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Bile salts and taurine as chemical stimuli for glass eels, *Anguilla anguilla*: a behavioural study

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Synopsis

The behaviour of migrating glass eels towards different concentrations of seven bile salts and taurine was investigated by binary-choice experiments. All substances attracted glass eels when presented at concentrations below 10^{-10}M. Glycocholate, taurodeoxycholate and taurine remained attractive at higher concentrations, while taurocholate, cholate, deoxycholate, glycochenodeoxycholate and taurochenodeoxycholate became repellent. A role of bile salts in grouping and orientation behaviour of glass eels is discussed.

Introduction

A considerable number of substances, many of which are catabolites, are involved in intraspecific communication among fish. Some substances can induce attraction or repulsion, favour grouping and reproductive behaviour or act as orientation signals (for review see Liley 1982).

The homing behaviour observed in adults of some salmonid species can also be explained by chemical trails laid down by conspecifics (Nordeng 1971, 1977).

Electrophysiological data have shown that some substances produced by fish are effective stimuli of the olfactory system of other specimens of the same species (Døving et al. 1973), and that the mucus secreted in large quantities from the epidermis is a strong chemical signal (Hara et al. 1984). Behavioural studies have shown that the urine and intestinal waste of the Arctic charr, *Salvelinus alpinus*, attract both young (Olsén 1987) and adult conspecifics (Selset & Døving 1980). Research on the young coho salmon, *Oncorhynchus kisutch*, has shown that recognition is extremely specific, and water which has been inhabited by siblings can be distinguished from that inhabited by nonsiblings (Quinn & Hara 1986).

Glass eels of *Anguilla anguilla* (L.) are evidently attracted by the odour of conspecifics rather than by that of other species (Pesaro et al. 1981). Glass eels of *A. rostrata* present a similar behaviour, being attracted by the odour of other glass eels (Sorensen 1986) and adult eels (Miles 1968).

Research to identify the attractant chemical substances involved in the grouping and orientation behaviour of fish has led to a number of studies on the electrophysiological and behavioural responses to different substances, including taurine and bile salts. Indeed, considerable quantities of taurine are found in the mucus of *Oncorhynchus mykiss* and *Coregonus clupeaformis* (Hara et al. 1984), and of *Anguilla anguilla* (Saglio & Fauconneau 1988), while bile salts are released through the urine and intestinal waste.

The physicochemical properties of the bile salts make them excellent short- and long-term chemical signals in aquatic environments. They present many molecular structures and can give rise to numerous compounds, some of which are very stable, while others degrade rapidly once released into the environment (Haslewood 1967).

Electrophysiological studies have shown that bile salts, especially taurine conjugates, can stimulate the olfactory system of *Thymallus thymallus*, *Salvelinus alpinus* (Døving et al. 1980) and *Oncorhynchus mykiss* (Hara et al. 1984).

The olfactory system of the eel species is very sensitive, but to date only limited data are available on behavioural reactions of *Anguilla rostrata* to the bile as a whole (Sorensen 1986). The present study analyses the reactions of glass eels of *Anguilla anguilla* towards taurine and individual bile salts which could be involved in some aspects of glass eel behaviour, such as grouping and predatory activity.

Material and methods

Experiments were carried out on glass eels, *Anguilla anguilla*, caught at sea off the north Tyrrhenian coast (Italy) every fifteen days from December 1991 to April 1992. The stage of development of the glass eels was established with reference to pigmentation, following Boëtius (1976). Glass eels caught between December and February were mostly unpigmented (stage A) or slightly pigmented (stage B). In those caught between March and April, the number of pigmented specimens (stages C and D) increased. In April, a small number of fully pigmented specimens (stage E) was also collected.

