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# A PC-Aided Video Based System for Behaviour Observation of Fish Larvae and Small Aquatic Invertebrates

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*ABSTRACT*

*A PC-aided video based behaviour observation system for fish larvae and small aquatic invertebrates is presented, tested, and discussed. A video camera is moved horizontally and vertically in front of a 90-litre observation aquarium by means of servo motors remotely controlled via a joystick. Camera focus is also controlled from the joystick. Camera movement and focusing cause voltage variations which are monitored by a PC and transformed into stereo coordinates and stored. Behaviour patterns are registered into the PC by the observer in real time. The aquarium is illuminated with infrared (IR) light to facilitate dark observations with an IR sensitive video camera. The system was tested with cod and plaice larvae and live prey. Results obtained compare well with literature data.*

## INTRODUCTION

Cultivation of marine fish species is presently undergoing rapid development. Sea bass (*Dicentrarchus labrax*) is already commercially established in several Mediterranean countries, and species like turbot (*Scophthalmus maximus*), sea bream (*Sparus aurata*), halibut (*Hippoglossus hippoglossus*) and cod (*Gadus morhua*) are on the verge of being commercialized. The bottleneck so far has been to escalate fry production. A substantial proportion of the scientific cultivation effort has been in feeding and larval nutrition, while relatively few investigations have dealt with the physical and biological environment in larval rearing.



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A number of fundamental behaviour studies of fish larvae have been carried out by means of direct visual observation (e.g. Blaxter & Staines, 1971; Hunter, 1972). However, detailed behaviour studies of small (<10 mm) fish larvae require magnification of the subjects. This normally means that either a small observation chamber has to be used to restrict the movement of the animal, a low magnification must be used to give a wide field of view, or with higher magnification the animal can only be studied as it passes the field of view. With a tracking system, a larger observation chamber can be used, and individual animals can be followed for long periods of time.

The ability to record behaviour on visual media for closer inspection or measurements is necessary for some types of investigation, e.g. locomotor patterns and swimming (burst) (Batty, 1981; Webb & Corolla, 1981; Fuiman, 1986), and helpful in most cases.

Dark-observation by means of infrared (IR) illumination was in early use by Baylor (1959), and was sophisticated by Batty (1983). Use of IR light facilitates experiments where behaviour is observed as a function of illumination.

The enumeration of behaviour data is often cumbersome and time consuming. The use of direct computer data acquisition can be very helpful and can facilitate studies which otherwise would not be possible (Kleerekoper, 1969; Kleerekoper *et al.*, 1970).

The present paper describes the design and application of an observation system for studying intra- and interspecific behaviour of fish larvae and their prey, ranging in size between 0·3 and 25 mm. The system was designed to be used in order to establish larval requirements for illumination, temperature, salinity, space, prey density, etc. The animals were tracked with a moveable video camera, and three dimensional coordinates were recorded in a computer, as were behaviour patterns. Care was taken in the design of the system to facilitate simulation of natural or culture environmental conditions through the use of comparatively large observation chambers, and IR light as a principal illumination source for the video observation system so that illumination visible to the observed organisms could be set freely. Real time automatic and manual data acquisition was applied to facilitate enumeration of large blocks of behaviour, so that experiments prohibitive with standard observation methods could be undertaken.

Two versions of the system have been used in several experiments which are published separately. In the present paper the results of the technical testing of the equipment will be presented and discussed, together with some obtained biological results which are compared with literature data in order to evaluate the system.

## MATERIAL AND METHODS

The system is outlined in Fig. 1. The camera rig was made of stainless steel and aluminium plates. A vertical main frame erected from a base plate supported an upper and a lower horizontal guide rod for the camera frame. The camera frame travelled horizontally on these guide rods. The camera mount travelled vertically on two guide rods mounted

