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# FLASH-EVOKED C-ESCAPE BEHAVIOUR FROM FREE SWIMMING CODFISH (*GADUS MORHUA*)

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**Bao-Quey Huang and Peter J. Fraser** (1989) Flash-evoked C-escape behaviour from free swimming codfish (*Gadus morhua*). *Bull. Inst. Zool., Academia Sinica* **28**(2): 97-106. Flash evoked startle behaviours were studied using video and high-speed cinematographic techniques on the freely swimming codfish (*Gadus morhua*). The fish was demonstrated to have a stereotyped response to a sudden brief flash with an initial fast body bend and subsequent rapid tail flip. The latencies of these two responses were 52.40±5.33 ms ($n$=32) and 126.99±16.02 ms ($n$=8). The curvature and angular velocity were also calculated from Cartesian coordinates showing the highest responses at 100~140 ms. There was good agreement between video (100~140 ms) and high speed cinematographical (126.99 ms) techniques.

**Key words:** Visually-evoked, C-escape, High-speed cine, TV-video.

The startle response of teleost fish to acoustic and vibratory stimulation has been investigated at many levels. It is believed to be mediated by a pair of giant interneurones, the Mauthner cells, located at the level of the eighth cranial nerve in the medulla oblongata (Zottoli, 1977; Eaton, *et al.*, 1977, 1981; Faber & Korn, 1978; Blaxter, 1981; Eaton, *et al.* 1984). Few studies have been done on visually evoked startle responses since their discovery. Eaton *et al.*, (1977) showed that a startle response in the goldfish could be elicited by the visual stimulus of a dropped golf ball. However, because it is impossible to know the exact time when the fish saw the dropping ball, the latency measurements of the visual startle response are uncertain.

The ecological, morphological and behavioural diversity of fish prevent a broad generalization throughout the teleosts of the functions of this behaviour (Zottoli, 1978a). The codfish was used in this experiment because it was considered that vision would be important in its natural habitat and hence it might show a strong vision dependent startle response. Above all, Huang (1986, 1987) has demonstrated that cod is unique in giving a response to a brief flash light with a stereotyped C-escape response and with conspicuous "missing heartbeat".

In this investigation, video and high-speed cinematographic techniques were used to quantitatively analyse the visually evoked startle response. The purpose was to gain insight into the neurophysiological basis and functional importance of the visually evoked startle behaviour. This work showed evidence of a sterotyped response pattern with fast-body-bending into C-shape and rapid-tail-flipping, which was believed to be involved, by the Mauthner cell. Zottoli *et al.* (1987) have demonstrated that visual information is relayed to the goldfish Mauthner cells through optic tectal input. In brief, this pathway presumably accounts for the demonstrated behavioural efficacy of visual stimuli in evoking a startle response.

## MATERIAL AND METHODS

Codfish (*Gadus morhua*), 28-32 cm in length, were caught from North Sea and kept in aquarium for at least 3 days. Random sampled individual was placed either in a tank, measuring 60×45 cm for video studies, or in a tank 30×50 cm for high-speed cinematographic work. Both tanks were filled with sea water to a depth of more than 15 cm. Water temperature of the experimental tank was kept close to that of the home aquarium (8-14°C).

For high-speed cinematographic studies, responses were recorded by setting a cine-camera (16 mm—IPL, Photosonic Inc.) about 1 meter above the tank floor to get a suitable photographic field. The evoked responses were recorded on 16 mm Kodak Video News colour film (High speed 7250 Tungsten, Eastman Kodak Co.) at the rate of 200 frames per second, with 1 msec exposure time. Four Intralux Optic Fibric Lights (Volpi) provided illumination, also sharp silhouettes were obtained by using a 'Scotchlite' reflex-reflector background beneath the tank to enhance the illumination. In general, fish were allowed to swim freely in the tank and were usually filmed in midwater at various orientations.

