

Influence of salinity on the early development in the spined loach, *Cobitis taenia*

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Abstract

Spined loach *Cobitis taenia* developed successfully between 0.12 and 4.80 ‰ salinity. At 6.00 ‰ S net production was strongly reduced, and development failed at or above 7.20 ‰. Below 0.12 ‰, net production became variable, indicating restrictive effects. In comparison with other primary freshwater fish species *C. taenia* has a low sensitivity to salinity. The upper limit for early development was equal to the highest salinity under which *C. taenia* adults are found along the Baltic coast. Therefore, salinity should not limit early development within the brackish habitats of spined loach.

Introduction

Life histories of several freshwater fishes include a facultative or obligate migration into brackish or marine waters. For example feeding migrations have been recorded for salmonids, sticklebacks, the cyprinids roach, *Rutilus rutilus* (L.), and ide, *Leuciscus idus* (L.) (MÖBIUS & HEINCKE, 1883; NELLEN, 1965; SCHÖFER, 1979; WHOOTON, 1984; JOBLING, 1995). During the non-reproductive season, young specimens and adults migrate into brackish water for feeding and growth but have to return into waters of lower salinity for spawning, because the high salinity of the environment does not allow successful development of the eggs. Generally, early life stages are much more sensitive to external physico-chemical stressors than are adult fish (HOLLIDAY, 1969). Therefore, the tolerances of the early life stages to factors such as ambient salinity restrict the habitats or areas in which successful reproduction is possible.

The influence of salinity on the early development of freshwater fish has been investigated using mainly secondary freshwater fish species like salmonids, coregonids, sticklebacks and percids (WHOOTON, 1984; KLINKHARDT & WINKLER, 1989; JOBLING, 1995). The only studies investigating primary freshwater fish were carried out on the cyprinids *R. rutilus*, carp, *Cyprinus carpio* L., and bream, *Abramis brama* (L.) (JÄGER ET AL., 1981; KLINKHARDT & WINKLER, 1989; OLIFAN, 1941). The last two authors concluded that cyprinids were less tolerant to salinity during early development than were percids, coregonids and the burbot, *Lota lota* L. No data on the effects of salinity on cobitids are available.

In some parts of the Baltic coast a representative of the family Cobitidae, the common spined loach *Cobitis taenia* L., occurs in brackish waters (WINKLER, 1996). It is not known whether this species undertakes migrations for reproduction or if natural reproduction occurs in brackish water. Therefore, it is not possible to decide whether the brackish habitats act as a feeding ground or if the spined loach is able to build up constant populations in this environment. Since the

spined loach is listed as an endangered species in many European countries (KOTUSZ, 1996), an evaluation of its requirements for spawning is necessary.

The aim of the present laboratory study was to assess the range of salinities at which the early development of *C. taenia* is possible, and to answer the question whether early development of spined loach in a brackish environment is limited by salinity. The results from spined loach are compared to those of other species and families of freshwater fish to determine whether tolerance to salinity is a species-specific or family-specific character.

Material and methods

Eggs of *C. taenia* were obtained by natural spawning from a laboratory stock kept in water with a conductivity of 250 to 350 $\mu\text{S}/\text{cm}$ (0.2 ‰). The eggs were sorted under a stereomicroscope to ensure that all eggs used in the experiments were fertilized and that development had not exceeded the blastula stage. Because of the nocturnal spawning habits of *C. taenia* no eggs could be obtained at earlier stages. For each experiment eggs from a single clutch were used and clutches from different females were used between the experiments. Depending on the number of eggs available, the clutch was divided into 7 to 13 equal subsamples (Table I), which were transferred gently to water of different salinity.