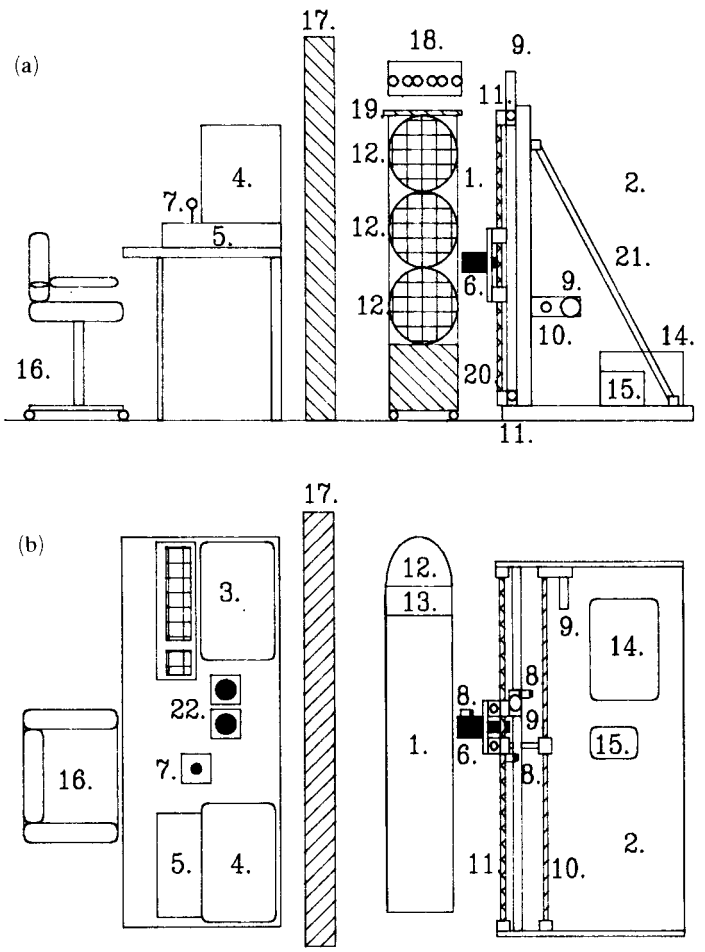


**Fig. 1.** System layout. (a) Horizontal view; (b) vertical view. (1) Aquarium; (2) camera rig assembly; (3) PC; (4) video monitor; (5) VCR; (6) video camera; (7) joystick; (8) potentiometers; (9) servo motors; (10) horizontal threaded bar; (11) horizontal guide bars; (12) IR lights; (13) water jacket; (14) main power supply; (15) potentiometer power supply; (16) operator chair; (17) wall; (18) fluorescent lamps; (19) diffusor plate; (20) vertical guide bars; (21) frame support bar; (22) light dimmers (IR and fluorescent).

vertically in the camera frame. Thus the camera could move horizontally by the movement of the camera frame in the main frame, and vertically by the movement of the camera mount in the camera frame. The movement was brought about by one horizontal and one vertical threaded rod rotated by servo motors. A non-rotating nut on each of the threaded bars was fastened to the camera frame and the camera mount respectively. When the threaded bars rotated, the nuts, and the camera frame and mount with them, were moved along the threaded rods guided and supported by the guide rods. The horizontal threaded rod and servo motor were fastened to the main frame, while the vertical threaded rod and servo motor were fastened to and travelled with the camera frame.

An earlier version of the system was based on the ruler system of a drawing board. The camera movement was brought about by servo motors and dented plastic belts pulling the camera mount vertically and horizontally. The vertical movement was aided by a counter weight balancing the mass of the lens and camera via a block.

The movement of the camera was in both systems controlled by a joystick mounted on the observer's table. The motor focus of the camera lens was also operated from the joystick by two push buttons. The maximum travelling distances of the centre of the camera field of view was 600 mm horizontally and 800 mm vertically. Rotating variable resistors (potentiometers) were mounted to the camera frame, the camera mount, and to the focusing ring of the lens. The wheels mounted on the axle of the potentiometers travelled along the main frame and the camera frame respectively when the camera frame and the camera mount moved. The movement of the lens focusing ring was transferred to the focus potentiometry by a rubber band. The three potentiometers were fed 10V DC from a separate power supply. The resultant voltage from each potentiometer was read by an analog/digital (A/D) converter card in an IBM PC AT. These readings were transformed into three dimensional coordinates by a simple Pythagorean algorithm in the observation data program, which was written in Turbo Pascal. The data were then transferred to a standard software package (RS/1, BBN Software Products Corporation, Cambridge, MA) for organization and analysis. The observation data program was also made to receive input of behaviour pattern categories from the keyboard. Position coordinates were either logged at given time intervals (0·5 or 1·0 s), or were logged each time a key was pressed on the computer keyboard.

