SHORT COMMUNICATION

THE RELATIONSHIP BETWEEN THE LENGTH OF THE CUPULAE OF FREE NEUROMASTS AND FEEDING ABILITY IN LARVAE OF THE WILLOW SHINER *GNATHOPOGON ELONGATUS CAERULESCENS* (TELEOSTEI, CYPRINIDAE)

YUKINORI MUKAI[1], HIROMASA YOSHIKAWA[2] AND HIROSHI KOBAYASHI[1]

[1]*Department of Fisheries, Faculty of Agriculture, Kinki University, Nakamachi, Nara 631, Japan* and [2]*Interdisciplinary Research Institute of Environmental Sciences, Nishi-iru, Hichihon-matsu, Itsutsuji-dori, Kamigyo-ku, Kyoto 602, Japan*

Free mechanosensory neuromasts of larval fishes have been described as playing a complementary role to vision in feeding behaviour (Disler, 1971; Iwai, 1972*a,b*). In certain species or under limited conditions, free neuromasts play a major role in detecting prey. The larvae of mottled sculpin *Cottus bairdi* can feed on *Artemia* in the dark by using free neuromasts (Jones and Janssen, 1992). Artificially blinded surface-feeding *Aplocheilus lineatus* can detect insects on the water surface by means of free neuromasts (Müller and Schwarts, 1982; Tittel *et al.* 1984; Bleckmann, 1988; Bleckmann *et al.* 1989). Furthermore, vibrations produced by swimming crustaceans are known to be a potent natural stimulus for the lateral line system in the Antarctic fish *Pagothenia borchgrevinki* (Montgomery and Macdonald, 1987; Montgomery, 1989). We found that larvae of a plankton feeder, the willow shiner *Gnathopogon elongatus caerulescens* (Sauvage) (Cypriniformes, Cyprinidae), fed on nauplii of *Artemia* in complete darkness. Ototoxic compounds, such as streptomycin, have been shown to disturb the function of the lateral line organ or free neuromasts (Kaus, 1987; Blaxter and Fuiman, 1989; Janssen, 1990; Jones and Janssen, 1992). Willow shiner larvae treated with streptomycin sulphate no longer feed on *Artemia* in the dark (Y. Mukai, in preparation).

The willow shiner inhabits calm lakes and feeds on zooplanktonic prey (Nakamura, 1949). The larvae show a high sensitivity to minute water displacements. From these observations and from our findings, it appears that larval willow shiner must feed on zooplankton by using free neuromasts in the dark. In larval willow shiner, the vane-like cupulae of the free neuromasts protrude from the body surface and the long cupulae are 100–250 μm in length (Mukai and Kobayashi, 1991). The prey is detected by the free neuromasts as a result of a slight bending of the cupula in response to local water movements. The shape of the cupula, especially its length, must therefore be related to the

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sensitivity of the free neuromast, as inferred from the results of Coombs and Janssen (1989) and van Netten and Kroese (1989).

A well-known anaesthetic, MS222 (tricaine methanesulphonate), has an unexpected action in that it dissolves the cupula. We previously reported that willow shiner larval cupulae treated with MS222 regrew at a constant rate of 8.7–10.6 μm h^{-1} (Mukai and Kobayashi, 1992). In the present study, the relationship between the length of the cupulae of free neuromasts and feeding ability was examined in a feeding experiment in the dark, using larval willow shiners with cupulae regenerating after treatment with MS222.

The larvae used in this study were 6.9–11.7 mm in total length 20–40 days after hatching. At this stage, larvae have only free neuromasts and their canal organ has not yet developed. Larvae were starved for 24 h before the feeding experiment.

