement volume. Self-calibration procedure of Wieneke (2008) was applied to correct and improve the fit of the mapping function. For the self-calibration procedure, we acquired 300 sets of simultaneous particle images (without fish and copepods), which were used to minimize disparity associated with particle triangulation. This step was also used to determine the effective volume thickness (z-dimension) where particle seeding intensities were sufficient for correcting disparity errors. The x and y dimensions were determined from the overlapped camera views.

iii) Volume reconstruction. Particle intensity volumes were reconstructed from sets of four images using five iterations of the MART algorithm implemented within DaVis 8.0 software. The voxel to pixel size ratio was set to 1, and the reconstructed volume was selected to cover -12.5 mm < x < 10 mm, -4 mm < y < 6.5 mm and 0 mm < z < 12 mm, which lay within the self-calibrated volume. Magnification was 0.02 mm / voxel.

(iv) Visual Hull Masking. The visual hull technique (Adhikari and Longmire 2012a) was applied to mask the reconstructed volumes. A 3-D mask is required to remove the artifacts, caused by the presence of the fish within the measurement volume, before cross correlating in order to obtain accurate velocity vector fields. The visual hull method will be elaborated in the next section.

(v) Volume cross-correlation. Volumetric velocity fields were determined using a multi-pass
approach started with interrogation volume of 128 voxel\(^3\) to final size of 96 voxel\(^3\) with 75\% overlap. The dimension of the resulting interrogation volume in all three directions was 2 mm, and grid spacing of the velocity vectors was 0.5 mm. This resulted in approximately 27,648 (48 × 24 × 24) grid points for each time step. Vectors were computed only on points located outside of the reconstructed object, and any vectors centered within the object were again masked during post-processing.

(v) Post-processing. After volume cross-correlation, outlier vectors were removed and recursively replaced by the average of nearest neighboring vectors (3 × 3 × 3 pixel window). Generally, fewer than 2\% of the vectors were replaced in 96 voxel\(^3\) correlations.

**Visual Hull Method**

Step (iv) is a recently proposed 3-D masking technique (known as visual hull) for tomographic PIV (Adhikari and Longmire 2012a). In this case, the implementation of the visual hull is a necessary step for tomographic PIV to eliminate the reconstruction artifacts caused by the existence of the fish. If these artifacts are not masked, fluid velocity vectors derived near the object may be contaminated such that they are biased or completely erroneous. Fig 2 shows a schematic of the process employed to obtain the visual hull of a fish. Fig 2a includes the four images acquired from the cameras. The silhouette of the fish (Fig 2b) within each field of view was extracted using the image processing sequence suggested by Adhikari and Longmire (2012a). After that, these silhouettes were back-projected using DaVis 8.0 multiplicative line-of-sight (MLOS) operation, to obtain the visual hull (Fig 2c) which constituted the 3-D masked applied to the reconstructed measurement volume.

![Fig. 2 Processing sequence from (a) image to (b) silhouette to (c) visual hull of the fish (blue iso-surface).](image-url)
The shape of the visual hull is highly dependent on the shape of the fish, orientation of the fish (i.e. direction of approach into the illuminated volume), and the camera arrangement (see Adhikari and Longmire 2012a). Since the camera arrangement and the fish shape used in the present work are constant, only the direction of approach of the fish into the illuminated volume influences the construction of the visual hull and the region where velocity vectors can be resolved. Fig 3 shows examples of a fish entering the illuminated volume parallel to the (a) x-y plane and (b) y-z plane. From the figure, it is observed that the fish travelling parallel to the y-z plane (fig. 3b) creates a visual hull with a larger volume in front of the fish mouth as compared to the fish travelling parallel to the x-y plane (fig. 3a). Although the volume in front of the fish mouth is visible in fig. 3b (raw image), the back-projection of the silhouette from the camera images causes the visual hull to form in front of the fish mouth. This visual hull is then used to mask the velocity vectors in that volume. The significant fluid volume masked in fig. 3b is obviously problematic for understanding velocities near the mouth. In contrast, the visual hull created when the fish is approaching from the x-y plane (fig. 3a) makes a tighter fit to the mouth of the fish, thus, providing more vectors in that region. Based on this observation, only results with fish trajectories orientated near the x-y plane were analyzed for this study.

Fig. 3 Schematic representation of the various approaches of fish into the measurement volume. (a) Fish enters from the side of the measurement volume (parallel to x-y plane); (b) fish enters the back of the volume (parallel to y-z plane).