Table I: Number of eggs used in experiments A to F

Experiment	Temp. °C	Salinity (‰)																			
		0.0 1	0.0 3	0.0 6	0.0 9	0.1 2	0.1 5	0.1 8	0.2 4	0.3 0	0.6 0	1.2 0	1.8 0	2.4 0	3.0 0	3.6 0	4.8 0	6.0 0	7.2 0	9.0 0	12.00 0
A	20-22	45		45		45			45	45	45	45									
B	20-23										40		40		40	40	40	40	40	40	40
C	21		24	24	24	24	24	24	21												
D	21		23	23	23	23	23	23	21												
E	24	19	20	19		20		20		21	19	20			19		22	21	22	21	
F	24	21	20	23		21		20		23	21	21			19		21	21	23	21	
Sum		85	87	134	47	133	47	87	42	89	85	126	45	40	38	40	83	82	85	82	40

Water of different salinity was created by dissolving commercial sea salt (Instant Ocean[®]) into reverse-osmosis water. Conductivity was measured during the experiments using an electronic conductivity meter (WTW LF92). Salinity was calculated from the conductivity data by the linear equation $S (\text{‰}) = 0.0006 * X (\mu\text{S}/\text{cm})$ estimated from a calibration curve. For example, conductivity of 10,000 $\mu\text{S}/\text{cm}$ corresponds to a salinity of 6 ‰. Temperature was 20 to 24° C

during the experiments (Table I). Occasional measurements showed that media were saturated with oxygen, that no NO₂ was detectable and that the pH values ranged from 6.6 to 7.5. The variations of the pH value can be explained by the very low buffering capacity of the medium at low salinities. Larvae were fed with nauplii of *Artemia* daily.

Eggs were incubated in plastic boxes containing 1 l of experimental water which was changed daily. After the larvae had started exogenous feeding (usually on day 6-7) the volume of water was increased to 2 l and the number of larvae was reduced to a maximum of 20 per treatment to minimize density effects.

The boxes were checked daily for dead or abnormal specimens. Since the presented study was induced by ecological rather than by physiological questions, all embryos and larvae with noticeable malformations were excluded from the experiment, because they would not be viable under natural conditions. When embryos started to hatch, the number of hatchlings per treatment was counted three to five times per day. In the first two experiments, A and B, the rates of survival and growth were recorded over a 35 days period. These experiments served to document the development of the parameters survival, time of hatching and growth during subsequent experiments. Based on the results of these first two experiments, the duration in the following experiments (C-F) was reduced to 20 days and length were measured only at hatching and at the end of the experiment. For further calculations the data from the end of the experiment were used.

In order to combine the effects observed from survival (including abnormal specimens) and growth into an ecologically meaningful parameter, the net production was calculated by multiplying the number of healthy survivors in each treatment by their mean length. The number of survivors and the length of specimens were reduced under non-optimal conditions. For the calculation of the relative net production in each experiment, the treatment with the highest net production was taken as the standard. Body length at the end of each experiment was compared between the treatments using one-way analysis of variance (ANOVA) and Bonferroni's test (SPSS software). Differences between the treatments in relative net production were found using a hierarchical cluster analysis.

Results

Experiments A and B

In experiments A and B the survival, time of hatching and growth of early *C. taenia* were recorded over a period of 35 days. Experiment A covers a range of salinity values from 0.01 to 1.80 ‰ while experiment B includes salinity values from 1.20 to 12.00 ‰ (Table I). There were no general differences between these results and those of subsequent experiments C-F, which covered salinity ranges between 0.01 and 9.00 ‰. Therefore, the results of the first two experiments are representative for mortality and growth during all experiments.

Mortality was observed until the 12th day of the experiment (Fig. 1), after which only occasional mortality occurred. Time of hatching was not affected by ambient salinity (Fig. 2). The only treatments with a prolonged period of hatching were those with lethal concentrations of salt. The slightly earlier hatch at 0.06 ‰ S in experiment A may have been due to frequent disturbances. Significant differences ($P < 0.01$) in the body length of the larvae between the treatments were recorded in both experiments (Fig. 3). In experiment A mean embryos length at 0.06 ‰ S was significantly smaller ($P < 0.01$) than that of the other embryos. In experiment B the embryos in the treatment with the highest non-lethal salinity (6.00 ‰ S) were significantly

smaller ($P < 0.01$) and the embryos in the lowest salinity (1.20 ‰) significantly larger ($P < 0.01$) than the embryos in the other treatments.