On the earlier version, a Luxor ABC-80 PC was used. This is an 8-bit computer with 8-bit parallel data transfer, giving a resolution of 255 points per dimension. For the vertical dimension this meant a metric

resolution of 600 mm/255 = 2·35 mm. With the 16-bit data transfer of the PC AT the same resolution was 600 mm/65 535 = 0·009 mm. For the other dimensions the resolution was even better, as the same number of points was available for a shorter travelling distance. Precision potentiometers were used. Care was taken to select potentiometers where the whole number of turns were utilized to cover the full distance of the dimension in question, in order to obtain maximal resolution.

A CCD (charge coupled device) camera (Phillips LDH 670) weighing 220 g and measuring 120 mm × 60 mm × 25 mm was used. The camera had a quantum efficiency at 800 nm (IR) of 30%. Sensitivity was 0·05 lux (100% video signal) and horizontal resolution was more than 500 lines. An autoiris motorzoom/motorfocus lens (Cosmicar C10Z1014M2EMA) with a focal length between 10·5 and 105 mm, and an extension ring of 22 mm, was used. Applying the motorzoom for focusing, a focusing range to 250 mm from the lens was obtained. A fish larva of 10 mm gave a picture on the monitor of 10% of the monitor diagonal. In the earlier version a Newicon tube camera (JVC) was used. The lens was similar to the present one, but without autoiris. High resolution 14 in monochrome monitors (600–1200 lines) were used in both systems. Both VHS and S-VHS video tape recorders were used. Comments were recorded directly on to the video tape through a headset microphone.

The aquarium had a volume of 96 litres, a depth of 800 mm, a width 600 mm, and a thickness of 200 mm. The back wall was white, and a black rubber mat could be placed on the bottom to avoid reflection. Three dimmable IR light sources illuminated the aquarium from the side. A water jacket was mounted between the light sources and the aquarium to absorb heat. Tungsten halogen bulbs (250 W) were used, and the IR filter cutoff wavelength was 780 nm. The aquarium was illuminated from above with six dimmable daylight fluorescent tubes, through a matted glass plate on top of it. Both illumination sources could be dimmed from the observer's table. The earlier system had no IR illumination, and was illuminated from above with two daylight fluorescent tubes. The aquarium and camera rig were placed in a room with temperature control between 0 and 30°C ± 0·5°C.

During observation the observer sat in the chair with one hand on the joystick and the other on the PC keyboard. The system was calibrated by starting the logging at an origin in the lower left corner. The larvae were tracked by moving and focusing the camera with the joystick so that the sharp image of the head of the larva coincided with a black dot in the centre of the monitor. Whenever the larva performed a behaviour pattern, the key corresponding to this behaviour pattern was pressed on

the PC keyboard, an action also causing a position update. All experiments were recorded on video tape, and the observer commented on to the videotape through the headset microphone.

The experiments took place at the Institute of Marine Research, Austevoll Aquaculture Research Station, Austevoll, Norway, in April, May and June 1989. The behaviour of cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*) larvae, with collected natural zooplankton, cultured rotifers (*Brachionus plicatilis*), and *Artemia nauplii* as prey organisms, was investigated in order to test the system. The cod larvae were hatched from eggs naturally spawned in the spawning pen at the Austevoll station. The eggs were incubated at ambient water temperature averaging 5·5°C in an open flow hatching cylinder. When about half the eggs had hatched, the remaining eggs were transferred to a different hatching cylinder in order to obtain a group with homogeneous age for observation. The plaice larvae were hatched from eggs stripped at SINTEF, Trondheim. The eggs were incubated at SINTEF until after gastrulation, and then sent in oxygenated ice cooled sea water by aeroplane to Austevoll. Hatching took place in open flow hatching cylinders at an average temperature of 6·5°C. Observation started at the day of 50% hatching for both species, which was designated day 0.

The aquarium was divided into nine units by drawing a grid on the front plate. One larva was observed in each unit in each observation period. Thus each observation period consisted of nine observations. Each observation lasted for at least 3 min. Within units the larvae were selected randomly for observation. Light regime in the aquarium room was 12 h light/dark. This light was turned off 1 h before dark observations. A surface illumination of 1200 lux was used in all light observations. Temperature was kept at 6°C in both experiments.