In the laboratory, animals caught in each period were divided into two groups and kept, one group in salt water (33‰), for 3 days and the other in fresh water for 6 days, at a constant temperature of 11°C in tanks equipped with an aerator and shelters. The glass eels were never kept in the laboratory for more than two weeks and were used for one trial only. In order to avoid any odours which could affect the experimental results, drinkable well-water devoid of any organic matter was used. Salt water was prepared by adding synthetic marine salt, produced with guaranteed pure salts (Tropic Marin Neu), to well-water.

The experiment was designed to record preferences towards two water flows, one without odours (blank) and the other containing a preset solution of bile salt. The linear flows were at a constant rate of 130 ml min^{-1} and at the same temperature (11°C) and salinity (fresh water or 33‰ salt water). Differences in salinity and temperature between the two flows, which could influence glass eel behaviour (Tongiorgi et al. 1986, Tosi et al. 1988, 1989, 1990), were avoided, making all experimental conditions strictly controlled.

The experimental device (Fig. 1), designed according to the behavioural characteristics of glass eels, consisted of a glass choice chamber (35×25×25 cm), on one side of which the mouths (∅ 6 cm) of two glass funnels opened. The funnels were inserted through a silicon cork into the neck of 250 ml filtering flasks so as to form a trap. On the wall opposite the traps, an out-flow ensured a constant level of 6 cm of water and constant circulation of the water in the tank. The efficiency and reliability of the experimental device had been established in previous studies (Tongiorgi et al. 1986, Tosi et al. 1990).

At the beginning of each experiment, 20 glass eels were placed in an enclosure in the choice chamber and left for 20 min at the temperature and salinity to which they had been previously acclimatized. The animals were then released, and left free to choose for the next 20 min. Responding to the positive rheotaxis, the glass eels moved against the flows and, choosing one of them, entered the flask where they remained trapped. At the end of the test, the number of animals in each trap was recorded. Each experiment was repeated at least twice and the position of the flows inverted. After each test, the apparatus was carefully cleaned with absolute ethyl alcohol and washed with well water to remove any traces of odours.

The two plain water flows were also tested both before and at random intervals during the stages of the experiment. The tendency of the animals to divide more or less equally between the two flows demonstrates that no other factors influence choices during the testing with the bile salts.

The responses were recorded to taurine (BDH, purity 99%) and the sodium salts of the most common bile acids: cholic, deoxycholic (BDH, purity 99%), taurocholic, taurodeoxycholic, taurochenodeoxycholic, glycocholic and glycochenodeoxy-

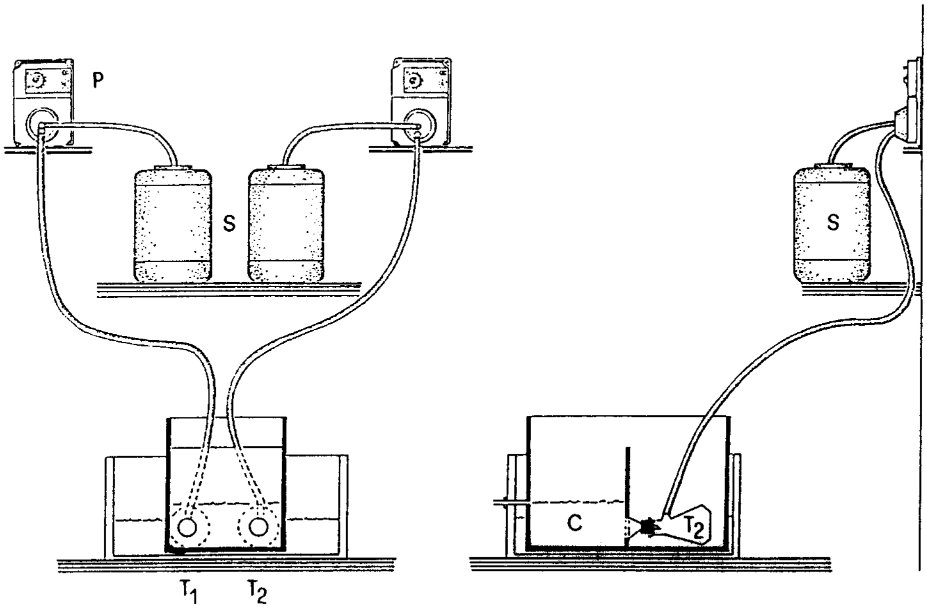

Fig. 1. Experimental device used to test preferences for a flow of plain water (blank) or a preset solution of bile salts or taurine. T_1, T_2 = traps; C = choice chamber; P = pumps; S = blank and bile salt solution (modified after Tongiorgi et al. 1986).