An electronic frame counter (Bowen Electronics Ltd.) provided a 100 Hz calibration signal recorded by a light pulse on the margin of the film to allow accurate timing of data. Responses were evoked by the electric flash gun (Vivitar Auto Thyristor 4600 system) in both high-speed cinematographic work and video studies. The duration of the flash was measured by a photodiode (R. S. components Ltd., No. 305-462) and oscilloscope. The measure duration was about 4.5 to 5.0 ms. Direct energy measurment with a conventional photometer was impossible, the energy of the flash corresponds to approximately 4 W $Sr^{-1}$ $M^{-2}$. (Anthony, 1981). Neither neutral attenuators nor spectral filters were used through the experiments. All the experiments were carried out under normal room illumination (about 100 lux). The position of the stimulus light and the intensity of the background light did not show significant effects on the responses. The flash stimulus interval was 20-30 minutes and between 10-20 stimuli were delivered to each animal. The pre-test acclimation times in the experimental tank (more than 1 hour) and interstimulus intervals were controlled to increase the probability of the startle responses. Experiments on each individual were continued until several startle responses had been photographed. Another fish was needed if it did not show response after 10 stimulus. Control experiments, without flash present, confirmed that fish did not respond to switching the camera, flash gun and other equipment.

For analysis of video-recording data, an analysis projector (NAC, DF1-16C) was used to draw and measure the projected images frame by frame. The displacement speed was determined by the position of the rostral extremity of the head in consecutive intervals. The video tape was analysed (Fig. 1) using a video cassette recorder (JVC Model CR-6600E), video disc system (VAS DIS36-500, Cameron Video System) and video monitor (Cameron Video System Type TVM1) which allows recordings from an image analyser (SP114 Hampton Video System) at constant 20 msec intervals. All measurements were from dorsal views of the

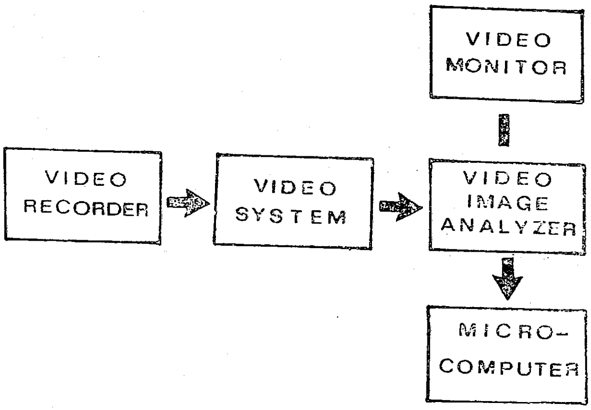

Fig. 1. Schematic experimental apparatus for video recording and behavioural analysis from freely swimming fish.

fish. The coordinates of each head and tail on a Cartesian grid from the digitizer were input to a microcomputer (NASCOM 3, Lucas Logic Ltd.) to calculate the displacements of the heads and tails and the curvature. The curve between head and tail was assumed to be circular and curvature was taken as the reciprocal of the radius of this circle.

## RESULTS AND DISCUSSION

### I. The Startle Response to Visual Stimulation

It has been widely demonstrated that fast-start kinematic patterns are divisible into three stages (a preparatory stoke, a main propulsive stroke and a variable third stage) (Webb, 1978). The first two stages were equivalent to the biphasic period of the Mauthner-initiated startle response (Eaton, *et al.* 1977). The present analysis of flash evoked startle responses was mainly done on the first two stages. The data from high-speed cinematographic and video techniques showed a characteristic pattern and latency which are similar to those described by others (Retzlaff, 1957; Eaton, 1977; Webb, 1978; Webb & Corolla, 1981) from various species of fish.

More than 40% of the 39 responses analysed from the TV technique (random sampling from 122 trials) showed maximum displacements of the head and tail at 82.10+12.06 ms ($n$=39, X±S.D.) after the flash stimulation (Fig. 2A). The mean peak of these displacement speeds was 8.0±2.0 B.L/S (Body lengths per second, Fig. 2B). Body length was more suitable for using as speed unit because the variable body lengths might affect the displacement distance. This can also be seen in Fig. 3 from high-speed cine film, the time to the maximum displacement speed after the initial movement of the head was 18.93±3.49 ms ($n$=5). It shows the startle responses were characterized by an initially high displacement speed corresponding to a pronounced lateral movement of the head and tail to the same side (Fig. 4). The mean latency of the flash evoked startle response from high-speed cine film was 52.40±5.33 ms ($n$=32). The expected time to the maximum speed from these data should be about 71.33 ms (18.93 ms + 52.40 ms), and the maximum speed derived from TV-video analyses was 82.10 ms. The difference between these two values could be ascribed to the lower resolution of the TV recording system.

As described by Webb (1976), it is convenient to refer to four different startle responses;

1) S-start without turn (the body being bent into a double flexture during the responses and adopting an S-shape);
2) L-start accelerated turn in the direction of the initial yawing of the anterior of the body;
3) L-start accelerated turn in the opposite direction to the initial yawing of the anterior of the body;
4) L-start without turn and acceleration forward as the tail moves laterally in the opposite direction.

