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The effect of salinity on the chemotaxis of glass eels, *Anguilla anguilla*, to organic earthy and green odorants

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Synopsis

The behaviour of upstream migrating glass eels to salt and brackish water solutions of 8 pure organic earthy and green odorants was investigated. In 35‰ salt water, IPMCET and D-MF are ineffective, while MMP, IBMP, MT, TMCE, ETMCE and L-MF are strongly repellent. Dissolved in brackish water, MMP and ETMCE become attractive. The strong attraction which glass eels show to earthy and green odorants emerges as the level of salinity is reduced, suggesting that these chemicals could be orienting cues in the last phase of glass eel migration.

Introduction

An ever-increasing number of studies have described the major role played by chemoreception in fish behaviour (see Liley 1982, Hara 1992, Olsén 1992, Smith 1992). An interesting example is that of glass eels, who are attracted by the odour of co-specifics (Pesaro et al. 1981), aquatic plants and detritus (Sorensen 1986), as well as by pure solutions of amino acids (Sola et al. 1993) and bile salts (Sola & Tosi 1993). Recent research has shown that glass eel behaviour is also affected by vegetable odorants. These organic compounds, which produce earthy, musty and green odours, are common in soil and inland water (Persson & Jüttner 1983, Jüttner 1984), while they have not be reported in oceanic waters. A chemical component of the odour of damp soil, termed geosmin (Gerber & Lechevalier 1965), was the first vegetable odour to be defined chemically (Gerber 1968), and it is among the most frequently studied. Broad-based research on glass eels responsiveness to geosmin has revealed that it is strongly attractive for glass eels when dissolved in freshwater, while it is repellent when associated to a salinity of 35‰ (Tosi & Sola 1993). A further eight, synthetic green and earthy odorants, some of which already reported to occur in appreciable quantities (ng l^{-1}) in inland waters (Hwang et al. 1984) have been shown to attract glass eels strongly when diluted in freshwater. In consequence, it has been suggested that these may act as kairomones during glass eel migration from the sea to the inland waters (Sola 1995).

Salinity has been cited among the environmental factors guiding glass eel upstream migration (Deelder 1973, Fontaine 1975, Tosi et al. 1989), while its fundamental influence on the orienting role of geosmin, which becomes even more attractive when associated with a progressively decreasing salinity, has also been described (Tosi & Sola 1993).

In this light, then, it would appear valuable to look more deeply at the chemotaxis of glass eels to vegetable odorants at different salinity levels, in order to understand behavioural changes as the animals pass from oceanic to inland waters.

Material and methods

The glass eels were caught off Leghorn, Italy, on the coast of the north Tyrrhenian Sea fortnightly during the 1992/1993 and 1993/1994 migration seasons, which in this region were between December and April. As upstream migrating eels usually present different stages of pigmentation (Boëtius 1976), specimens ranging from stage A (i.e., unpigmented) to stage E (melanophores over the entire body surface) were caught and used.

In the laboratory, the animals were acclimated for three days in salt water at the same salinity as that in which they were captured (35‰) or for six days in brackish water (salinity 30, 25, 20, 15, 10 or 5‰), at a constant temperature of 11° C in tanks equipped with an aerator and shelters. Specimens were used for the experiments within fifteen days and then released.

The chemotaxis of the eels was tested using a two-choice flowing water apparatus consisting of a glass choice chamber (35 × 25 × 25 cm), on one side of which the mouths of two glass funnels opened (Ø 6 cm). The funnels were inserted through a silicon cork into the neck of 250 ml filtering flasks so as to form traps. On the wall opposite the traps, an out-flow ensured a constant water level in the tank of 6 cm (for further details see Tongiorgi et al. 1986 and Sola & Tosi 1993). Experiments were performed by testing the glass eels preference for two water flows at the same salinity, one without odour (blank) and the other containing a preset solution of pure odorant. The water of the flows, as that used for acclimation, was prepared by adding pure synthetic marine salt (Tropic Marin Neu produced by Tropicarium Buchschlag) to distilled water.