cholic acids (SIGMA, purity 98%). In fresh water, concentrations ranging from 10^{-7}M down to the threshold of behavioural response were tested. Concentrations higher than 10^{-7}M were not used because of the possible irritation caused by the bile salts. In salt water, all concentrations which elicited a response in fresh water were tested, so as to establish if there were any differences in the choices. Testing solutions were prepared immediately before each test using sterilized glass balloons and automatic Eppendorf pipettes.

The results are plotted in semi-logarithmic graphs as the ratio between the number of observed preferences for the bile salt solution (ON) and the expected frequency that all specimens prefer the odorous flow (EF). This value can theoretically vary between 0 (total repulsion) and +1 (total attraction). If there is no preference, the choices equalize and the theoretical value of ON/EF is 0.5. Since some specimens did not enter the traps, the frequency with which the animals did not show any preference (i.e., the number of eels which remained in the choice chamber/the number of tested eels) produces a real value of no preference (n.p. in Figs. 2–4) which is in the neighbourhood of 0.5. Values of ON/EF within the n.p. range were not included in the statistical analysis, as it was considered unwise to reject the null hypothesis. The significance of the responses was tested with the χ^2 test, assuming the expected frequency of an equal number of preferences for the two flows, as occurred when plain water was used in both flows. χ^2 reported in the figures was calculated as the sum of the χ^2 for each point.

Results

Taurodeoxycholate and glycocholate were attracting eels both in fresh water and in salt water solutions, with a threshold concentration of 10^{-11} and 10^{-10}M, respectively. In fresh water, taurine is attractive down to 10^{-12}, while in salt water the threshold of the behavioural response is one thousand times greater (Fig. 2). Taurocholate in fresh water is strongly attractive at 10^{-10} and 10^{-11}M, while at higher concentrations the choices between the two flows equalize. In salt water, taurocholate is ineffective, as is also shown by the limited number of choices. On the other hand, cholate evokes stronger reactions in salt water than in fresh water. Fresh water solutions are slightly attractive only at 10^{-11}M, while higher concentrations are avoided. In salt water, at-