In the last three, the anterior body

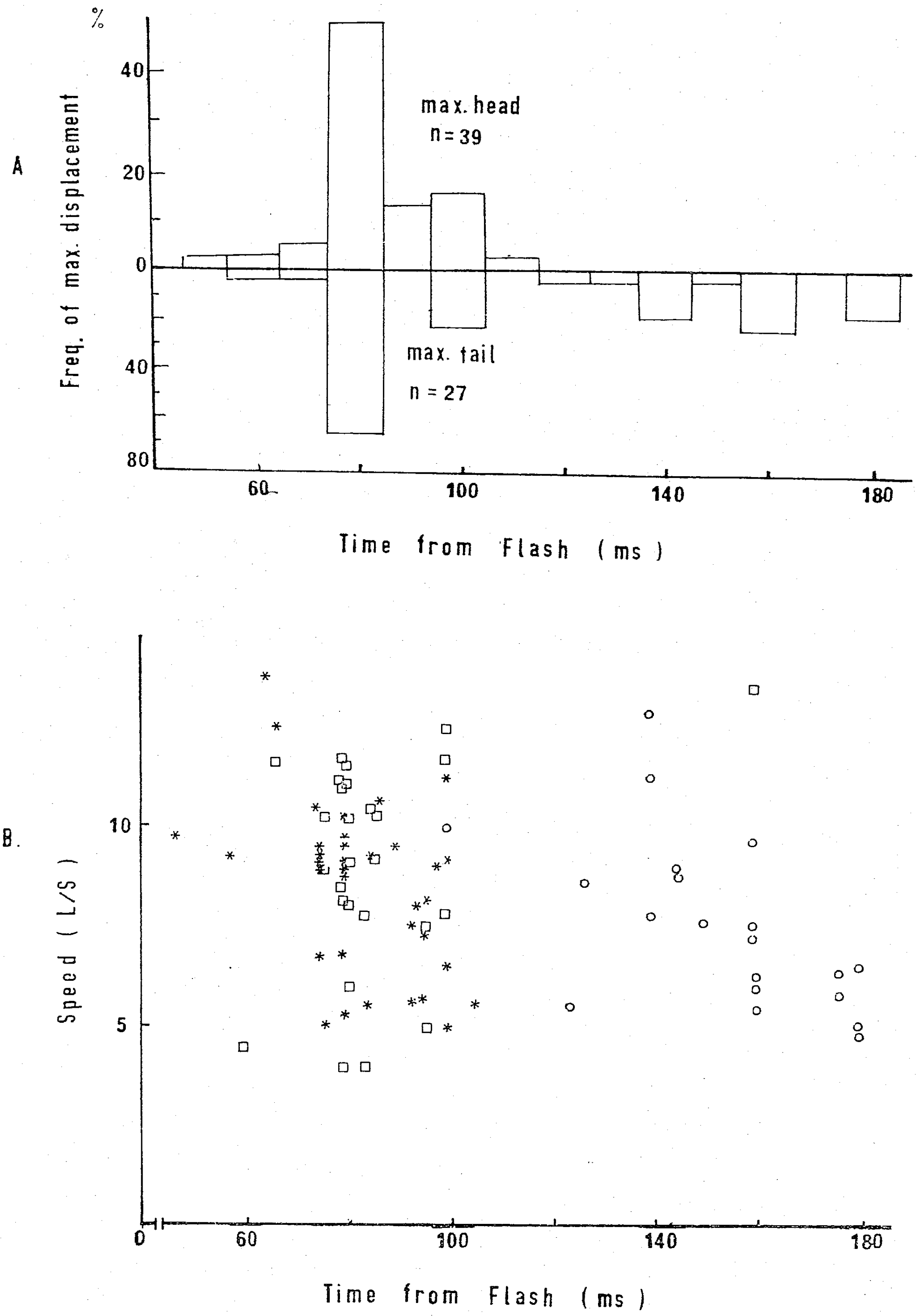

Fig. 2. The timing of maximum displacements of head and tail. (A) Frequency distribution of random samples from 122 analyzed trials of head displscement (upper) and tail displacement (lower). (B) Maxmum speeds of head and tail plotted against their time of occurrence. Maximal head displacement (*) is about 10 body lengths per second (L/S). Note that the peak speed of head and tail (□) are achieved simultaneously. ○ shows the peak speed of the second tail beat.