At the beginning of each experiment, twenty glass eels were placed in an enclosure in the choice chamber and left for twenty minutes to acclimatise. Some minutes before the glass eels were released, the two water flows were activated in order to replace the water in the tank. A multi-channel cartridge metering pump (Masterflex, Cole-Palmer) ensured constant flows of 150 ml min^{-1}. After being released, the animals were left to choose for the next twenty minutes. At the end of the test, the number of animals in each trap was recorded. After each test, the apparatus was carefully cleaned with absolute ethyl alcohol and washed with dechlorinated water to remove any traces of odour. Each experiment was repeated at least twice, and the position of the flows reversed. The same specimens were never used for more than one trial. Twenty control tests with two flows of plain water were also performed randomly.

Two experimental series were carried out using the same odorants employed in a previous study testing chemotaxis evoked by freshwater solutions (Sola 1995). The odorants were 2-methyl-3-methoxypyrazine (MMP), 2-isobutyl-3-methoxypyrazine (IBMP), 1,2,2,6-tetramethylcyclohexanol (TMCE), 1-ethyl-2,2,6-trimethylcyclohexanol (ETMCE), 4-methylthiazole (MT), 4-isopropyl-7-methylcyclohexathiazole (IPMCET) and (L) and (D) 2-methylfenchol (MF). All the substances were pure chemicals synthesised by Paolo Pelosi of the Instituto di Industrie Agrarie, University of Pisa and purity was checked as greater than 99% by GLC on a 25 m OV-1 capillary column (for further information, see Finato et al. 1992).

Odorant solutions were prepared immediately prior to the test in carefully washed glassware. The first series of experiments tested the response of glass eels acclimated to a salinity of 35‰ to different concentrations of odorants dissolved in salt water. The second series of experiments tested the response of glass eels acclimated to brackish water to preset solutions (10^{-13}, 10^{-9} and 10^{-5} mg l^{-1}) of two selected odorants (MMP and ETMCE), presented at different salinities (30, 25, 20, 15, 10 and 5‰). The salinity used was the same for both the odorous and the blank flow.

The χ^2 test was used to assess the significance of the differences between the choices of the blank and odorous flow, assuming the null hypothesis of an equal number of preferences for the two flows. The χ^2 reported in the Figure 1 is the sum of the χ^2 at different concentrations.

Results

As soon as they were released, the glass eels began to swim freely in the choice chamber. Once they had located the flows coming from the funnels, they swam against the current towards one or other of the flasks where they remained trapped. Only a limited number of animals did not effect a choice, remaining in the initial chamber. Indeed, in 190 experiments (116 in the first series and 74 in the second), the number of individual choosing one or other the flows was frequently above 90% and never below 75%. Furthermore, in the 20 control tests, the mean number (± s.d.) of specimens choosing the left (9.5 ± 1.96) or the right (8.95 ± 2.19) trap was statistically equivalent.

Experiments in salt water

When dissolved in water at a salinity of 35‰, the odorous substances were avoided (Fig. 1). The avoidance of IPMCET (Fig. 1f) and D-MF (Fig. 1h) was modest and not statistically significant, while in the case of the other substances the repulsion was clear. The maximum repellent effect with the two pyrazines (IBMP with a threshold of 10^{-9}, and MMP, perceived down to 10^{-13} mg l^{-1}) was observed at a concentration of 10^{-9} mg l^{-1} (Fig. 1a, b). With ETMCE, one of the strongest substances, the repulsion was relatively constant from a concentration of 10^{-5} down to 10^{-13} mg l^{-1}, the response threshold (Fig. 1d). Constant repulsion was also seen with L-MF (Fig. 1g), which was perceived down to a concentration of 10^{-12} mg l^{-1}. TMCE (Fig. 1c) had a dose-dependent effect, with repulsion falling as the concentration was reduced down to 10^{-11} mg l^{-1}. Finally, MT was effective down to a concentration of 10^{-1} mg l^{-1}, but was particularly repellent at a concentration of 10^{-7} mg l^{-1} (Fig. 1e).