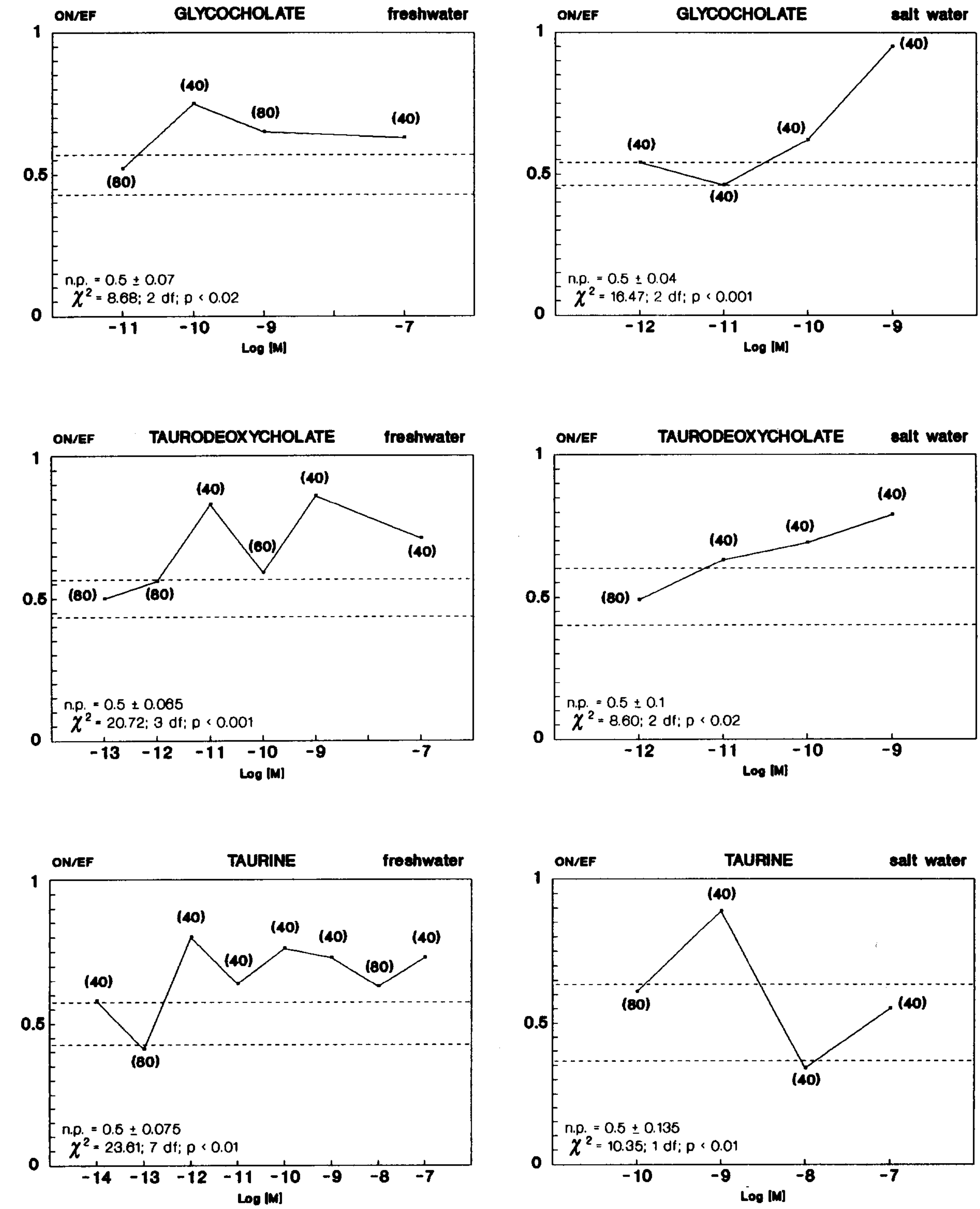

Fig. 2. Choices by groups of fresh water (left) and salt water (right) acclimatized glass eels in respect of different solutions of taurine and two bile salts which induce attraction. The x-axis shows the tested solutions and the y-axis the entries into the traps, expressed as the ratio between the observed number of preferences for the bile salt (ON) and the expected frequency (EF). The number of glass eels tested is given in parentheses. The frequency with which the glass eels did not enter the traps (n.p.), measured with respect to the total number of eels tested, is indicated as a value in the neighbourhood of 0.5 and delimited by the dotted lines.