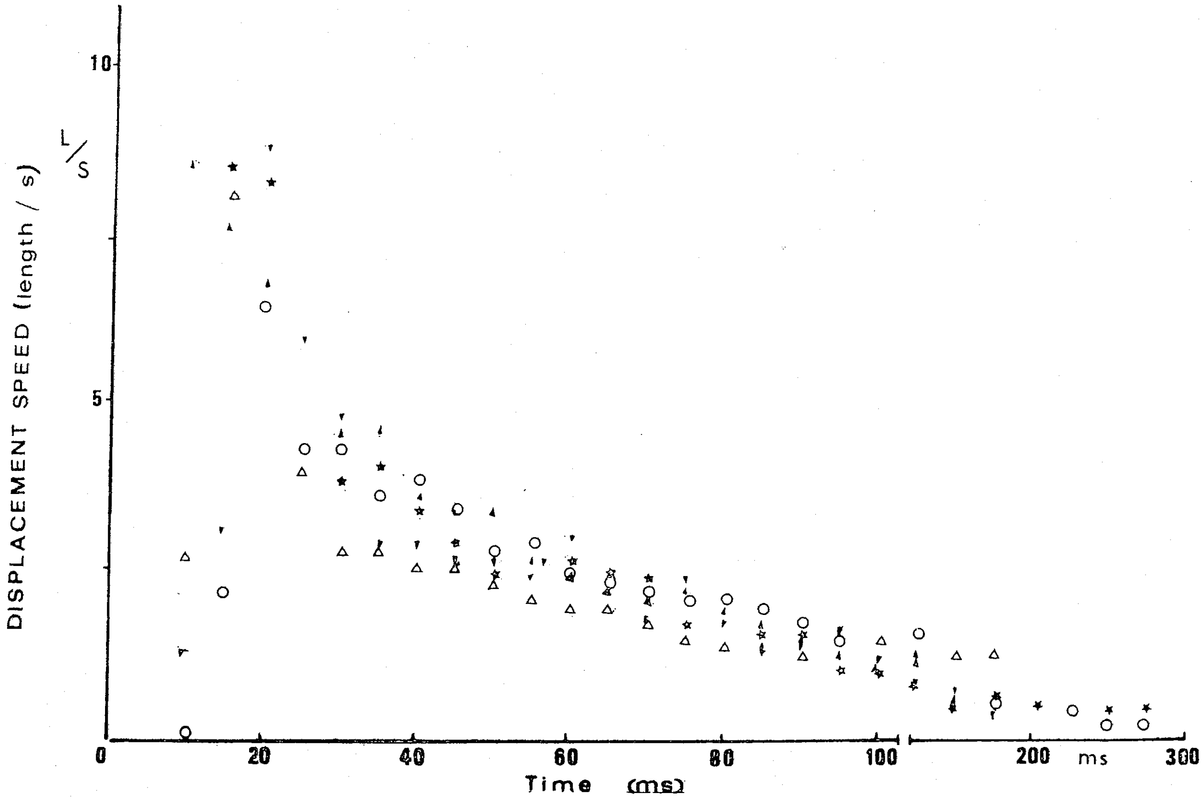

Fig. 3. Displacement speed of 5 freely swimming cod during the flash evoked C-startle response. 0 ms is taken from the onset of the flash. The maximum speed was 18.93±3.49 ms ($n=5$) (high speed cine film). Different symbols for different trials.

and the tail are bent into L- or C-shape during the biphasic period of the Mauthner initiated response. Analysis of 122 trials from TV-viedeo and more than 40 trials from high-speed cine film showed that the C-start without turn was the only response to flash stimulation in cod. As shown in the sampled tracing from high-speed cine record (Fig. 4), the visually evoked startle response constitutes stereotypically a preparatory stroke at frame 8 counted from the onset of the flash (mean=52.40±5.33 ms, $n=32$ after flash), followed by the main propulsive stroke at frame 21 (mean=126.99±16.02 ms, $n=8$). The first bend of the fish stopped at 85.12±6.23 ms ($n=12$) then the head straightened and reversed its direction of movement while the tail continued moving as before. This occurred at about 30 ms after the start of the response (85.12 ms−52.40 ms) and before the start of the tail flip. During this stage, both the head and tail contracted to the same side and presented an L- or C-like shape (Fig. 4). This fast body bend was followed by the next stage consisting of a variable movement pattern and resulting in the straightening of the head and a flip back of the tail. Fig. 5 shows some sampled tracing of the head displacement from high-speed cine film and demonstrates the stereotyped startle responses during several trials at the first stage (involving the starting head movement and head straightening, 52.40 ms−85.12 ms). The initial 30 ms of this pathway (Fig. 5) reveals predominantly overlapping patterns, but after that, the fish turned in different directions. Although