On experimental day 35, differences between the treatments in relative net production were found using a hierarchical cluster analysis. In experiment A differences were evident between 0.01 ‰ and 0.06 ‰, between 0.06 ‰ and 0.12 ‰ and between 1.20 ‰ and 1.80 ‰ salinity (Fig. 4). In experiment B there were differences between all treatments from 0.01 to 4.80 ‰. No difference was found between the treatments in the range from 6.0 to 12.0 ‰. It became evident from these first two experiments that the effects were expressed up to the 10 th day and did not change qualitatively afterwards. Therefore, the result of an experiment could be estimated by focussing on any single day after the 11 th day of the experiment.

Net production (experiments A-F)

The relative net production of early development of all experiments was calculated and correlated with the salinity of the experimental water (Fig. 5). Differences between the repetitions were caused mainly by differences in the rate of survival. No differences in the relative net production between the replicates incubated at 21° C (experiments C and D) and 24° C (experiments E and F) was found. Over the range from 0.12 and 4.80 ‰ salinity the net production was > 60 . At 6.00 ‰ salinity the net production dropped markedly and at 7.20 ‰ and higher no early *C. taenia* survived. In contrast to this distinct upper limit, the effect of water with very low salinity was less obvious. Instead of a lethal lower limit, there was only an increase of the variability of net production between the experiments. At 0.60 ‰ salinity the net production was reduced slightly but significantly ($P < 0.01$) compared with that at salinities of 0.30 ‰

Discussion

Experiments A and B showed that the main effects of salinity on the early stages of *C. taenia* occurred during the first two weeks of development. A declining sensitivity of fish embryos with increasing age was also reported by SCHÖFER (1979) for *R. rutilus*. HOLLYDAY (1969) suggested the development of the osmoregulatory organs in fish larvae was responsible for the decline of sensitivity.

In contrast to the findings of SCHÖFER (1979) and JÄGER et al (1981) on the cyprinid *R. rutilus*, *C. taenia* did not hatch at earlier developmental stages with increasing salinity. Such an effect may be masked due to the early hatching in *C. taenia* 2.5 days after fertilisation (at 20-24° C) while in roach it takes 10 to 17 days (at 10-14° C, SCHÖFER, 1979). Effects on the time of hatching was observed only in some treatments with lethally high salinities and obviously damaged embryos. In these cases, the hatching was delayed but never accelerated.

Body length was affected significantly by salinity but mainly in the marginal parts of the salinity range tested. However, survival was a more sensitive indicator for effects than was growth.

The combined results from all experiments showed early stages of *C. taenia* to be able to develop under a broad range of salinities. Successful development of at least some eggs was possible between 0.01 and 6.00 ‰ S. Within this range, there was no correlation between net production and salinity: net production is high over the whole range of tolerance. There was a slight but significant ($P < 0.01$) depression of net production at 0.60 ‰ salinity. HEUTS (1947) found a similar, bimodal curve of hatching success in *Gasterosteus aculeatus* L. when eggs were

