Automated analysis of eye-body coordination during prey captures by newborn live-bearing fish.

Martin Lankheet, Twan Stoffers, Bart Pollux

University of Wageningen, Department of Animal Sciences, Wageningen, the Netherlands

Live bearing fish

Compared to egg-laying fish, live-bearing species invest much energy in the production of fewer, yet relatively more developed offspring. Although in many live-bearing species (e.g. mammals) viviparity is combined with extensive brood care after birth, this is not the case in the Poeciliidae, a family of freshwater fish that includes the well-known guppy (*Poecilia reticulata*), mollies (subgenus *Mollienesia*) and swordtails (genus *Xiphophorus*). The lack of post-natal parental care makes it critically important for newborn Poeciliids to quickly develop the required skills for survival, such as the ability to capture prey and escape predators.

In many of these behaviors visual perception and eye movements play a crucial role. To study the development of such behavior it is therefore critical to measure both swimming behavior and eye movements. In this paper we will show the development of prey capture behavior in newborn live-bearing fish, including changes in their use of eye movements for detecting and approaching prey. Since prey capture events are in many respects highly variable we recorded over 2000 capture events using high-speed video and developed fully automated analysis protocols for both body and eye movements.

Prey capture behavior

We studied ontogenetic changes in prey capture behavior during the first three days of life in the live-bearing fish *Girardinus metallicus* (Poeciliidae). Newborn fish need to start hunting immediately after being born in order to survive. It is not known, however, to what degree these behaviors are innately present at birth or acquired through learning during the first days of life. To measure these potential changes, and the underlying mechanisms, we have fed 28 newborn *G. metallicus* one baby brine shrimp (*Artemia nauplii*) at a time until satisfaction for a period of three days and recorded the strike and pre-strike behavior of every prey-capture event. During these three days the fish increased their food intake considerably (both in terms of the total amount of consumed prey and the rate of prey intake) and greatly reduced the rate of failures in catching prey. The question we address here is how the fish change their swimming pattern and eye movements while improving their hunting skills during this critical three-day period after birth.

Figure 1. Example frames at 4 ms apart from a prey capture event, just before ingestion of the prey (artemia).

High-speed movies

Prey capture events were filmed dorso-ventrally with a Mikrotron EoSens MC1362 high-speed camera at 500 frames per second and 1280x1024 spatial resolution. The fish were kept in petri dishes at 24 ºC temperature and a normal day-night light regime. LED arrays provided lighting from below (white LEDs) and from the sides (circular arrays of RGB LEDs), controlled independently by means of PWM signals generated by a MatLab controlled Arduino-based LED driver. Adjustment of different light sources allowed us to obtain the required contrast for body and fins relative to the background, and for the eyes relative to the body. Fig. 1 shows three examples of images from a recorded movie. In total we recorded and analyzed over 2000 capture events for 28 fish divided over the first three days of their lives. All experiments were approved by the ethical committee at Wageningen University (the Netherlands).

Data analysis

We used MatLab (including the image analysis toolbox) to analyze body, pectoral fins and eye orientations from the recorded frames. In addition, we tracked the position of the prey throughout the movie, to establish eye and head parameters relative to the prey.