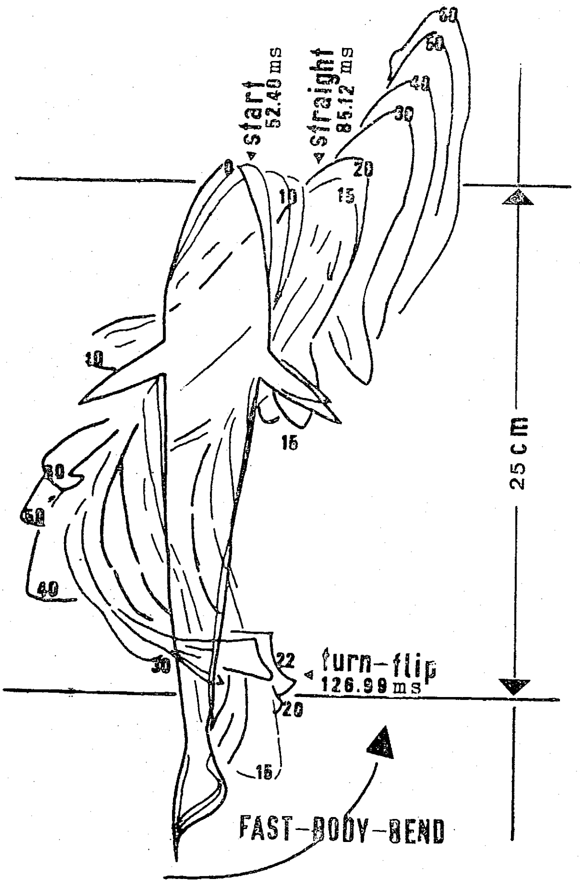

Fig. 4. One displacement sequence of the whole fish during flash evoked startle response showing C-like shape. The "0" marks the position of the fish at the onset of the flash. Figures represent frame numbers (6 msec per frame).

the positions varied from the starting point, the covered distances of their travel were almost equal. From Fig. 5, 300 ms after the flash, the heads were located at the periphery of the same circle.

### II. Effects of Startle Evoked C-Shape Curvature on Fish Motion

Webb and Corolla (1981) have discussed the effects of swimming path curvature on the energetics of fish motion. In the cod, the effects of curvature on the swimming speed during

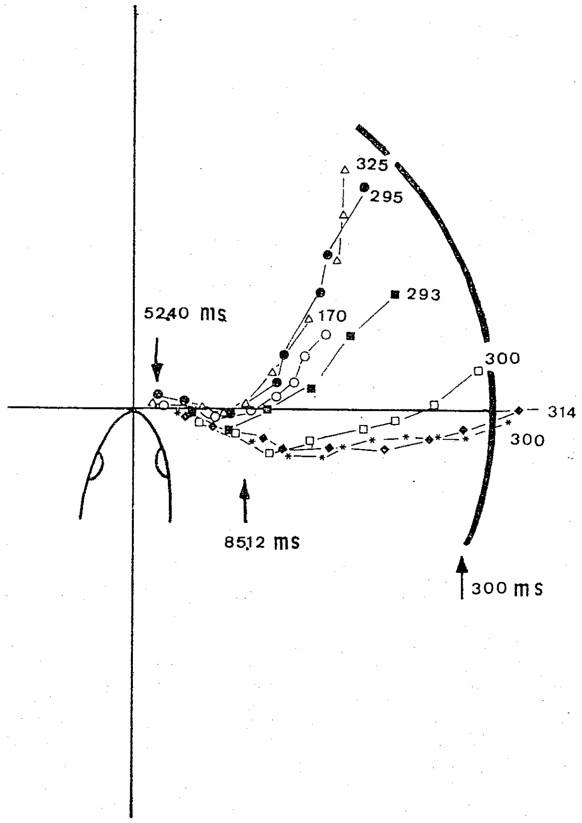

Fig. 5. Head displacements in 7 trials (each shown with different symbols or lines). The body length, direction of the body-bend and the initial orientation at the beginning of the response are standardized and pathways are determined by marking successive head positions. After the flash, the fish starts the fast-body-bend. During the initial 30 msec period (betewen 52.40 and 85.12 ms), the pathways are essentially identical among trials. During the return-tail-flip, the pathways show variability among trials, leading to different directions of escape.