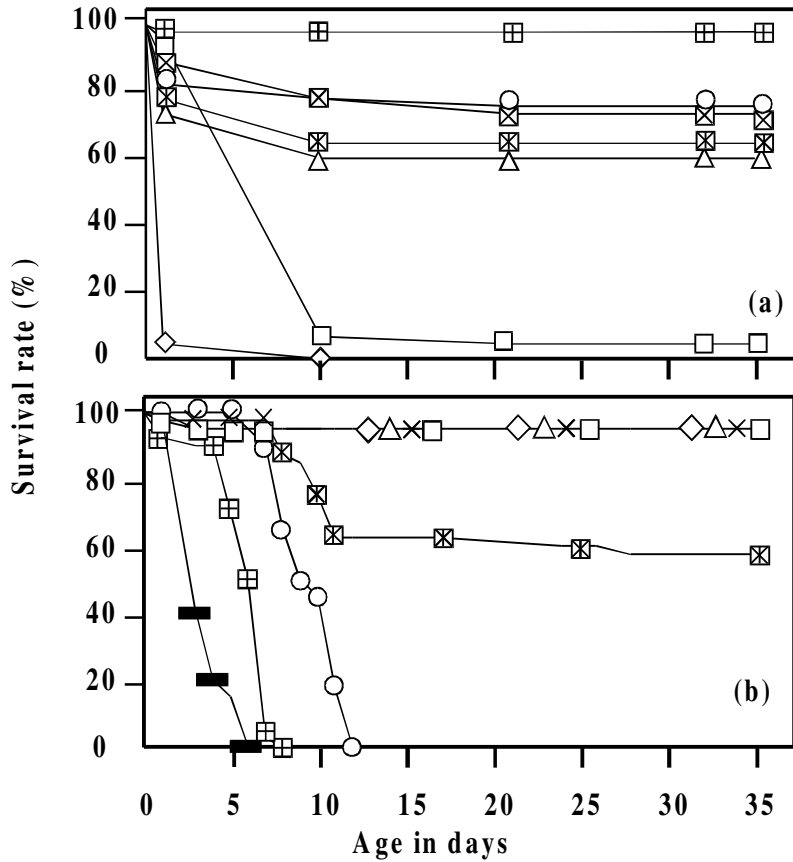


FIG. 1: Survival of early stages of *C. taenia* at different salinity values (a) 0.01 - 1.80 ‰ (b) 1.2 - 12.0 ‰. Nearly all lethal effects were expressed prior to day 13. salinity (in ‰): (a) \diamond , 0.01; \square , 0.06; \triangle , 0.12; \times , 0.30; $*$, 0.60; \circ , 1.20; $+$, 1.80; (b) \diamond , 1.20; \square , 2.40; \triangle , 3.60; \times , 4.80; $*$, 6.00; \circ , 7.20; $+$, 9.00; \blacksquare , 12.00.