## RESULTS

Experience from use of the latest version of the system has so far been obtained in experiments with plaice and cod larvae, while the earlier version also was used for turbot (*Scopthalmus maximus*) and halibut (*Hippoglossus hippoglossus*) larvae, as well as for predation studies on fish larvae by ctenophors and medusae. CCD cameras were used with the latest version, while Newicon tube cameras were used with the earlier version. Both VHS and S-VHS video cassette recorders (VCRs) were used in the different studies. Experience from these experiments and with these components form the basis of the discussion of the technical performance.

With the 22 mm extension ring used in the present study, rotifers and nauplii could be dicerned in good illumination throughout the focusing range. Copepodites were easily seen even in dim light, but were only seen close to the light sources with IR illumination only. The picture quality with IR illumination only did not normally allow detailed studies of larval behaviour like jaw or fin movement, but presented no problems in tracking the larvae.

Figure 2 shows development in average swimming speeds (mm $s^{-1}$) for plaice larvae in darkness and in light. The lines are sixth-order polynomial fits. The bold line is in light. Swimming speeds were significantly higher in light up to day 7, the day after *Artemia nauplii* and rotifers had been introduced. From day 7 to day 11 average swimming speeds in light and darkness were not significantly different. Swimming speeds the first week peaked on day 5 in both groups.

Figure 3 shows development in average swimming speed for starving (bold points and curve) and feeding cod larvae in light. The lines are sixth-order polynomial fits. Live zooplankton was introduced in the

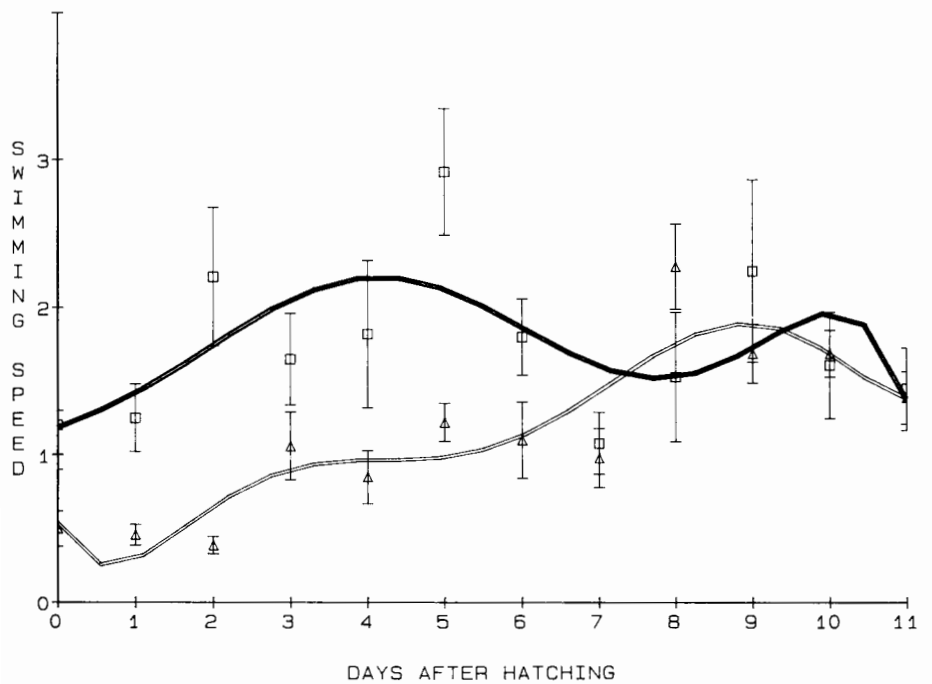


**Fig. 2.** Swimming speed (mm $s^{-1}$) of plaice larvae in light (squares, bold line) and dark (triangles). Lines are sixth-order polynomial fits. Each point represents the mean of nine larvae.