Experiments in brackish water

The repellent effect seen in marine water disappeared when the two substances tested (MMP and ETMCE) were presented in brackish water. The change in the preferences at minimum (10^{-13} mg l^{-1}), intermediate (10^{-9} mg l^{-1}) or maximum (10^{-5} mg l^{-1}) odorant concentration began at salinities only a little below (30‰) that of the open sea (Fig. 2).

However, the attraction of the odorants emerged at different salinities, depending on the concentration used. Threshold concentrations (10^{-13} mg l^{-1}) of both substances were strongly attractive even in relatively high salinity (25‰) solutions (Fig. 2a, b), while stronger concentrations only exerted attraction at lower salinities. Solutions of MMP at a concentration of 10^{-9} mg l^{-1} attracted glass eels when associated with salinity less than or equal to 15‰ (Fig. 2c), while ETMCE at a similar concentration was only attractive at or below 10‰ (Fig. 2d). As the concentration of the odorant increased further, so salinity had to be further reduced: both odorants at a concentration of 10^{-5} mg l^{-1} were attractive only if dissolved in slightly brackish water (5‰) (Fig. 2e, f).

Discussion

A previous paper testing the behaviour of glass eels towards earthy and green odorants showed that these substances are strong stimulants and are attractive when dissolved in freshwater (Sola 1995). In contrast, present results show that when dissolved in salt water these odorants induce a completely different behaviour. Four compounds (MMP, ETMCE, TMCE and L-MF) have behavioural activation thresholds equal to that registered in freshwater but induce clear repulsion, two (MT and IBMP) induce a statistically significant response only when presented at a concentration, respectively, at least 10 or 100 times higher than that required in freshwater, and the final two (IPMCET, D-MF) seem to exert little effect.

The relationship between salinity and the positive and negative chemotaxis of the glass eels is evident. An explanation of this generalized repulsion of glass eels to salt water solutions of odorants, which has emerged previously with the earthy odour geosmin (Tosi & Sola 1993), might be that this is a laboratory artifact, as these inland substances would not be normally encountered in the open ocean. Consequently, glass eels refrain from following this incorrect message, and their behavioural response appears confused. The situation is different in coastal waters fed by inland flows, where a certain amount of earthy and green odorants is still present. Indeed, glass eels are clearly attracted by an odorous flow appropriately combined with brackish water.