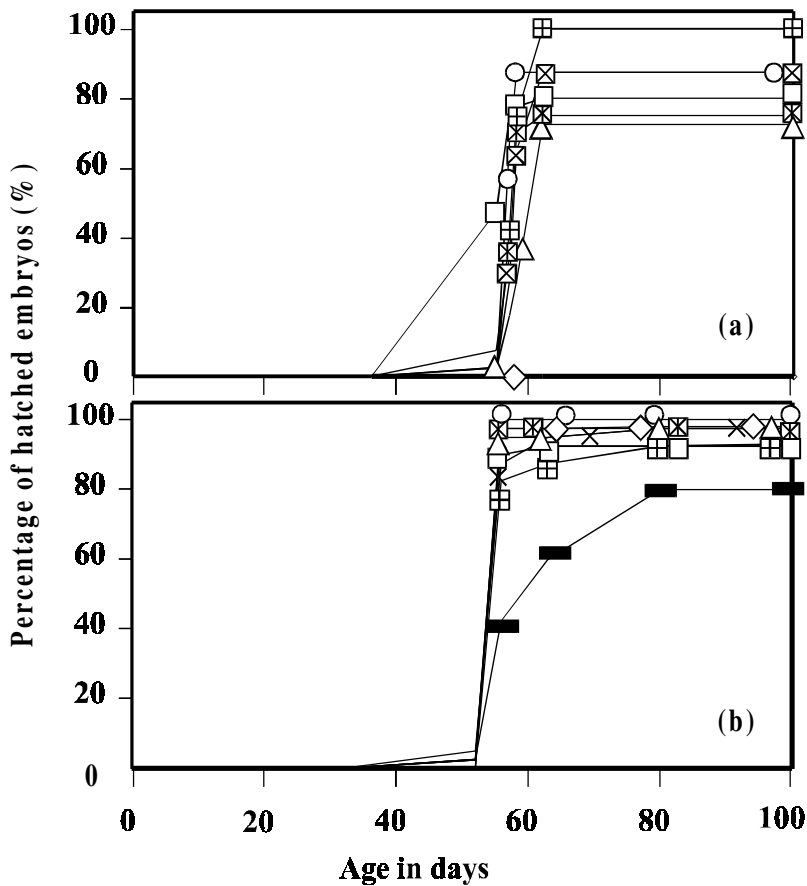


FIG. 2: Time of hatching of *C. taenia* at different salinity values: (a) 0.01 - 1.80 ‰ (b) 1.2 - 12.0 ‰. The treatments with lethal salinities (in experiment A treatments with 0.01 and 0.06 ‰ S, in experiment B with 12.00 ‰ S.) were the only ones which deviate from the main timing. Symbols indicate different salinities as in Fig. 1.

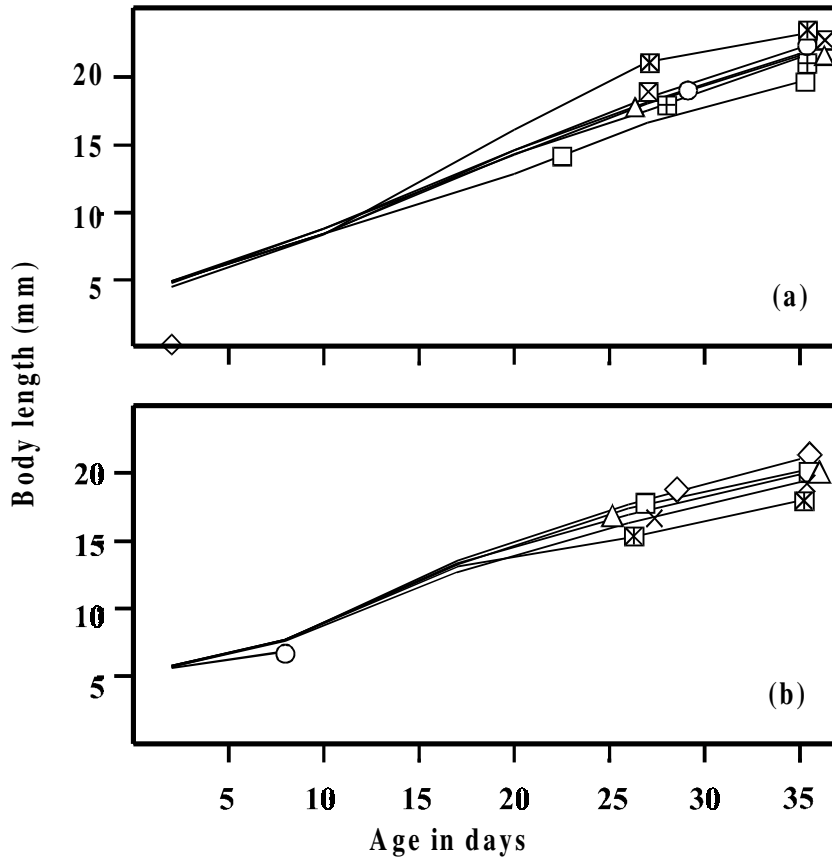


FIG. 3: Length of early stages of *C. taenia* under different salinity values (a) 0.01 - 1.80 ‰ (b) 1.2 - 12.0 ‰ Symbols indicate salinities as in Fig. 1.