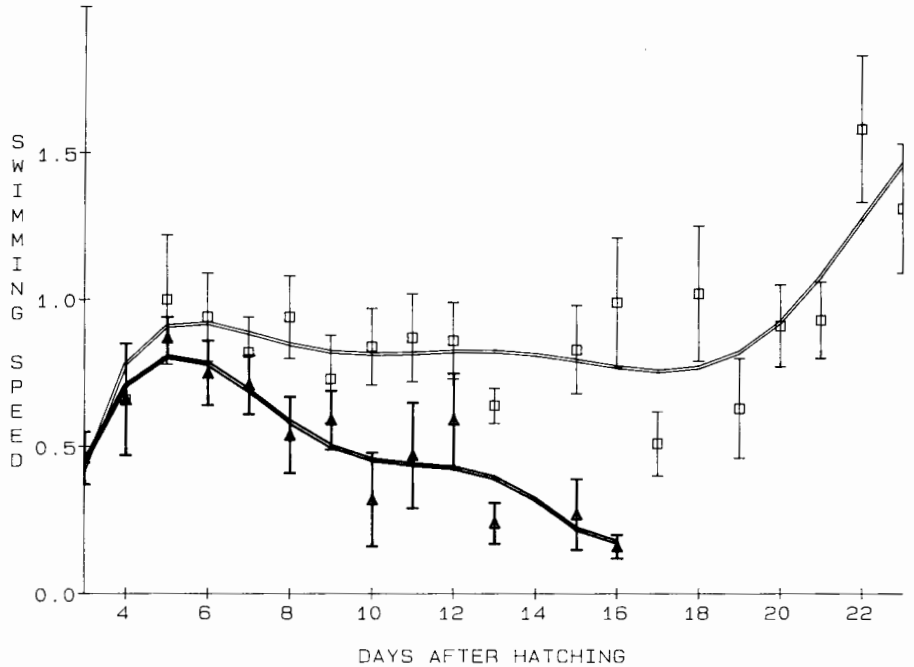


**Fig. 3.** Swimming speed (mm $s^{-1}$) of starving (triangles, bold line) and feeding (squares) cod larvae. Lines are sixth-order polynomial fits. Each point represents the mean of nine larvae.

feeding group on day 5. Swimming speed during first feeding peaked on day 5 with a subsequent slow decline to day 13. In the starving group swimming speed also peaked on day 5, with a subsequent decline to a moribund state on day 16, and total mortality on day 19.

Figures 2 and 3 show mean swimming speeds over the whole observation periods including pauses in swimming, and intermittent activity. Thus the figures do not show actual swimming velocities, but rather average distance travelled per second of observation time. Each point represents the average of nine larvae.

## DISCUSSION

The standard method for recording behaviour of fish and fish larvae has been photographic filming with a resolution varying between 24 and 1150 frames per second (Hunter, 1972; Batty, 1981; Webb & Corolla, 1981; Milinski, 1984; Fuiman, 1986; Drost, 1987). With the rapid development of video technology, however, such systems are being more

and more used (Buchanan *et al.*, 1982; Batty, 1983, 1987; Blaxter & Batty, 1985, 1987; Batty *et al.*, 1986; Fuiman & Webb, 1988). The limitation of video is mainly in resolution, as maximal number of frames per second for ordinary systems is 60. Where very high resolution ( > 60 frames per second) is required, high speed film cameras are still the best choice.

In the present system it was important to keep camera mass at a minimum, giving no alternatives but a CCD video camera, and a resolution of 60 frames per second is sufficient for anything but the most resolution critical applications. An apparent improvement of resolution can be obtained by producing 'frozen images'. This was done by Batty (1983) using an IR light emitting diode as illumination source, strobed in very short pulses (0·01 ms) in synchrony with the video field pulse. The same effect can be achieved using a video camera with very short shutter opening time (down to 0·1 ms). Some high end consumer camcorder cameras have such specifications. The real resolution in terms of frames per second is not enhanced this way, but each frame presents a sharp image of even a rapidly moving object. As for tube cameras versus CCD cameras, tube cameras still (1989) are superior in most aspects. The best tube cameras produce less noise, are more light sensitive, and have better resolution than CCD cameras. As for size and shock tolerance, however, CCD cameras are superior, and they also generally produce less afterglow and picture smearing in moving objects.