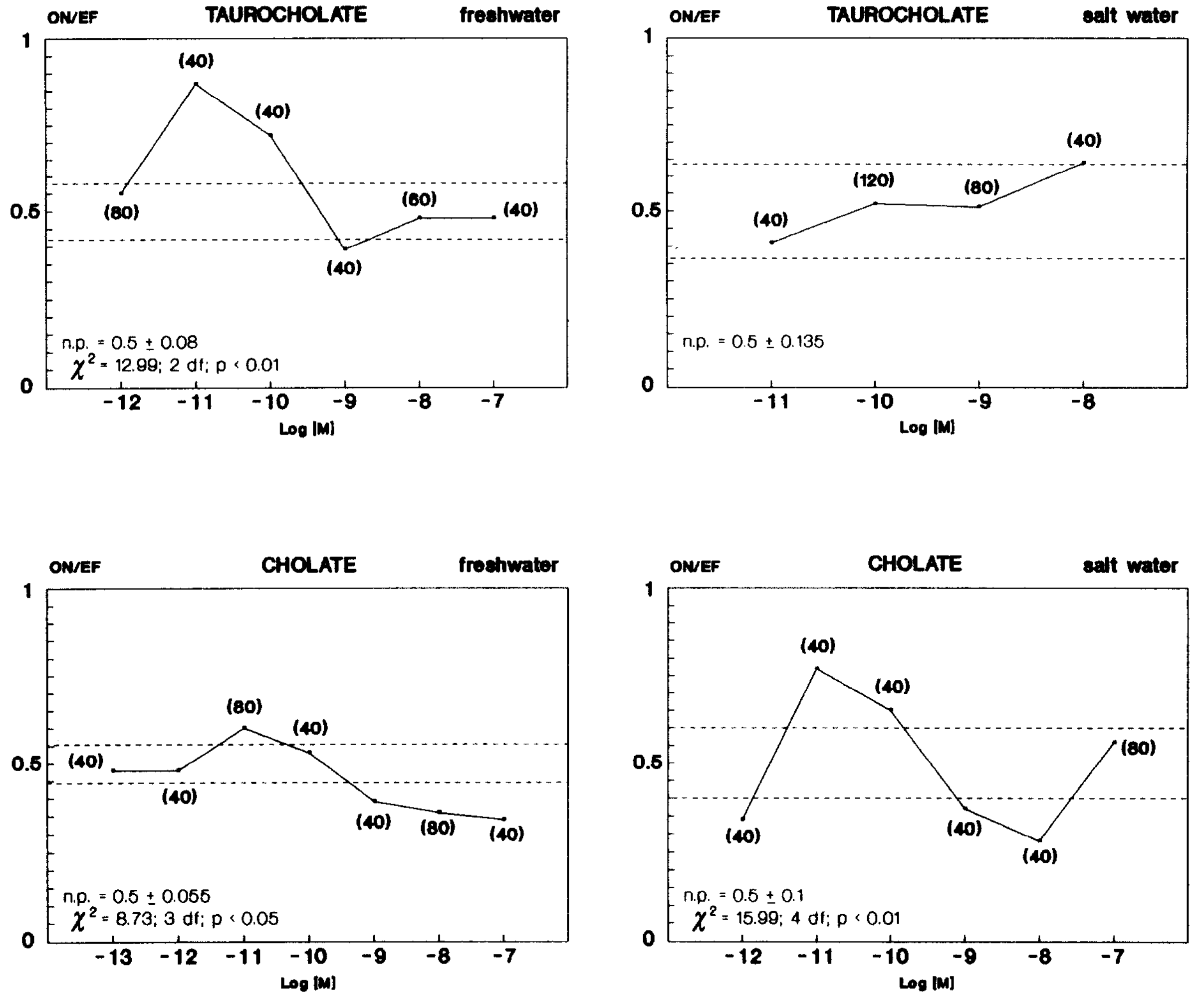

Fig. 3. Choices by groups of fresh water (left) and salt water (right) acclimatized glass eels in respect of different solutions of two bile salts inducing weak avoidance at high concentrations and attraction at more diluted solutions. Keys as in Fig. 2.

traction is also evident at 10^{-10} M. The limited number of preferences for cholic acid at a concentration of 10^{-12} M is to be seen in the context of the overall low number of choices recorded at this concentration (Fig. 3).

At concentrations below 10^{-9} M, fresh water solutions of deoxycholate, glycochenodeoxycholate and taurochenodeoxycholate are strongly attractive, while at higher concentrations these substances elicit clear avoidance. In salt water, fewer choices are made; attraction persists, while avoidance is only slightly evident (Fig. 4).