**Animal Motion tracking**

The procedure for motion tracking consists of two steps: (1) locating a point on the organism in the images, and (2) triangulating the point into a 3-D location based on two or more camera images. In order to track the fish motion, the center of the fish eye was selected from each camera image. A Circular Hough Transform (CHT) scheme, as implemented by Schreiner (2011), was applied to automatically identify and locate the center of the eye in all of the images. Since the prey was small (10 - 40 pixels), the head of the prey was identified manually in all the image sequences, and its coordinates were recorded.
After identifying the location of the fish eye and prey within the planar images, a separate image file consisting of only the identified point location ('particle') was generated. Thereafter, a 3-D particle tracking velocimetry (3-D PTV) operation within LaVision DaVis 8.0 was applied to reconstruct the volumetric location of each 'particle' at different times within an acquisition sequence.

3. Results and Discussion

The described measurement technique was employed recently by Adhikari and Longmire (2012b) where they reported that the infrared laser and the particles used in the experiment did not affect the natural behavior of the fish. In addition, the signal-to-noise ratio of the reconstructed measurement volume in tomographic PIV was demonstrated to be acceptable with the use of infrared illumination. Adhikari and Longmire (2012b) reported that the zebrafish applies sucking action to capture a non-evasive prey (suction feeding), and uses sudden acceleration to capture evasive copepods (ram feeding). In this paper, the measurement system is used to analyze and compare cases of successful and failed captures of copepods by the zebrafish.

First, the entire image sequence of the zebrafish attempting to capture a copepod was analyzed visually based on individual images with time interval $\Delta t = 2$ ms. The time, $t = 0$ ms is set when the copepod first starts to move, which is less than 2ms after the initial acceleration of the fish. Each volumetric velocity field (masked by a visual hull) of $22.5 \text{ mm} \times 10.5 \text{ mm} \times 12 \text{ mm}$ was obtained after applying tomographic PIV processes. The location of the fish eye and copepod were obtained separately, and were superposed into the same vector field. These locations of the predator and prey are represented as spheres or dots (see red, green and yellow spheres in fig. 4 and 5).

Volumetric Velocity Field

Fig. 4 shows a raw image (fig. 4a) from one of the cameras and the corresponding volumetric vector field (fig. 4b) of the zebrafish, as it is about to capture a copepod successfully at $t = 12$ ms. In the entire image sequence (not shown in the figure), the fish is observed to first approach the prey, and then execute a sudden acceleration to capture it (ram feeding). Fig. 4 shows only an instance of the ram feeding just before the copepod was captured. The vector field around the mouth of the fish during ram feeding is generally diverging (see fig. 4b). This high velocity lunge causes the flow around the mouth of the fish to attain velocity of about $0.014 - 0.02$ m/s, mainly directed away from the fish mouth. From the initial and final location of the prey (see green and red dots in fig. 4b), it is observed that the prey moves away from the mouth of the predator (see x-y plane), and the direction of motion does not follow the direction of the fluid velocity (see y-z plane). Thus, the evasive copepod is observed to move away from the fish mouth independent to the direction and magnitude of the flow field. This observation is possible due to the capability of the system to measure 3-D flow field and location of the organism.
Fig. 4 Volumetric velocity field of the fish capturing evasive prey (copepod) at $t = 12$ ms. (a) Image and (b) volumetric vector field. Red dot depicts the location of the prey, and yellow dot depicts the center of the fish eye used to track its motion. Green dot depicts the initial ($t = 0$ ms) location of the prey.

Fig. 5 shows a result from a case where the zebrafish fails to capture the copepod. Fig. 5a shows an image, and fig. 5b shows the corresponding velocity field when the fish forages on the copepod. This instance corresponds to the same time, $t = 12$ ms, as the successful prey capture in fig. 4, and it is also the time that the fish mouth comes closest to the copepod. For this case, the velocity field around the mouth of the fish is also generally diverging. The magnitude of the velocity field around the fish is about $0.014 - 0.02$ m/s, and mainly directed away from the mouth of the fish. This velocity field magnitude is similar to that observed in fig. 4. The prey trajectory (fig. 5b) shows that the prey moves away from the fish in both x-y and y-z planes. This suggests that, while volumetric velocity vectors may provide the flow field around the interaction, no obvious difference in the field is observed for predation success and failure (fig. 4 and 5).
Fig. 5 An instance of volumetric velocity field of the fish failing to capture evasive prey (copepod) at $t = 12$ ms. (a) Image and (b) volumetric vector field. Red dot depicts the location of the prey, and yellow dot depicts the center of the fish eye used track its motion. Green dot depicts the initial location of the prey.

