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A note on seasonality in anoxia tolerance of crucian carp (*Carassius carassius* (L.)) in the laboratory

Jorma Piironen & Ismo J. Holopainen

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Young crucian carps from a natural pond were enclosed in jars with anoxic water and the survival times at different temperatures were recorded. Considerable seasonal differences in the survival times were demonstrated.

The year of the crucian carp can be divided into three periods. 1) the *reproductive period* in spring and early summer. This starts from the melting of the ice (in April—May) and during its first three weeks the high tolerance to anoxia is lost. 2) the start of *reserve build up* from late summer to autumn is further characterized by only short survival (approx. 1 day) in anoxia. Then, accompanied by a final accumulation of winter reserves (glycogen), a longer survival time of up to 10 days or more at low temperatures is attained. 3) *wintering* is the period characterized by very long survival times in anoxia.

The tolerance of juveniles (age group 0+) seems to develop a month or two later than that of older fish. The long survival times (up to 4.5 months at +3°C) in winter are suggestive of even longer tolerance to natural anoxia at the low temperatures prevailing in local ponds.

J. Piironen & I. J. Holopainen, Department of Biology, University of Joensuu, Box 111, 80101 Joensuu, Finland.

1. Introduction

The ability to tolerate prolonged environmental anoxia is rare in vertebrates, as recently reviewed by Hochachka (1980) and Hochachka & Somero (1984).

Goldfish (*Carassius auratus* L.), however, are able to live from several days to two weeks without oxygen (Walker & Johansen 1977, van den Thillart et al. 1983) and the anoxic metabolism of this species is most well known (e.g. Shoubridge & Hochachka 1980, 1981, van den Thillart 1982, van den Thillart et al. 1982, 1983). An equal anoxic metabolism has been suggested for its close relative, the crucian carp (*Carassius carassius* (L.)) (Johnston 1975, Johnston & Bernard 1983, Holopainen & Hyvärinen 1985).

After the pioneering works of Blažka (1958, 1960), the tolerance to long-term anoxia of crucian carp was not confirmed until the work of Hyvärinen & Holopainen 1983. In fact recently doubt has been cast on it, e.g. by van den Thillart et al. (1983, p. 299). As part of our attempt to gain a better understanding of the biology of this species (Holopainen & Hyvärinen 1985, Holopainen & Pitkänen 1985, Hyvärinen et al. 1985, Holopainen et al. 1986) we now publish a short note on the seasonal dynamics of anoxia tolerance at different temperatures in the laboratory. As background data, the seasonal dynamics of some other physiological characteristics based on the recent results of our research group are summarized in Fig. 1.

2. Material and methods

Crucian carps were caught with hoop nets (fry <4 cm) or traps (young 6—10 cm) from a natural pond (Hermanninlampi) in the vicinity of the town of Joensuu, eastern Finland (for description of the pond and its fish population see Holopainen & Pitkänen 1985). At each season groups of 1—3 fish of approx. 10 g each were sealed in small Erlenmayer flasks with 300—320 ml pond water (see Fig. 2). The water was deoxygenated by nitrogen bubbling for 20—30 min before, and for 5 min after, the beginning of the test, which lowered the oxygen concentration to about 0.5 mg/l. The bottles were immersed in thermostatically controlled baths and kept in darkness.