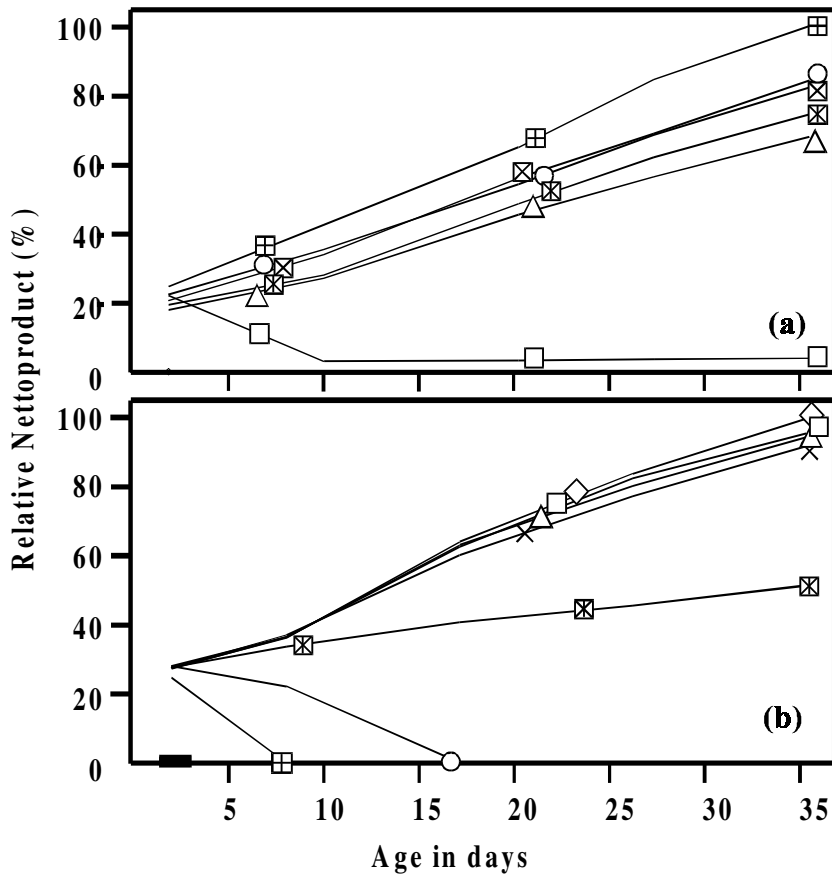


FIG. 4: Relative net production of early *C. taenia* under different salinities during the experiments A and B. Symbols indicate salinities as in Fig. 1.

low salinity may provide a huge potential spawning habitat for the endangered spined loach. Moreover, reproducing populations in the brackish waters may act as a source for colonization of freshwater habitats. Careful field investigations are needed to clarify this hypothesis.

Secondary freshwater fish like burbot, sticklebacks, salmonids, coregonids and percids have higher salinity limits than spined loach (JÄGER ET AL., 1981; WHOOTON, 1984; KLINKHARDT & WINKLER, 1989). Compared with primary freshwater fish, *C. taenia* has rather high limits, similar to the cyprinids carp and bream. Roach is notably less tolerant (JÄGER ET AL., 1981; KLINKHARDT & WINKLER, 1989). Within the percids, coregonids and cyprinids (KLINKHARDT & WINKLER, 1989) as well as in sticklebacks (WHOOTON, 1984) there are obvious strong variations regarding the sensitivity to salinity. This indicates rather stronger species-specific than family-specific adaptations to salinity. CHLEBOVIC (1968, cited by KLINKHARDT & WINKLER, 1989) stated that a salinity of 5 to 6 ‰ acts as a general ecophysiological barrier for the development of freshwater fish eggs. *C. taenia* reaches the upper limit of this range which corresponds to the tolerances of bream, carp and pikeperch, *Stizostedion lucioperca* (L.).

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This article was published in *Journal of Fish Biology*, 55, J. Bohlen, Influence of salinity on the early development in the spined loach, *Cobitis taenia*. 189-198, 1999, and is posted with the permission from Elsevier.