**Velocity Magnitude**

The velocity magnitudes of the predator, prey, and the fluid velocity at the location of the prey are shown in fig 6a and 6b for predation success and failure, respectively. For both cases, $t = 0$ ms is designated as the time when the copepod first starts to move, which is less than 2 ms (or one data frame) after the initial acceleration of the fish. Note that the velocities of the fish and copepod are much higher than the local flow velocity.

For the case of predation success (fig. 6a), the maximum velocity of the fish is $\sim 0.55$ m/s, while the maximum velocity of the copepod occurs earlier with a magnitude of $\sim 0.32$ m/s. In the same figure, it is also noted that the copepod is captured when the velocity of the fish is maximum. On the contrary, in the case of predation failure, the maximum velocity of the copepod is $\sim 0.5$ m/s, which is approximately the same as the maximum velocity of the fish (fig. 6b). Also, the copepod velocity at the time of closest approach (dashed green line) is larger than when the other copepod was captured. This may intuitively suggest that higher prey velocity can lead to predation failure.
**Fig. 6** The velocity magnitude of predator (solid line; ●), prey (dashed line; ■), and local flow velocity at the location of prey (dotted line; ▲). (a) Predation success, (b) Predation failure.

### 3-D Trajectories of Predator and Prey

Trajectories of the fish and copepod are shown in figs. 7 ($t = 0 - 12$ ms) and 8 ($t = 0 - 14$ ms) for the cases of predation success and failure, respectively. From fig. 7, we observe that the fish trajectory is mainly in the x-y plane with limited displacement in the z-direction. However, the copepod moves with a positive z-displacement component towards the fish. Although the copepod maximum velocity is higher than fish at $t = 2 - 5$ ms (as observed in fig. 6a), its motion towards the z-location of the fish contributes to the predation success.

**Fig. 7** Predator and prey tracks with $\Delta t = 2$ ms time intervals for successful prey capture by ram feeding. Final location of the prey is shown by the read sphere, while final location of the predator is shown by the yellow sphere. The initial location of both predator and prey is given by the green sphere.

Fig. 8 shows a different trajectory of the fish and copepod as compared to fig. 7. In fig. 8, the fish moves in the positive z direction (+ ~1 mm), while the copepod moves in the negative z direction (- ~2 mm). This trajectory combination, combined with the higher maximum velocity of the copepod suggests that predation failure in this case may be due to strategic combination of the maximum velocity of the copepod and the direction at which it escapes relative to the fish. The
copepod is able to spring away from the fish by choosing a better escape direction.

![Fig. 8 Predator and prey tracks with Δt = 2 ms time intervals for failed prey capture by ram feeding. Final location of the prey is shown by the read sphere, while final location of the predator is shown by the yellow sphere. The initial location of both predator and prey is given by the green sphere.]

### 4. Conclusion

Infrared tomographic PIV has been applied successfully to study aquatic predator-prey interaction. In addition, predator and prey tracks are also obtained, and combined with the volumetric velocity field for simultaneous analysis of fluid motion and organism trajectories.

Comparison of flow fields and organism trajectories was carried out for cases of predation success and failure. The zebrafish was observed to employ a sudden acceleration to feed on the copepod successfully, and the flow field around the mouth of the zebrafish was observed to be divergent. A similar flow field was also observed for failed predation.

Further analysis on the velocity of the fish and the copepod during the interaction showed that the maximum velocity of the copepod was higher for the case of predation failure. In addition, the trajectory of the fish and the copepod moved away relative to each other in the z-direction for it failed predation to occur. This study suggests that the 3-D trajectory and velocity of the predator and prey may play a dominant role in determining predation success.

In future work, we plan to develop an improved, automated routine for identifying the relatively small copepod species within image sequences. In addition, we plan to extend the capabilities of the identification routines to include species orientation in order to resolve rotational as well as translational motion. This work is part of a larger investigation aimed at understanding the effects of flow currents and turbulent eddies on predation success rates.

### 5. Acknowledgements

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### 6. References


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