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An Analysis of the Effect of Rearing Temperature on the Prevalences of Myxosporea in Experimentally Infected Common Carp (*Cyprinus carpio* L.)

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Abstract

Experimentally reared 10-day-old common carp (*Cyprinus carpio* L.) were exposed to natural infection by Myxosporea by holding for 14 days in a pond known to be highly infective. They were then incubated in cement tanks at three predetermined temperatures of 25.3 ± 0.4°C, 28.0 ± 0.4°C, and 31.1 ± 0.6°C. Four species of Myxosporea were recovered: *Myxobolus koi* Kudo, 1920; *Myxobolus toyamai* Kudo, 1915; *Myxobolus artus* Akhmerov, 1960; and *Thelohanellus callisporis* Ha Ky, 1971. Results revealed that with the possible exception of *M. toyamai*, which occurred only with extremely low prevalences, all other species developed at all temperatures tested. Prevalences of infection, however, varied with species and rearing temperature, *M. koi* and *T. callisporis* having significantly lower prevalences at higher temperature, whereas prevalences of *M. artus* did not differ significantly among the three temperatures tested.

Introduction

Common carp, *Cyprinus carpio* L., has been reported to harbor a wide variety of parasites (Lom et al. 1976). Some of these may behave as commensals, while others may cause adverse effects to infected fish and be considered as pathogens. In Indonesia, *Myxobolus koi* Kudo, 1920 and *Myxobolus artus* Akhmerov, 1960 have been reported to infect common carp and have caused 90% mortalities among cultured carp fry and fingerlings (Djajadiredja et al. 1983).

tissues were dissected and scanned for cysts under a dissecting microscope. The cysts, if any, were then isolated, and fresh mounts observed under a phase-contrast microscope. In addition, smear preparations of gill and muscle tissues were made and observed for spores under a phase-contrast microscope. Since specific determination of Myxosporea cannot be made from plasmodial stages, fish were only considered infected if spores were observed.

Prevalences of myxosporean infections are calculated from the number of fish infected over the number of fish examined, expressed as a percentage. A randomized complete design was used in this study. Statistical analysis of the data was conducted after arcsin transformation of the raw data to reduce variability (Gomez and Gomez 1976). Analysis of variance was applied to evaluate differences in the prevalences of infection for a given parasite between the rearing temperatures tested. If the tests gave significant value, Duncan's Multiple Range Test was employed to examine for differences between mean levels of infection (see Steel and Torrie 1981).

Results

Examinations showed that the fish were infected by four species of Myxosporea. Two of these, *Myxobolus koi* Kudo, 1920 and *Myxobolus toyamai* Kudo, 1915, infect the gills; one species, *Thelohanellus callisporis* Ha Ky, 1971, infects both gill and muscle; and one species, *Myxobolus artus* Akhmerov, 1960, infects the muscle. The prevalences of infection, and the results of arcsin transformation and statistical analysis are shown in Tables 1 and 2.

Myxobolus koi and *M. toyamai* were not found in fish from some experimental tanks. *Myxobolus toyamai* especially was not encountered in any tank assigned a water temperature of 31°C. Prevalences for *M. toyamai* were extremely low in all tanks, ranging from 0.0% to a maximum of only 6.3%. Prevalences for *M. koi* ranged from 0.0% to 73.3%. *Thelohanellus callisporis* and *M. artus,* on the other hand, were found in all experimental tanks, their range of prevalences being 23.3%-63.3% and 33.3%-80.0%, respectively.

The prevalences of *M. koi* and *T. callisporis* were different for each assigned temperature, being higher at low temperatures ($P < 0.05$). The prevalences of *M. artus,* on the other hand, were not significantly different for all three temperatures ($P > 0.05$).

So far no chemotherapeutic agents have been proven to be effective against myxozoan infections. At present, the only way to prevent the infection of fish by Myxosporea is through rearing uninfected fish in clean water (see Bower and Margolis 1985). Dana (1988) observed that the prevalence of *M. koi* in a place with a relatively high temperature was lower than that occurring at a place with lower temperature. No experimental evidence, however, is available to support this observation.

This paper presents the results of a controlled experiment to determine the effect of rearing temperature on the prevalences of myxosporean infections in common carp.

Materials and Methods

Ten-day-old, myxosporean-free common carp fry were used as source material for this experiment. Eggs, obtained by artificial fertilization, were hatched in glass aquaria which were supplied with recirculated water. The aquaria were disinfected with 50 ppm $KMnO_4$ before being used. Two days after hatching, the fry were fed a suspension of soybean mixed with vitamin B complex and nauplii of *Artemia*.

Transmission of myxosporean infections was carried out by stocking 23,000 fry of size 0.7 ± 0.1 cm in an earthen pond which was previously shown to harbor fish highly infected with these parasites. Tubificids, which have recently been suggested as intermediate hosts of Myxobolidae (see Markiw and Wolf 1983) were also present in the pond. Fish were exposed in the pond for 14 days, during which they were fed fine rice bran.

After exposure, the fish were incubated in nine concrete tanks at temperatures of 25.3 ± 0.4°C, 28.0 ± 0.4°C and 31.1 ± 0.6°C, each temperature being run in triplicate. These temperatures represent the average water temperatures in low-, medium-, and highland areas of West Java in which common carp are cultured. To maintain the water temperatures, immersable automatic water heaters, set at the predetermined temperatures of 25°C, 28°C, and 31°C, were used. A total of 150 fish were stocked into each tank. During the incubation period of 50 days the fish were fed crushed pelleted feed.