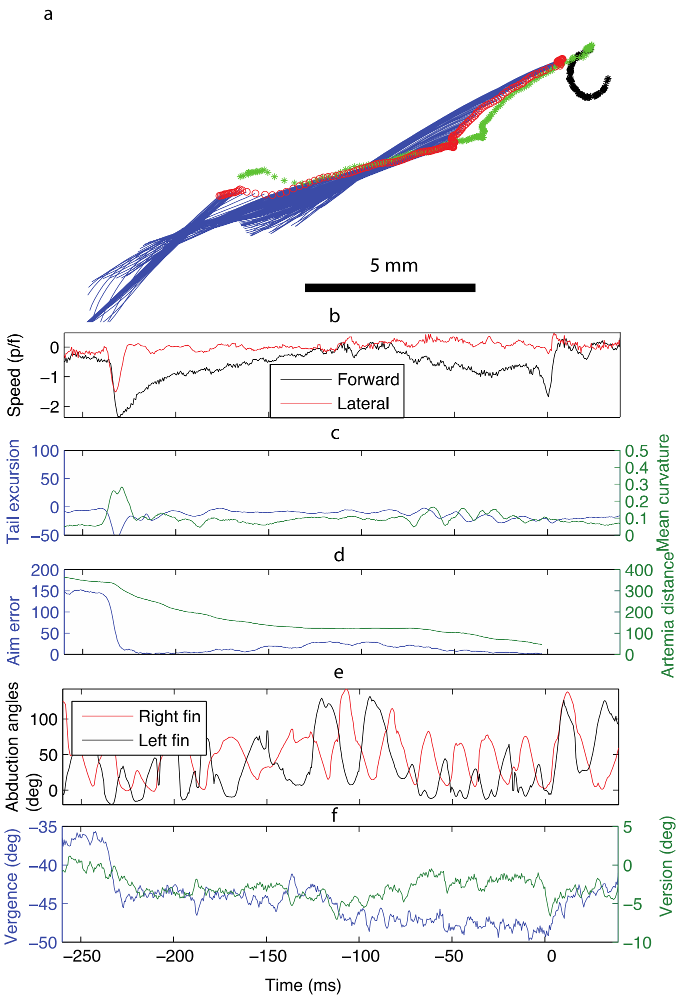

Figure 2. Quantifying prey capture events. a) body axes, midpoint of the eyes (red) and tip of the snout (green) in x-y coordinates, artemia in black. b-f behavioral parameters as a function of time relative to the strike (T=0); b) forward and lateral speed of the head. c) tail excursion and body mean curvature (arbitrary units). d) aim error of head relative to the artemia and distance to artemia. e) left and right fin abduction angles. f) eye vergence and version angles.

Previous studies on eye movements in, for example, larval zebra fish (*1*) have used the orientation of the main axis of the eyes to quantify viewing direction. For our images of live-bearing fish this was not optimal. In many movies the contrast between the eyes and the body was critically low and variations affected the length and or width measurement of the eyes. Such variations directly affected the estimate of viewing direction. To obtain a more robust measure we extracted the (mean) orientation of the flat, ‘lateral’ surface of the eye. First, the centroids of the eyes were determined by applying an adaptive threshold. Second, the center of the eye was used to define the outline of the fish that corresponded to the flat lateral surface of the eye. We determined the required outline of the fish by means of standard edge detection. Finally, eye orientations were quantified as the orientation of a straight line fitted to this part of the outline (i.e., orthogonal to viewing direction).

In addition to vergence (differences in orientations between the eyes) and version angles (mean viewing direction for the two eyes) we also determined body kinematics (axis curvature) and pectoral fin movements. The latter proved essential in explaining capture behavior, especially during the final strike. Pectoral fins were detected using edge-detection in a limited circle around the center-of-mass of the fish silhouette, followed by subtracting the silhouette. From the image of the fins we determined the fin abduction angles as the angle of the front-most edge relative to the midline through the head.

To compare measures for all recorded movies and between day 1, 2 and three we aligned all movies in time taking the last frame before the prey disappears into the mouth as the reference frame. This corresponds to the middle frame in Figure 1 and time T=0 in Figure 2.

Results

Figure 2 shows an example of analyzed data for one capture event. The maneuver starts with a C-bend and fast acceleration that aligns the head more or less with the prey (Fig 2d). Subsequently the fish slows down in the final approach to ingest the prey during a fast, forward acceleration, which is immediately followed by a strong deceleration. While approaching the prey the fish continuously increases its vergence angle, thus enlarging its binocular visual field. Version angles, on the other hand, are small and more or less constant during the approach.

Throughout the final approach the fish makes alternating left and right pectoral fin strokes. The changes in speed during the final strike are not accompanied by any obvious tail movements or changes in mean curvature. Instead, these speed changes strongly correlate with a change in the coordination of fin movements: Just before the strike the fish changes from alternating left-right strokes to a complete, simultaneous adduction (bringing both fins backward to align with the body). This symmetrical adduction causes a large forward thrust. The adduction is followed by a large amplitude, symmetrical abduction (moving fins outward and forward) that causes the nearly instantaneous break after the strike.

Based on a comparison of similar data obtained for day 1, 2 and 3 we conclude that the use of pectoral fins during the strike is highly stereotypical and invariant with age. The fish however, quickly learn to approach prey at higher speeds, while at the same time making less extreme eye vergence movements. Vergence angles show a similar rate of change, but maximum angles reduce significantly from day 1 to 2 and to 3.

Discussion

Fully automatic image analysis allowed us to quantitatively compare the performance and skills of newborn fish in prey capture events. It allows one to distinguish innate skills from skills that require development and calibration after birth. In zebra fish reflexes that control eye movements develop and become functional during the first days after fertilization (*2, 3*). Presumably, the gestation period in live bearing fish such as *Gerardinus metallica* is long enough to complete the circuitry for eye-body coordination. However, our data show that the final calibration to fine-tune eye-body coordination takes place after birth and presumably require visual input or practice. Our study further shows that other skills, such as coordinated movement of pectoral fins for final steering and for fast acceleration and deceleration during the strike, are already present and fully functional at birth.

1. I. H. Bianco, A. R. Kampff, F. Engert, Prey capture behavior evoked by simple visual stimuli in larval zebrafish. *Front Syst Neurosci* **5**, 101 (2011)10.3389/fnsys.2011.00101).
2. S. S. Easter Jr, G. N. Nicola, The development of vision in the zebrafish (Danio rerio). *Developmental Biology* **180**, 646-663 (1996)10.1006/dbio.1996.0335).
3. S. S. Easter Jr, G. N. Nicola, The Development of Eye Movements in the Zebrafish (Danio rerio). *Developmental Psychobiology* **31**, 267-276 (1997).