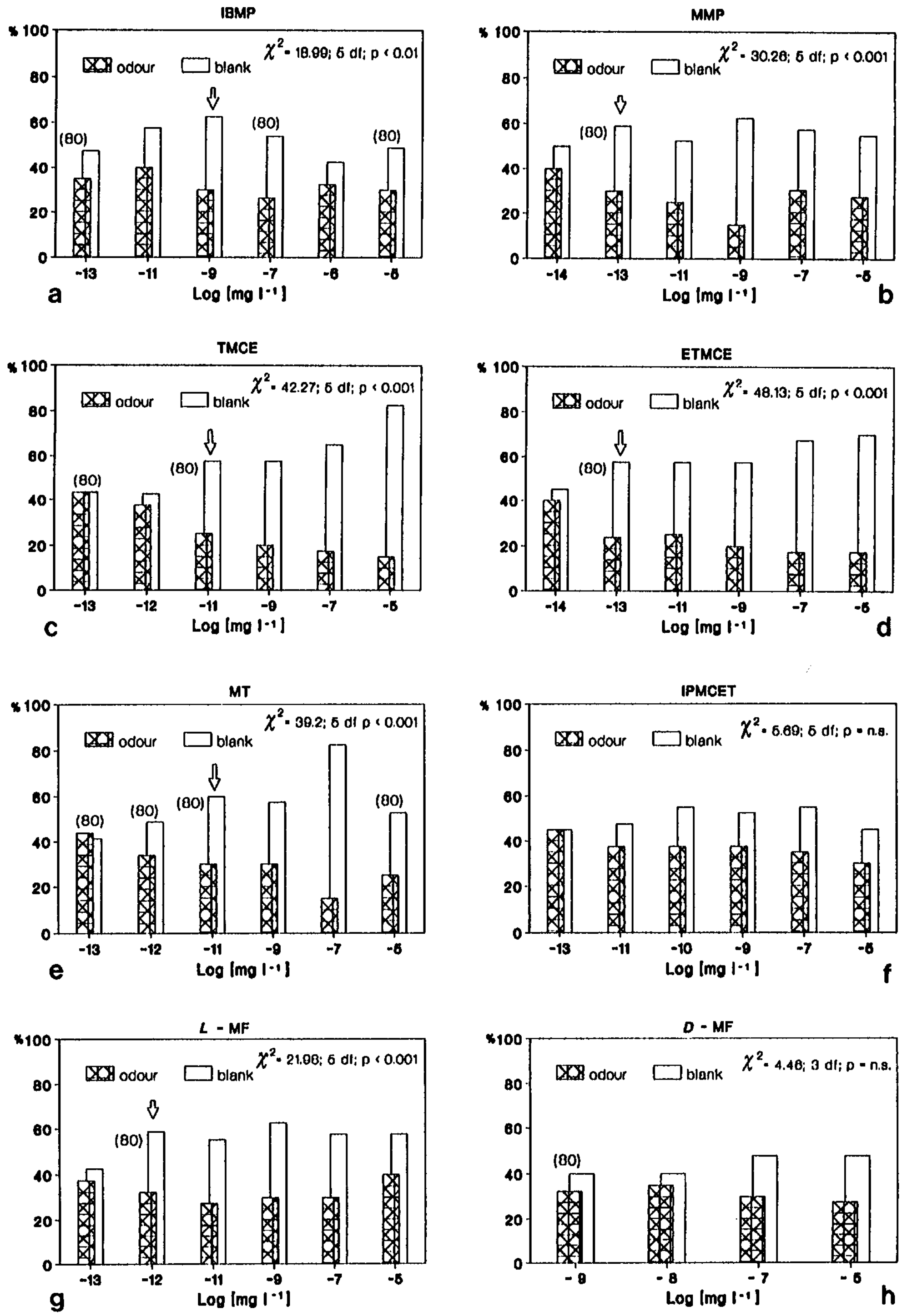

Fig. 1. Choices (% of tested eels) of blank flows or odorant solutions in 35‰ salt water at different concentrations. Unless otherwise indicated, the response to each concentration was independently tested with two groups of 20 glass eels. The arrow indicates the behavioural response threshold.