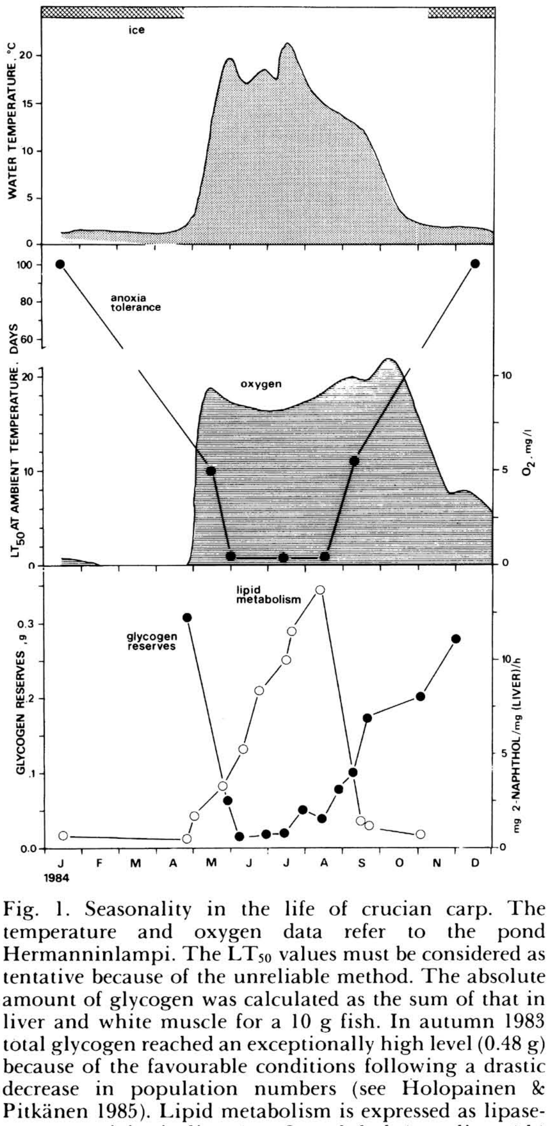

Fig. 1. Seasonality in the life of crucian carp. The temperature and oxygen data refer to the pond Hermanninlampi. The LT_{50} values must be considered as tentative because of the unreliable method. The absolute amount of glycogen was calculated as the sum of that in liver and white muscle for a 10 g fish. In autumn 1983 total glycogen reached an exceptionally high level (0.48 g) because of the favourable conditions following a drastic decrease in population numbers (see Holopainen & Pitkänen 1985). Lipid metabolism is expressed as lipase-esterase activity in liver (mg 2-naphthol / mg liver / h) (Hyvärinen et al. 1985).

Table 1. The LT_{50} values ($\log_e$ days) for crucian carp fry (age group 0+, length less than 4 cm) in their first autumn for comparison with the results for young fish (age group 1+) in Fig. 3. *N* denotes the number of fish at each temperature.

Date	*N*	Temperature (°C)			
		2	5	10	15
15.8	9	–	2.9	3.1	–
23.8–1.9	9	–	3.2	2.4	2.6
13.9	5	–	3.7	4.0	3.7
4.10	5	5.2	4.2	3.5	–

Tests were begun within two days of catching and at least one temperature was within the limits of 2°C of the ambient water temperature. In winter some fishes were kept in 0.5 m^3 laboratory tanks at 2°C for up to two weeks before use. Control groups with 1–3 fish (2–3 replicates at each temperature) in open glass jars with 0.5 l pond water were always used. The mortality of control fishes was approx. 12 % in young fish, 20 % in fry, and randomly distributed among tests and temperatures. The fish were considered dead after the loss of any activity or reaction even after vigorous agitation of the test bottle. The survival times were considered satisfactory for comparative purposes and indicative of natural tolerance even when attained in unnatural conditions.

The data for outdoor temperatures as well as fish for glycogen measurements were collected mainly during 1984 from the same pond (for the methods see Holopainen & Hyvärinen 1985). The material for lipid metabolism is partly taken from Hyvärinen et al. (1985).

3. Results and discussion

The year of the crucian carp can be divided into three main periods: reproduction in early summer, reserve build-up in late summer, and wintering. In eastern Finland the first period starts with the melting of the ice in April–May and is characterized by the use of the winter reserves (Fig. 1), by gonad maturation, and by increasing lipid metabolism (Hyvärinen et al. 1985). The metabolic rearrangement occurs within one month, during which the fish lose their tolerance to anoxia (Figs. 1–2). The rest of this period, up to the end of July, is characterized by periods of reproduction (this species is a fractional spawner) and active metabolism with only low tolerance to anoxia.

Reserve build-up starts with the falling water temperature in August and may last up to late December (Fig. 1 and Hyvärinen et al. 1985). Reserves are stored as glycogen mainly in the liver and white muscles. The liver size increases to up to 12–15 % of the body weight and its glycogen content to 30 % of the fresh weight (Hyvärinen et al. 1985). The absolute amount of glycogen in white muscles is high, too, and may increase up to one third of that in the liver.

At the end of August lipid metabolism suddenly diminishes (Fig. 1) and tolerance to anoxia begins to develop again. The high tolerance appears to be gained later in juveniles than in older fish (Table 1 and Fig. 3). This might be the effect of a later start to reserve build-up owing to the need to grow as large as possible before the hard period of wintering.

Over-wintering is a critical phase in the life of the crucian carp. Many of the small sheltered ponds, commonly inhabited by crucian

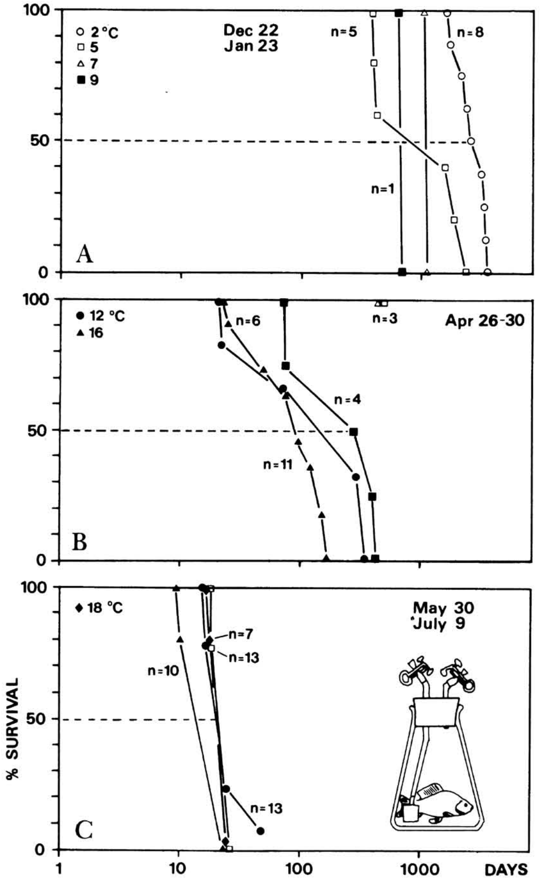

Fig. 2. Seasonal anoxia tolerance of crucian carp at different temperatures. — A. High tolerance in winter. — B. Diminishing tolerance in spring. — C. Low tolerance in summer.