Discussion

The bile salts are very effective chemical stimuli for several species of fishes, mainly salmonids. Electrophysiological studies have shown that the threshold of olfactive perception of these substances is comparable to or even lower than that observed with the amino acids. The olfactory system of Arctic charr, *Salvelinus alpinus*, grayling, *Thymallus thymallus* (Døving et al. 1980) and rainbow trout, *Oncorhynchus mykiss* (Hara et al. 1984) respond to 10^{-9} M concentrations of bile salts. The response to some amino acids is evident down to a concentration of 10^{-8} M or, in the rainbow trout (Hara et al.

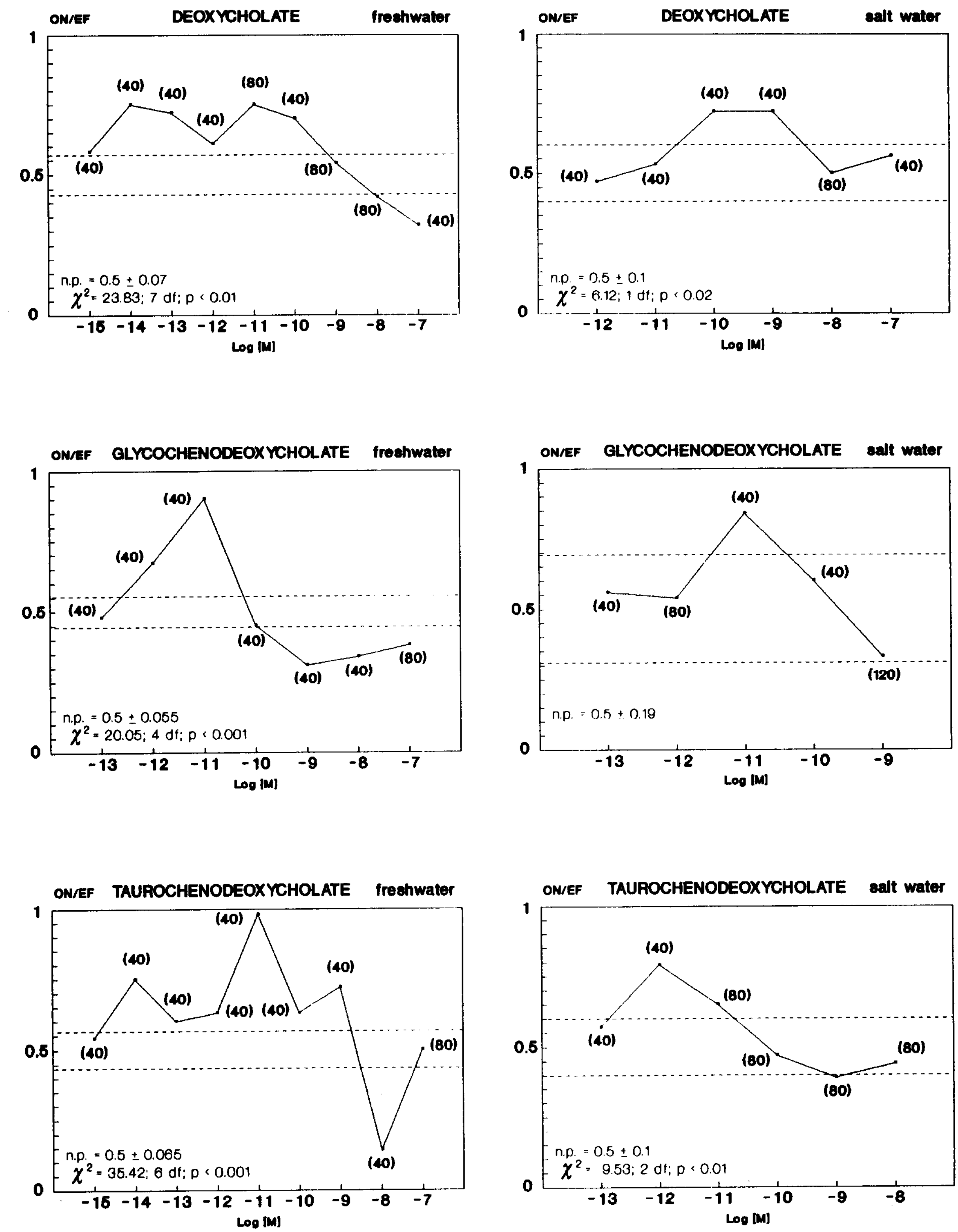

Fig. 4. Choices by groups of fresh water (left) and salt water (right) acclimatized glass eels in respect to different solutions of three bile salts inducing clear avoidance at high concentrations and strong attraction at more diluted solutions. Keys as in Fig. 2.

1984) and in the Arctic charr (Sveinsson & Hara 1990) 10^{-9}M, while the threshold for methionine in the Arctic charr and in the grayling is not below 10^{-6}M (Døving et al. 1980). The Arctic charr, the grayling and the rainbow trout are particularly sensitive to taurine conjugates, while taurine itself is an effective stimulus for the first two species only at concentrations above 10^{-5}M (Døving et al. 1980) and has no effect in the rainbow trout (Hara et al. 1984).

The present behavioural study shows that the glass eels of *Anguilla anguilla* can perceive bile salts: down to a concentration of 10^{-14}M in the case of deoxycholate and taurochenodeoxycholate and as low as 10^{-12}M with taurine. The avoidance induced by the highest concentrations tested of cholate, deoxycholate, glycochenodeoxycholate and taurochenodeoxycholate is in agreement with that seen with high concentrations ($5 \cdot 10^{-2}$ ml $\cdot$ l^{-1}) of whole bile in glass eels of *A. rostrata* (Sorensen 1986).

The behavioural approach allows the integrated response of different sensory systems to be studied. Indeed, bile salts can activate taste as well as smell. Hara et al. (1984) have shown that in rainbow trout the electrophysiological limit of the taste response to taurolithocolic acid is even lower than that of the olfactive response. The cod, *Gadus morhua*, exhibits snapping behaviour linked to feeding and induced by taurocholate, even when deprived of the sense of smell, although the solution necessary to induce the response is ten times stronger (70nM) than that required when both sensory systems are available (Hellstrøm & Døving 1986).