To remove the cupulae, larvae (*N*=96) were subjected to turbulence from air bubbles blown through a solution of MS222 (200 p.p.m., pH 4.2) for 3 min at 17–18 °C. Groups of six larvae were put into fresh water in a Petri dish (14 cm in diameter). After 1, 3, 6, 9 and 12 h, these Petri dishes were placed in a completely dark room for 20 min. Each group was given 120 nauplii of *Artemia salina* for 10 min at a water temperature of 18–21 °C. To count the number of *Artemia* in the gut, larvae were anaesthetized with ethyl carbamate (2 %); this anaesthetic did not cause peristalsis which would have voided *Artemia* from the gut and did not affect the cupulae. At the same time, the whole body surface in three treated larvae, randomly selected at 1, 3, 6, 9 and 12 h, was recorded on video tape with a video recorder (TK-1070, Victor) through a phase-contrast microscope (Optiphoto XF-NT, Nikon) for subsequent measurement of the cupular length. For the recording, each larva was held in the normal posture on a glass slide with a hollow by a small pin (Serrfin) attached to the caudal fin. The cupular length was determined from 22–30 long cupulae in three larvae.

Fig. 1 shows the relationship between the length of cupulae and the number of *Artemia* eaten per larva after the treatment with MS222 (pH 4.2). The cupulae grew with time, elongating to 17±3.5 μm (mean ± S.D.; *N*=22) at 1 h, 35±2.9 μm (*N*=30) at 3 h, 66±3.8 μm (*N*=30) at 6 h, 99±13.0 μm (*N*=30) at 9 h and 133±8.0 μm (*N*=30) at 12 h after the treatment. The growth rate of the cupulae was 10.6 μm h^{-1} (*r*=0.984), calculated from 142 measurements taken 1–12 h after the treatment. This rate was considerably faster than the value of 1.4–4.1 μm h^{-1} reported for herring larvae (*Clupea harengus*, Blaxter, 1984), but is comparable to the range 8.7–10.6 μm h^{-1} measured for willow shiner in a previous study (Mukai and Kobayashi, 1992). Moreover, this rate was almost the same as those for other cyprinid larvae; 8.9 μm h^{-1} for pale chub *Zacco platypus* and 10.3–11.3 μm h^{-1} for crucian carp *Carassius auratus grandoculis* (Mukai and Kobayashi, 1992). The number of *Artemia* eaten increased with elongation of the cupulae. The regression curve showed that the number of *Artemia* eaten per larva was proportional to the cupular length and saturated at cupular lengths above approximately 100 μm. Disler (1971) also reported that *Carassius carassius* larvae could not respond to a mechanical stimulus 1 h after the cupulae had been removed by scrubbing with filter paper, and that the larvae began to hunt plankton again after 2.5–3.0 h. It is likely that the cupulae also regenerated in *C. carassius*, because we have found cupular regeneration in the closely related species *Carassius auratus grandoculis* (Mukai and Kobayashi, 1992).
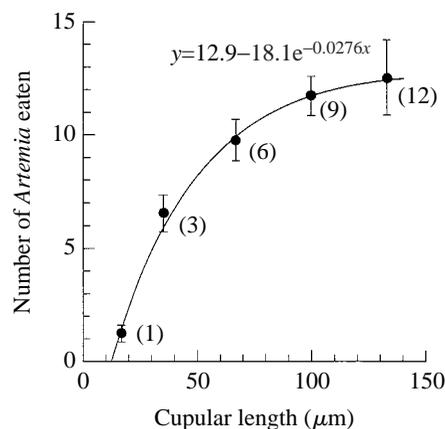
Fig. 1. The relationship between cupular length and the number of *Artemia* eaten in the dark by willow shiner larvae treated with MS222. Larvae were subjected to turbulence by bubbling with air in MS222 solution (200 p.p.m., pH 4.2). Numbers in parentheses indicate time (in hours) after the treatment. The regression curve was obtained by the difference equation method. The numbers of larvae in the feeding experiment were 30, 30, 24, 6 and 12 at 1, 3, 6, 9 and 12 h, respectively. Cupular length was determined from 22–30 long cupulae in three larvae randomly selected for each time after the treatment. The number of *Artemia* is expressed as mean ± S.E.M.; the S.E.M. for cupular length is within the symbols.