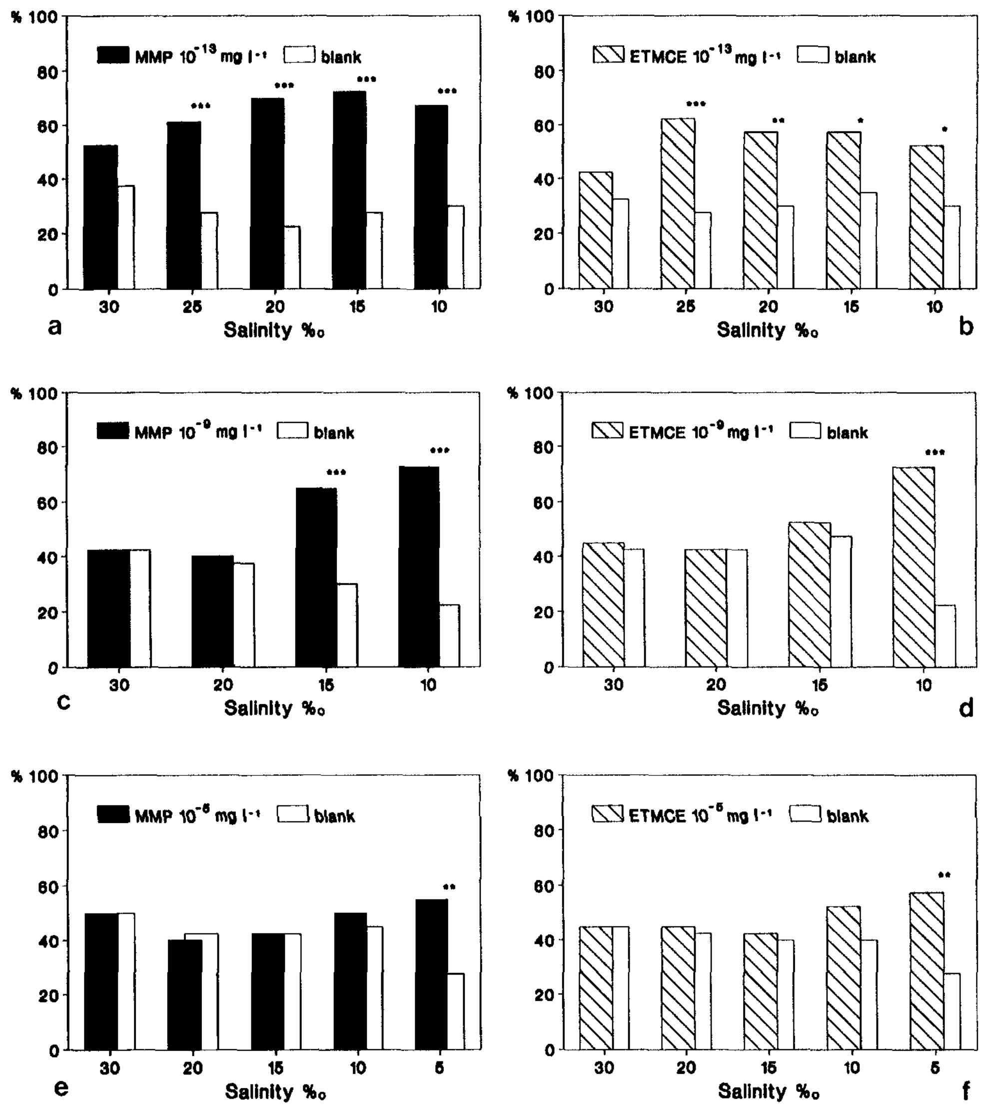

Fig. 2. Choices (% of tested specimens) by groups of 20 glass eels acclimated to brackish water of blank and odorous flows at the concentrations and salinity indicated. Glass eels tested: a, 280; b, 280; c, 260; d, 260; e, 200; f, 200, χ^2 test. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

A possible interaction between the respective concentrations of salinity and odorants is particularly interesting. Attraction is obtained in high salinity flows as long as the concentration of the odorant is extremely low, while at high odorant concentrations, salinity must be lowered. In nature, as animals move from the open sea towards the river mouths, the negative salinity gradient and the parallel increase in the concentration of odorants could come to form a single positive stimulus which becomes more precise and acts as an orientation signal for the glass eels. Indeed, glass eels swimming towards the river mouth remain close to the haloclyne (McCleave & Kleckner 1982), and so not only continuously detect differing salinity, but also the steep gradient of oceanic and inland odours.

Various experimental reports have indicated the influence of surface water odours (Creutzberg 1961, Miles 1968) and the interaction of these with salinity (Tosi et al. 1990) on glass eel migratory behaviour.

The strong interaction between salinity and the green earthy odorants evidenced in this study supports the hypothesis advanced in preliminary work (Sola & Tongiorgi 1994) that these odorants may be among the cues directing glass eels towards the coast.

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