startle behaviour are predicted as follows. From equation (10) of Appendix 1, the following equation could be obtained:

$$\left(\frac{U_t}{U_m}\right)^4 = \frac{1}{1-B}$$

$$\left(B = \frac{1}{R} \cdot \frac{2m\left(1+\lambda\frac{\rho_w}{\rho_f}\right)}{\rho_w A f\ C_{\text{max.}}}\right)$$

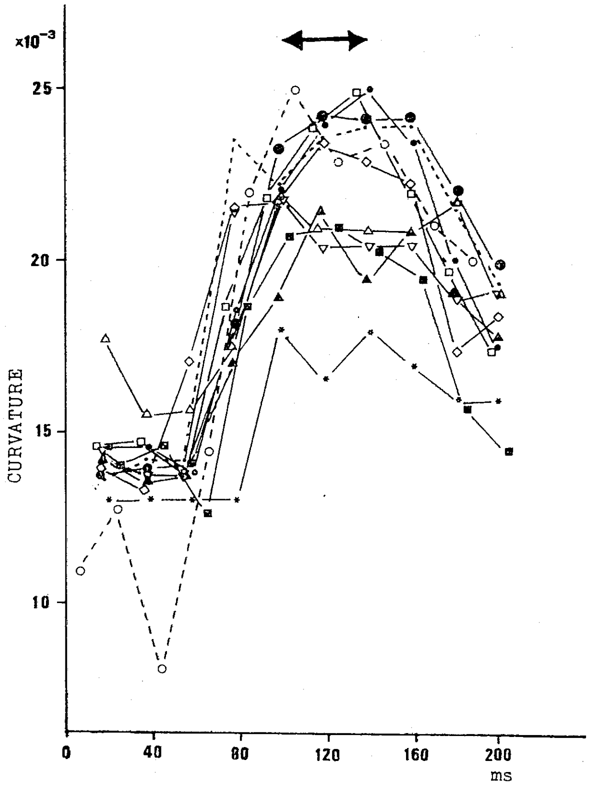

Fig. 6. Quantitative analysis of the curvature in degrees of 10 trials (each shown with different symbols or different lines) randomly sampled from 122 trials during C-startle response evoked by the flash stimulation. "0" msec in the X-axis shows the onset of the flash on.
↔ marks the duration of the highest curvature.

Explanation of symbols in Appendix 1. Considering the equation, when the radius of the curved path ($R$) increases to infinity, then $B$ approaches to zero and so the speed for the turning path ($U_t$) approches to that for moving in a straight line.

Conversely, when the radius $R$ decreases (i.e. curvature increases), the ratio between turning speed and straight-swimming speed increases. Theoretically, curved path swimming actually demands more energy to reach the higher speed. The high curvature movement of the startle response allows the fish to reach peak speed to escape from the danger.

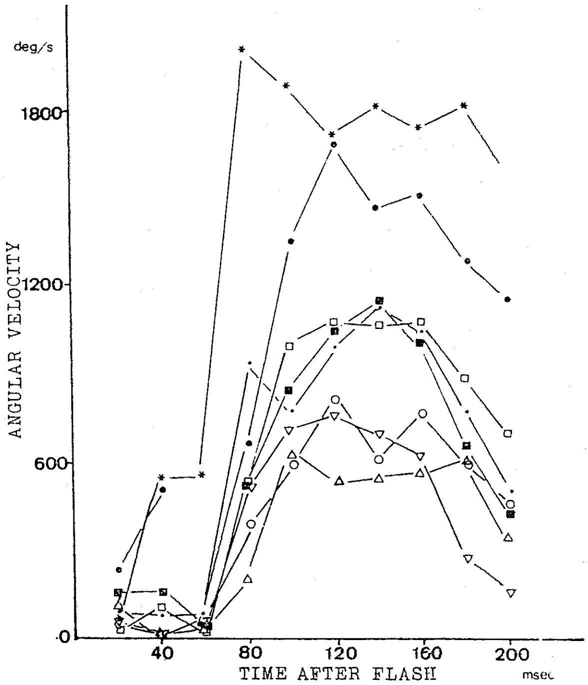

Fig. 7. Quantitative analysis of the angular velocity of 8 trials (each shown with different symbols or different lines) randomly sampled from the tested 5 fish during C-startle response evoked by the flash stimulation. X-axis marking 0 msec shows the flash time. The first frame starts at 20 msec after flash.