Following incubation 90 fish were sampled from each treatment. Examinations were carried out on fresh preparations and concentrated on the gills and musculature. The gills and muscle

Table 1. The prevalences of four myxosporeans in common carp (*Cyprinus carpio* L.) reared at different temperatures.

Myxosporea	Temperature[1] (°C)	Replicate 1	2	3	Average
Myxobolus koi	31.1 ± 0.6	0.0	6.7	3.3	3.3
	28.0 ± 0.4	3.3	3.3	13.3	6.6
	25.3 ± 0.4	13.3	73.3	50.0	45.5
Myxobolus toyamai	31.1 ± 0.6	0.0	0.0	0.0	0.0
	28.0 ± 0.4	3.3	6.7	0.0	3.3
	25.3 ± 0.4	3.3	3.3	0.0	2.2
Thelohanellus callisporis	31.1 ± 0.6	23.3	26.7	23.3	24.4
	28.0 ± 0.4	43.3	36.7	36.7	38.9
	25.3 ± 0.4	46.7	53.3	63.3	54.4
Myxobolus artus	31.1 ± 0.6	50.0	46.7	63.3	53.3
	28.0 ± 0.4	33.3	53.3	66.7	51.1
	25.3 ± 0.4	73.3	80.0	56.7	70.0

[1] n=50 for all temperature measurements.

Table 2. Arcsin transformation of prevalences and the results of statistical analysis of the treatment of three myxosporean infections.

Myxosporea	Temperature[1] (°C)	Arcsin y 1	2	3	Average	Significance[2]
Myxobolus koi	31.1 ± 0.6	0.6	15.0	10.5	8.7	F = 6.24
	28.0 ± 0.4	10.5	10.5	21.4	14.7	P < 0.05
	25.3 ± 0.4	21.4	58.9	45.0	41.8	
Thelohanellus callisporis	31.1 ± 0.6	28.9	31.1	28.9	29.6	F = 24.44
	28.0 ± 0.4	41.2	37.3	37.3	38.6	P < 0.05
	25.3 ± 0.4	43.2	46.9	52.7	47.6	
Myxobolus artus	31.1 ± 0.6	45.0	43.1	52.7	46.9	F = 1.97
	28.0 ± 0.4	35.4	46.9	54.7	45.6	P > 0.05
	25.3 ± 0.4	58.9	63.4	48.9	57.1	

[1] n = 50 for all temperature measurements
[2] $F_{0.05(1),2,6} = 5.14$

The water quality parameters during the experiment are presented in Table 3.

Discussion

This study has demonstrated that increased rearing temperature results in decreased prevalences of infection for two *(M. koi* and *T. callisporis)* of the four species of Myxosporea tested. Prevalences of one species *(M. artus)* appear to be independent within the range of

Table 3. Water quality in transmission pond and incubation tanks.

	Temperature (°C)	DO (ppm)	pH
Transmission pond	23.9 ± 3.2 (n=8)	5.9 ± 0.7 (n=8)	8.1 ± 0.2 (n=8)
Incubation tanks	1. 25.3 ± 0.4	6.1 ± 0.3	7.4 ± 0.5
	2. 28.0 ± 0.4	5.8 ± 0.2	7.4 ± 0.4
	3. 31.1 ± 0.6 (n=50)	5.6 ± 0.1 (n=8)	7.6 ± 0.4 (n=8)

temperatures studied, while prevalences of a fourth species *(M. toyamai)* were too low to permit analysis.

The effects of water temperature on the development and growth of a number of fish parasites have well been documented (Weber and Knispel 1977; Moller 1978; Ratliff 1983; Woo et al. 1983; Ching and Munday 1984). Schafer (1968) considered that infections of *Ceratomyxa shasta* Noble, 1950, a myxosporean parasite of salmonids, occur only at temperatures above 10°C. Ching and Munday (1984), however, found that infectivity of *C. shasta* remained at 4-6°C, and its prevalence of infection could reach up to 90%. It seems that infections of *C. shasta* can develop over a wide range of natural temperatures. The same is true for *M. koi, T. callisporis* and *M. artus,* as indicated by the present study. Whether development of *M. toyamai* can occur in fish reared at 31°C is not clear, since prevalences of infection at other temperatures were extremely low (Table 1).

The effects of temperature could be manifested directly on the growth and development of the parasites or indirectly, through enhancement of the host's defence mechanisms (Lom 1973; Woo et al. 1983). Woo et al. (1983) have suggested the absence of the flagellate *Cryptobia salmositica* Katz, 1951 in trout maintained at 20°C to be a direct effect of temperature on the survival of the parasite, since *C. salmositica* is active and infective at 5-10°C. It hard to say what mechanisms are behind the differences in prevalences of *M. koi* and *T. callisporis* in the present study, since the initial doses of infection and the course of development of these parasites during the experiment were not determined. Further studies should be carried out to clarify the exact mechanisms by which temperature affects the development of these species. The use of temperature as a means to control myxosporean infections in fish culture facilities is promising and deserves further exploration.

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