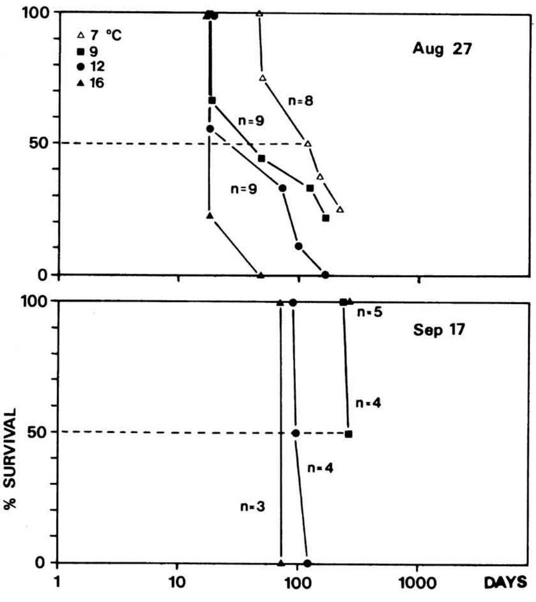

Fig. 3. Developing tolerance of young fish in the autumn.

carp, are ice-bound for about 5—7 months in Finland and suffer from a total oxygen lack for several months (2—3 months in our case). This period is characterized by high tolerance to anoxia (Figs. 1—2) based on the large glycogen reserves, the use of alternative metabolic pathways (with CO_2 and ethanol as the main end products) and low locomotory activity or none at all at the prevailing low water temperatures (Holopainen & Hyvärinen 1985, Hyvärinen et al. 1985, Holopainen et al. 1986).

Our laboratory data indirectly confirm the results of Blažka (1958, 1960), who was the first to observe that crucian carp can survive up to 5.5 months anoxia in natural ponds, and those of Holopainen & Hyvärinen (1985) who report four months winter anoxia in ponds with a large population of crucian carp. In our laboratory tests, with maximum survival times as long as 4.5 months, the fish are considered to be more stressed than in the wild and our results to suggest even longer natural survival. The accumulation of metabolic end products as well as other conditions in the small test bottles must have some adverse effects on fish and tend to lower their tolerance. These effects may become more pronounced in long-term anoxia during the wintertime than in short-term tests in summer, despite the higher temperatures (Fig 2). This is suggested, e.g. by the high ethanol content of water (up to 1 %) and the lowered pH in the long-term bottles (Holopainen et al. 1985). The high control mortality may also explain some of the variation in anoxic capacity (Figs 2—3) and suggest a selective winter mortality caused by prolonged anoxia in the natural environment, too.

The dependence of anoxia tolerance on the temperature is clear (Figs. 1—3) and has been demonstrated earlier for goldfish (Walker & Johansen 1977, van den Thillart et al. 1983) and for crucian carp by Blažka (1958), who reported a tolerance of up to two months at 5°C but less than two days at 15—20°C. The low tolerance of winter fish at high temperatures can be simply due to the inadequacy of the anaerobic energy yield for the unnaturally high demands set by the temperature. In summer the total lack of carbohydrate reserves and the necessary rearrangement of enzyme activity patterns are enough to keep the anoxia tolerance very low.

Information concerning the seasonality of anoxia tolerance in fish is generally lacking. Blažka (1958) reported differences in anoxia tolerance between crucian carp caught in early summer and in late autumn (at 15–20°C and at 5°C the fish lost their balance in 2–3 or 33 hours, respectively). Walker & Johansen (1977) found that winterfish (goldfish) survived better in anoxia than fish kept for three months in the laboratory before the experiments.

The maintenance and development of the amazing capacity to survive for months without oxygen has helped the crucian carp, even as an unsuccessful competitor and vulnerable to predators, to become a ruling species with high densities in an empty habitat island — the numerous seasonally anoxic ponds.

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Errata

Piironen, J. & Holopainen, I. J. 1986: A note on seasonality in anoxia tolerance of crucian carp (*Carassius carassius* (L.)) in the laboratory. —Ann. Zool. Fennici 23:335–338.

Page 336, Table 1: ($\log_e$ days) should read ($\log_e$ hours).
Page 337, Fig. 2 and 3: label on horizontal axis should read HOURS.
Page 337, right column of text: (up to 1 %) should read (up to 1 ‰).