Fig. 6 shows the curvature during the flash evoked startle response, the highest curvature happened at 100–140 ms and the highest angular velocity as shown in Fig. 7 also occurred at this time (the tail-flip occurred at 126.99 ms). The curvatures and angular velocities were calculated from the projected images every 20 msec interval of TV-video film. By geometry, the angle could be calculated by:

$$\frac{\sin\theta/2}{\theta}=\frac{D}{2L}$$

when $D$ is the cord, $L$ is the arc, is the radius angle. In this study, the distance of the head and tail, $D$ is very easy to calculate from the Cartesian coordinate of the image analyser. And also $L$ (the fish body length) was recorded before the startle response. From Fig. 7 angular velocity increased from a time 60 ms after the flash to 2000–3000 deg/sec at about 100–140 ms. These event times agree well with high-speed cinematographic records which show that the head movement started at 52.40±5.33 ms, and the turning tail-flip started at 126.99 ms. The shorter duration and the higher angular velocity of the goldfish visually evoked startle response (Eaton *et al.*, 1981) may be explained by the shorter body length of the goldfish.

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## REFERENCES

Anthony, P. D. (1981) Visully contrast thresholds in the cod, *Gadus morhua* L. *J. Fish. Biol.*, **19**: 87–103..

Blaxter, J. H. S. (1981) Startle response in herring: the effect of sound stimulus frequency, size of fish and selective interference with the acoustic-lateralis system. *J. Mar. Biol. Ass. U. K.* **61**: 870–879.

Eaton, R. C., R. A. Bombardier and D. L. Meyer (1977) The Mauthner initiated startle response in teleost fish. *J. Exp. Biol.* **66**: 65–81.

Eaton R. C., W. A. Lavender and C. M. Wieland (1981) Identification of Mauthner-initiated response patterns in goldfish: evidence from simultaneous cinematography and electrophysiology. *J. Comp. Physiol.* **144**: 521–531.

Eaton R. C., J. Nissanov and C. M. Wieland (1984) Differential activation of Mauthner and Non-Mauthner startle circuits in the zebrafish: implications for functional substitution. *J. Comp. Physiol. A.* **155**: 813–820.

Faber D. S. and H. Korn (1978) Neurobiology of the Mauthner cell. Raven Press.

Huang, B. Q. (1986) Visually evoked startle responses in the cod (Gadus morhua) Ph. D. thesis, University of Aberdeen, U. K.

Huang B. Q. (1987) Visually evoked startle response in teleosts. I. Electrocardiographic recordings. *J. Fish. Soc. Taiwan* **14**(2): 33–45.

Retzlaff E. (1957) A mechanism for excitation and inhibition of the Mauthner cell in teleosts: a histological neurophysiological study. *J. Comp. Neur.* **107**: 209–225.

Webb P. W. (1976) The effect of size on the fast-start performance of rainbow trout *Salmo gairdneri* and a consideration of piscivorous predator-prey interactions. *J. Exp. Biol.* **65**: 157–177.

Webb P. W. (1978) Fast-start performance and body form in 7 species of teleost fish. *J. Exp. Biol.* **74**: 211–226.

Webb P. W. and R. T. Corolla (1981) Burst swimming performance of northern anchovy, *Engranlis mordax* Rarvae. *Fish Bull.* **79**: 143–176.

Zottoli S. J. (1977) Correlation of the startle reflex and Mauthner cell auditory responses in unrestrained goldfish. *J. Exp. Biol.* **66**: 243–254.

Zottoli S. J. (1978a) Comparison of Mauthner cell size in teleosts. *J. Comp. Neur.* **178**: 741–758.

Zottoli S. J., A. R. Hordes and D. S. Faber (1987) Localization of optic tectal input to the ventral dendrite of the goldfish Mauthner cell. *Brain Res.* **401**: 113–121.

