Flødevigen rapportser., 1, 1984. ISSN 0333-2594 The Propagation of Cod *Gadus morhua* L.

NEUROMASTS AND CUPULAR GROWTH OF COD LARVAE

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#### ABSTRACT

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Use of phase contrast microscopy and vital staining showed that newly hatched cod larvae had five free neuromasts on either side of the body and four on the head. About three weeks later, when feeding was established and the larvae had reached 5-6 mm body length, there were six on either side of the body, six scattered over the head and a row of four above the mouth. By use of staining under anaesthetic, followed by recovery, it was possible to measure the growth of the cupulae which averaged 2.4  $\mu\text{m/h}$ .

#### INTRODUCTION

Free neuromasts are found on the body and head of many species of fish larvae (Iwai, 1967). Being transparent they are difficult to see without the use of phase contrast microscopy or vital staining. This is especially true of the cupulae which are destroyed by most fixatives. Recently Blaxter et al. (1983) used phase contrast and the vital stain Janus Green to follow the development and distribution of free neuromasts in herring larvae and similar methods are described here for larvae of cod. Gadus morhua L.

## MATERIALS AND METHODS

Cod eggs were obtained from captive broodstock held by the Sealife Centre, Oban, and the Marine Laboratory, Aberdeen at their field site in Loch Ewe. They were hatched at temperatures of  $8\text{-}10^{\,\text{O}}\text{C}$  and after about one week the larvae were fed on rotifers and later brine shrimp nauplii of Brazilian origin. The neuromasts were investigated during the yolk sac stage when the larvae were 3-4 mm long and 2-3 weeks after the start of feeding when they were 5-6 mm long.

The larvae were anaesthetized in 200 ppm benzocaine and stained in a bath of 0.1% Janus Green made up in 50% seawater containing some anaesthetic. Usually 5-10 min in the stain was sufficient, the depth of staining often improving once the larvae had been returned to the anaesthetic for further examination. The neuromast organs were best seen from the dorsal or ventral aspect by supporting the larvae with fine pins. Microscopy and photomicrography were done with a fibre-optic light source which kept the larvae cool.

Some larvae were allowed to recover from the anaesthetic after staining and returned to a holding tank. They were then recaptured and re-examined over a period of 25 h to see whether there was any evidence of growth of the cupulae. In some cases the larvae were re-stained before re-examination.

## RESULTS

The neuromast sensory cells and especially the cupulae are easily dislodged during handling and only those larvae treated with great care proved of value in the investigation. The sensory cells were found to stain pale blue (as did the nasal pits) while the cupulae stained a more intense blue.

The free neuromast organ consists of a hump of sensory cells about 40  $\mu m$  in diameter with a gelatinous cupula projecting at right angles from the cells. The cupula was variable in length but rarely exceeded 40  $\mu m$ . Many neuromasts had no cupulae and it was not certain whether they had been dislodged

or shed as a result of the handling of the larvae before and during staining.

The distribution of neuromasts is shown in Fig. 1 for the early yolk sac stage and 2-3 weeks later after feeding has been established. The lateral body neuromasts only increased from

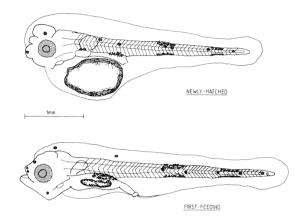


Fig. 1. Drawings of cod larvae soon after hatching and at first feeding showing the distribution of neuromasts (black spots). There is some variability between larvae.

five to six during this time. On the head the neuromasts increased from four to ten, four of these appearing in a row along the central margin of the upper jaw. Photomicrographs of body neuromasts are shown in Fig. 2A, B. It became very difficult to follow subsequent development by external examination as the body thickened and became more pigmented.

Where the larvae were allowed to recover after staining and were then re-examined some hours later, fading of the cupula had occurred but in some neuromasts a band of transparent tissue could be seen at the base of the cupula. This band increased in width the longer the larvae were left before re-examination indicating that growth of cupular material was occurring after staining. It was also possible to restain the larvae before re-examination and when this was done a sharply defined boundary could be seen between the darkly stained new

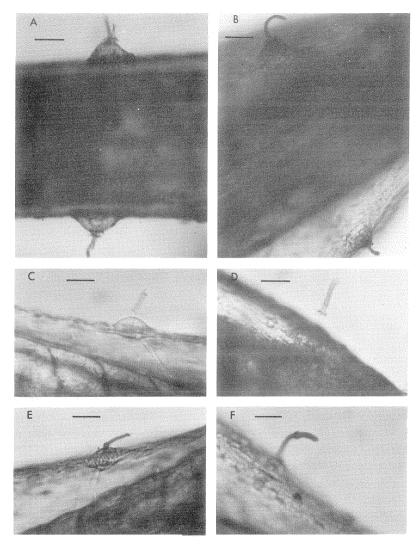


Fig. 2. Body neuromasts of cod larvae 2-3 weeks post-hatching stained with Janus Green. A, B, immediately after staining. C, 2 1/2 h after staining and recovery. D, 18 1/2 h after staining and recovery (note new growth continues below line of body). E, restained 5 h, and F, restained 25 h after initial staining and recovery. Scale bars 50  $\mu m$ .

tissue and doubly stained old tissue of the cupula. Examples are shown in Fig. 2 C-F.

The extent of the new growth was measured where possible and the results plotted in Fig. 3. The average growth was 2.4  $\mu$ m/h.

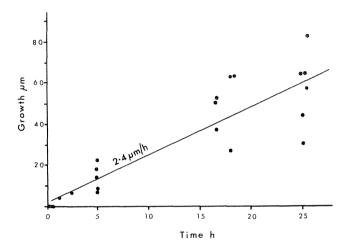


Fig. 3. Graph showing cupular growth with time. The line is fitted by regression analysis and the slope is 2.4  $\mu m/h$ .

# DISCUSSION

The position of the neuromasts confirms those shown by Hognestad (personal communication) and Fridgeirsson (1978) for cod larvae except that a larger number were located using vital staining. The proliferation of neuromast organs is rather slower in cod than herring (see Blaxter et al., 1983). Hognestad also confirmed this in cod larvae 7.5 mm long, 40 days after hatching when there were still only nine pairs on the trunk. This may be in part explained by the more shortened body form of cod larvae. Growth and replacement of cupulae affected by staining can be demonstrated clearly but it is less certain whether cupulae normally grow and are then shed and replaced

when they reach a certain length.

The variation in length of the cupulae suggests that growth and replacement is a normal event. Since the cupulae are delicate and easily dislodged it seems likely that the replacement process is necessary under natural conditions where the larvae may be subjected to violent stimuli, for example from predators.

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