Furthermore, the same chemical stimulus can elicit a different behavioural response depending on whether the stimulus is perceived by taste or by smell (Atema 1977, Herbert & Atema 1977, Little 1978).

The presence of inputs from both the taste and olfactory systems could explain the differences in preference at varying concentrations. The general attraction to bile salts at low concentrations ($<10^{-9}$M) could result from the response of the olfactory system alone. At higher concentrations, it is reasonable to assume that taste is also involved, and consequently the integrated response of the two systems could produce a different chemical 'image', resulting in a change in the behavioural response.

Sibling recognition, proved in some salmon species, could result from the particular mix of bile salts which siblings release into the water (Quinn & Hara 1986), and could therefore play a role in homing behaviour. Migration of glass eels to fresh water is not, obviously, a process comparable to homing, although it has also been demonstrated that migrating glass eels can recognise conspecifics (Pesaro et al. 1981). The strong attraction that the bile salts induce in glass eels, particularly in fresh water, suggests that the presence of these compounds constitutes an important stimulus for species-specific recognition. The presence of these substances should encourage glass eel entry into streams already populated by conspecifics, and it is likely that taurine contained in the mucus and bile salts present in the intestinal waste play an important role in intraspecific communication and, in particular, in the grouping behaviour displayed by these animals in the last phase of ascent when they gather near river mouths. Sensitivity to bile salts could also be important later, both for locating sources of food after onset of the trophic phase, as occurs in other predators (Hellstrøm & Døving 1986), and for dispersion when grouping behaviour subsides (Knights 1987).

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