# APPENDIX 1

### 9.4 Curvature Effects

Effects of C-startle curvature on fish motion

$$W=\frac{1}{2} pw\, Af\, C_{\max}\, U_m^2 \tag{1}$$

$$P=\frac{1}{2} pw\, Af\, C_{\max}\, U_t^2 \tag{2}$$

$$P=\sqrt{W^2+F^2}$$

$$\text{i.e. } \frac{P}{W}=\sqrt{1+\left(\frac{F}{W}\right)^2} \tag{3}$$

$$\text{from (2)/(1)} \quad U_t^2/U_m^2=P/W \tag{4}$$

$$\because\ F=m^1 U_t^2/R=V U_t^2/R\,(pf+pw) \tag{5}$$

and

$$\begin{aligned} m^1&=V(pf+pw\lambda)\\ &=V\cdot pf\left(1+\lambda\frac{pw}{pf}\right)\\ &=m\left(1+\lambda\frac{pw}{pf}\right) \end{aligned} \tag{6}$$

substituting Eq. (3), (5) in Eq. (4):

$$\begin{aligned} U_t^2/U_m^2&=P/W=\sqrt{1+(F/W)^2}\\ &=\sqrt{1+\left(\frac{m^1 U_t^2}{RW}\right)^2} \end{aligned} \tag{7}$$

$$\begin{aligned} \left(\frac{U_t}{U_m}\right)^4&=1+\left(\frac{m^1 U_t^2}{RW}\right)^2\\ &=1+\left(\frac{m^1 U_t^2}{R\cdot\frac{1}{2}pw\cdot Af\cdot C_{\max}\, U_m^2}\right)^2\\ &=1+\left(\frac{2_m(1+\lambda\cdot pw/pf)}{R\cdot pw\cdot Af\cdot C_{\max}}\cdot\frac{U_t^2}{U_m^2}\right)^2\\ &=1+\left(\frac{2_m(1+\lambda\cdot pw/pf)}{R\cdot pw\cdot Af\cdot C_{\max}}\right)^2\cdot\left(\frac{U_t}{U_m}\right)^4 \end{aligned} \tag{8}$$

$$\text{let} \quad \frac{1}{R}\cdot\frac{2_m(1+\lambda\cdot pw/pf)}{pw\, Af\, C_{\max}}=B \tag{9}$$

$$\therefore\ (U_t/U_m)^4=1+B^2\cdot(U_t/U_m)^4>0$$

$$\text{i.e.} \quad (U_t/U_m)^4=1/(1-B^2)>0 \tag{10}$$

$$0<B<1$$

(1) when radius ($R$) increases to infinity, then $B$ approaches 0, from Eq. (10) $U_t=U_m$ i.e. It is in straight motion.

(2) when radius decreases, then $B$ increases, i.e. $1>1-B^2$ and $U_t>U_m$ for all finite radius, ∴ the minimum speed for a horizontal turn, $U_t$ grows as the curvature increases.

$W$: submerged weight of the fish; $C_{\max}$: highest possible lift coefficient; $U_m$: minimum speed; $U_t$: minimum speed for a horizontal turn; $Af$: fin lifting area; $pw$: density of water; $pf$: average density of fish; $F$: centripetal force; $V$: volume of the fish; $\lambda$: longitudinal added mass coefficient; $m^1$: mass of the fish; $R$: instantaneous radius of the curved path. (modified from Webb and Corolla, 1981).

# 閃光誘致自由游泳鱈魚之逃避行爲

黃寶貴　符瑞瑟

利用電視錄影(TV-Video)及高速攝影裝置研究自由游泳鱈魚被閃光激發之驚嚇行爲，實驗結果顯示魚呈固定型式(Stereotypic)之反應，此反應包括(1)快速軀幹彎曲(fast-body-bending)，卽頭與尾部快速往同側彎曲，使魚體呈C-型，(2)尾部擺囘(return-tail-flip)，卽尾部快速往另一側擺囘。兩者之潛伏期分別爲52.40±5.33 ms ($n$=32)及126.99±16.02 ms ($n$=8)。高速攝影分析所得之魚體運動最大速度發生在刺激後71.33 ms，而電視錄影所得爲82.10 ms，結果兩者呈現極高之符合性，且前者顯示較高之準確性，利用電視錄影及廸卡爾座標(Cartesiancoordinate)作影像分析(image analysis)計算此行爲之曲度(curvature)及角速度(angular velocity)之變化，結果顯示兩值均在閃光後100–140 ms呈最高值，此與尾部擺囘之潛伏期(126.99±16.02 ms)極爲吻合，卽魚體呈C型時爲其呈最大曲度及具最快角速度之同時。