The increased horizontal resolution from 240 in the VHS video recording format to more than 400 lines in S-VHS gave a significant improvement of apparent picture quality which was especially important in slow motion studies. The picture quality of S-VHS recordings made at the slowest speed (1·1 cm $s^{-1}$) was still better than normal VHS recordings, giving a total recording time per cassette of 6 h. The 8 mm high-band format was not tested, but with a horizontal resolution of more than 400 lines it should perform similarly to S-VHS.

The visual part of the system functioned well, but better picture quality with IR illumination only would have facilitated an even wider range of experiments. Also the contrast conditions in the aquarium were not optimal either for observation or for the observed organisms. The white reflecting surface seemed to attract fish larvae, causing substantial wall interference, and the larvae and prey were often difficult to observe in detail. 'Scotchlite' (Blaxter & Batty, 1987) background was also tested, but produced too varying contrast conditions to be applied when tracking. This material should, however, be further tested.

The electromechanical part of the system functioned well with some exceptions. In the older version the heavy tube camera and the somewhat

underdimensioned mechanics of the drawing board caused picture vibration during movement. Also, the plastic belts pulling the camera mount and the vertical and horizontal potentiometers were stretched during operation to cause unprecise position readings. In the present system the mechanical components functioned well. However, the wheel drives of the horizontal and vertical potentiometers had a tendency to track slightly sideways, creating an offset necessitating frequent recalibration. The system will now be modified using digital rulers as found in modern lathes for the horizontal and vertical positioning.

Three 250 W IR light sources with a filter cutoff wavelength of 780 nm had to be applied to obtain a usable video picture in all parts of the aquarium. Even though the IR lights were always dimmed as much as possible, and despite the cooling water jacket, a slight ($<1$ mm $s^{-1}$) convection current could occasionally be observed along the lamp wall.

No phototaxis responses were observed in cod or plaice larvae using only IR illumination, even when the IR light was switched on and off at short intervals. From the data of Blaxter (1968*a*, *b*) and Anthony and Hawkins (1983), cod and plaice larvae could be expected to have a spectral vision range to 650 nm or less. The spectral response of the IR filters was not measured, but even if a small amount of light of lower wavelength than 780 nm may have been let through, the separation gap between the filter cutoff wavelength and the spectral sensitivity of the fish larvae should be wide enough to avoid visual stimulation, which is corroborated by the lack of phototactic response.

Blaxter and Staines (1971) found that first feeding plaice larvae swam with a speed of 10 cm $min^{-1}$, which is in good accordance with the present data. Blaxter and Staines, however, only registered larvae that were apparently searching for food. Thus, the increase in swimming speed is somewhat slower in the present study, as the larvae were randomly selected, and no larvae were excluded. This was done to reflect a culture situation, where wall and surface effects are problems that have to be taken into account.

Blaxter (1968*a*) showed that feeding in plaice larvae was greatly reduced in the dark. The present data also show that plaice larvae are significantly more active the first week of life in light than in the dark irrespective of the presence of food.

Solberg and Tilseth (1984) found a peak in swimming speed of cod larvae at day 6, as did Skiftesvik and Huse (1987). In this study the peak appeared on day 5. This can be attributed to the higher temperature (6°C) in this study than the others (5°C or lower). Solberg and Tilseth (1984) found a swimming speed at day 6 of 2 mm $s^{-1}$. Skiftesvik and Huse (1987) found 0·3 mm $s^{-1}$, and in the present study 0·8–1·0 mm $s^{-1}$.

Solberg and Tilseth (1984) measured free swimming larvae only, and larvae were transferred to a comparatively small container for each test. This resulted in a frequency of swim bursts several times higher than found in this study and by Skiftesvik and Huse (1987), a difference which also of course will cause a proportionally higher swimming speed. In both the latter studies the larvae remained in the observation aquaria for the whole experimental period. The difference between the present study and Skiftesvik and Huse (1987) can be accredited to the higher temperature and possibly to higher illumination in the present study (1200 compared with 300 lux).

## CONCLUSION

Apart from requiring frequent position offset calibration, and sub-optimal picture quality at very low light and with IR illumination, the presented system functioned well. Obtained results also comply well with literature data. The data acquisition and enumeration was easy compared with other relevant methods, facilitating studies which would otherwise be too resource consuming. The price of the complete system is similar to the cost of a scintillation counter or a gas chromatograph.

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