However, the increase in the number of *Artemia* eaten per larva might be dependent on recovery from a reduced larval appetite induced by MS222 or by the bubbling treatment. In order to investigate this possibility, the number of *Artemia* eaten by larvae treated with MS222 (pH 4.2) and by intact larvae was examined under light conditions. The larvae of both groups ate on average approximately 18 *Artemia* within 10 min at 1 h after the treatment (18.2±8.6 in treated larvae; 17.7±9.8 in intact larvae; $N=6$ in each group). Furthermore, to examine the influence of handling stress, the feeding experiment in the dark was carried out on intact larvae ($N=72$). The numbers of *Artemia* eaten by intact larvae in 10 min were unchanged with time after putting the larvae in the Petri dish: 10.3±4.0, 13.2±5.7, 11.3±4.6, 10.8±6.1 and 12.1±5.2 at 1, 3, 6, 9 and 12 h, respectively. The increase in the number of *Artemia* eaten per larva was evidently due to elongation of the cupulae, but not to recovery from the MS222 or bubbling treatments or to handling stress.

Previously, we suggested that the effect of MS222 on the cupula was the result of the low pH (Mukai and Kobayashi, 1992). According to Blaxter and Fuiman (1989), the cupulae of herring larvae were damaged simply by bubbling air. Therefore, to reconfirm the influence of low pH and to examine the influence of aeration, larvae were subjected for 3 min to turbulence caused by bubbling air in the following solutions: dechlorinated tap water at pH 7.3 ($N=6$), MS222 solution at pH 4.2 ($N=30$) and MS222 solution neutralized with NaOH ($N=30$). At 1 h after these treatments, the feeding experiment was repeated in fresh water in the dark. A statistical analysis of the numbers of *Artemia* eaten per larva was performed using the Kruskal–Wallis test among the different treatments.
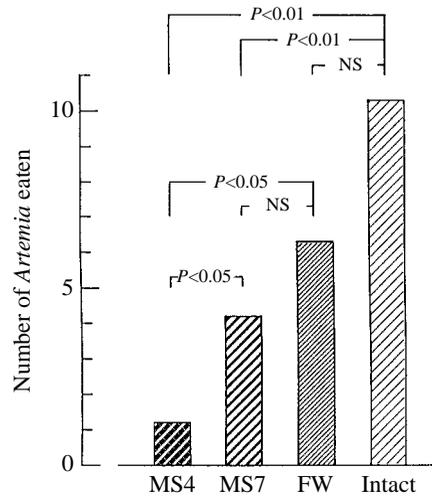
Fig. 2. The mean number of *Artemia* eaten per larva at 1 h after different treatments in the dark. Larvae were left intact or were subjected to 3 min of turbulence by bubbling with air in the following solutions: fresh water at pH 7.3 (FW), neutralized MS222 solution (MS7, 200 p.p.m.) and MS222 solution at pH 4.2 (MS4, 200 p.p.m.). Statistical analysis was carried out using the Kruskal–Wallis test. NS, not significant.

Fig. 2 shows the mean number of *Artemia* eaten per larva 1 h after different treatments in the dark. Intact larvae ate approximately ten *Artemia* (Intact, mean 10.3), which was significantly more than the number eaten by the larva subjected to turbulence by bubbling air in the neutralized MS222 solution (MS7, mean 4.2) or in the MS222 solution at pH 4.2 (MS4, mean 1.2). The number of *Artemia* eaten by intact larvae was not significantly different from that eaten in FW (mean 6.3). The number of *Artemia* eaten in FW was significantly more than the number eaten in MS4, but not from that eaten in MS7. It appears that MS222 itself does not affect larval feeding. The significant difference between the numbers of larvae eaten in MS4 and MS7 is therefore assumed to be due to the shortening of the cupulae caused by the low pH.

Under dark conditions, intact larvae ($N=6$) as well as larvae treated with MS222 ($N=6$, pH 4.2) ate no more than one dead frozen *Artemia*. These findings suggest that the larvae locate *Artemia*, which produce vibrations as they move, using free neuromasts with functional cupulae, but not by olfaction or taste. Moreover, the larvae may be able to feed on planktonic prey more accurately using vision than using free neuromasts, because the intact larvae ate approximately ten *Artemia* using free neuromasts in the dark and the treated larvae ate approximately 18 *Artemia* using vision in the light.

In conclusion, the cupular length seems to be proportional to the ability of larval willow shiner to detect and capture *Artemia*.

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