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Field and laboratory investigations of the thermal influence on tissue-specific Hsp70 levels in common carp (*Cyprinus carpio*)

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Abstract

Thermal discharge from power stations can affect normal environmental conditions and change in heat shock proteins expression of native fish with increasing temperature. In this study, we investigated levels of Hsp70 in the heart, kidney, brain and gill of the common carp *Cyprinus carpio* both in long-term heat discharge environment and after 24 h acute heat shock exposure. In laboratory exposure experiments, fish acclimated at 10 °C were exposed to various elevated temperatures (20, 24 and 28 °C). Hsp70 concentrations were determined in tissues by Western blotting analysis after one dimensional SDS-PAGE separation. In the field study, the level of Hsp70 in the gill of the carp remained at control values, and Hsp70 expression in the heart, kidney and brain underwent a 2.8 to 3.7-fold increase. A lower thermal sensitivity of the Hsp70 response of the brain, compared with the heart, kidney and gill, was observed in the laboratory experiments. Our data show that these tissues had different levels of Hsp70 responses to thermal influence both in acute exposure and long-term acclimation. The pattern of tissue Hsp70 expression may have a close relationship with the thermal tolerance of the carp and allows the fish to survive long-term thermal pollution.

Keywords: Biomarker; Common carp; Fish; Heat stress; Hsp70; Thermal pollution

1. Introduction

Temperature has been recognized as an important factor influencing biological systems at numerous levels of organization (Selvakumar and Geraldine, 2005). Many eurythermal fish have evolved a number of behavioural, biochemical and molecular mechanisms to assist in their continuous adjustment to naturally occurring temperature variations (Hochachka and Somero, 2002). When the water used to cool power plants is discharged into streams, rivers, and lakes, the addition of heat could stress biota residing in the receiving waters including macrophytes, phytoplankton, zooplankton, insects and fish. This is known as thermal pollution or thermal discharge, which can affect native fish species and cause a wide range of impacts on the aquatic ecosystem (Lardicci et al., 1999).

Recently, several studies into the effects of thermal discharges on heat shock response of fishes have primarily focused on changes in heat shock or stress protein (Hsps) expression with increasing temperature. As molecular chaperones to bind to folding intermediates and prevent misfolded proteins from irreversible denaturation, Hsps are a super-family of highly conserved intracellular proteins in response to various stressors including heat shock, hyperthermia exposure (Feder and Hofmann, 1999; Hamer et al., 2004). Of all of the Hsps families that have been studied, the 70 kDa protein family (Hsp70) has been most widely used as a biomarker due to its rapid and significant increase during a wide range of environmental stressors (Jonsson et al., 2006). Tissue-specificity of Hsp70 proteins was shown to occur both in vertebrates and invertebrates. In fish, a number of studies have demonstrated that the expression of Hsp70 is quite variable in different types of tissues following heat shock exposure (Dyer et al., 1991; Wood et al., 1999; Cara et al., 2005).

Abbreviations: Hsp70, 70-kDa heat shock protein; HS, heat shock; HSR, heat shock recovery; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

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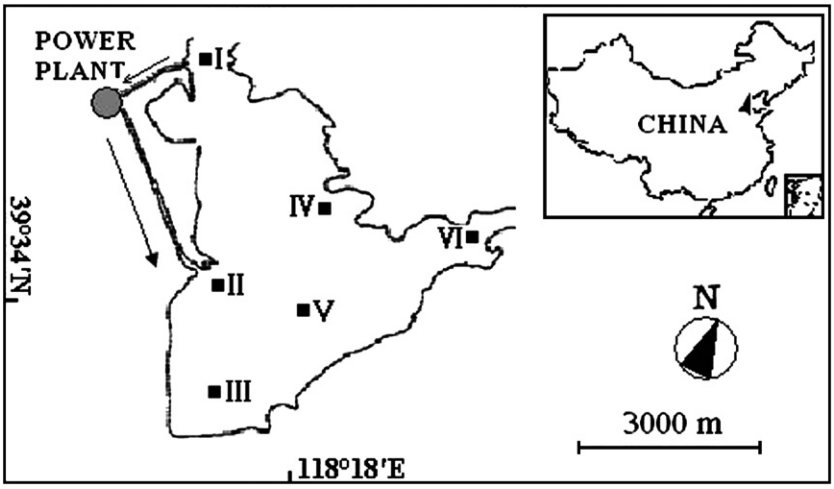

Fig. 1. Map of the Douhe Reservoir, receiving heated effluents by Tangshan power plant, located in the Hebei Province of China. Carp were collected in site V and the physico-chemical characteristics of sites I–VI were determined in Table 1.

Although an elevated level of Hsp70 has previously been reported in teleosts as a result of adaptive heat shock response (Jonsson et al., 2006), little is known about the effects of different temperatures on the expression of Hsp70 in tissues of the common carp *Cyprinus carpio* (De Wachter et al., 1998). *C. carpio* is one of the most economically important freshwater fish in North China and is widely aquacultured all over Asia, in most parts of Europe and on a small scale in some countries of Africa and Latin America (Aydín and Köpücü, 2005). In this study, the common carp was utilized to determine that field long-term thermal influence and *in vivo* 24 h acute heat shock induce Hsp70 expression in the heart, kidney, brain and gill. The area under our field investigation was the Douhe Reservoir, receiving heated effluents from the Tangshan power plant, located in the Hebei Province of China. The focus of the present study is on the differences in the induction level of Hsp70 in the various tissues of common carp by heat stress under field and laboratory conditions. Furthermore, we expected that this fish could be useful models in linking the ecological and physiological aspects of the thermal biology of fish.

2. Materials and methods

2.1. Field study

The Tangshan power plant began operating in 1976 and now uses 165 m^3/s of water from the Douhe Reservoir with a total area of 17.8 km^2 for cooling (Fig. 1). The thermal effluent produces a mean increase of 4 °C in the Douhe Reservoir, and the highest water temperature was measured in July of the year. The space distribution of water temperature in the three dimensions is homogeneous, so it is obvious that carp were exposed to thermal stress. In July 2006, adult common carp (*C. carpio*, 29–40 cm in length and 600–800 g in mass) were collected from the Douhe Reservoir. We also obtained some carp as controls from an aquaculture facility near the Douhe Reservoir. Fish were dissected and samples of each tissue, including heart, kidney, head and gill, were taken, immediately frozen in liquid nitrogen in the field, and stored at−80 °C for later Hsp70 analysis. The water quality in six representative sites of the reservoir was measured during sampling (Table 1). All physico-chemical parameters of the reservoir, determined following the techniques of APHA (1992) and using analytical-grade reagents, measure up to the fishery water standard of PR China (GB 11607-89).

2.2. Heat shock exposure in the laboratory

2.2.1. Fish acclimation

Carps (weighing about 700 g) were obtained from the same aquaculture of the field control fish, when the water temperature of this aquaculture was about 10 °C. All fish were kept at 10 °C for at least one week prior to the experiments into aerated tanks with a 12 h dark/12 h light photoperiod and fed

Table 1
The physico-chemical characteristics of each representative site in the Douhe Reservoir in July 2006. Sites I–VI within the Douhe Reservoir are shown in Fig. 1

Sample site	I	II	III	IV	V	VI
Water temperature (°C)	30.8	37.3	32.9	31.2	32.1	30.1
pH	8.56±0.03	8.82±0.06	8.67±0.02	8.63±0.04[a]	8.81±0.02[a]	8.40±0.01[a]
Dissolved oxygen (mg L^{-1})	7.18±0.33	6.26±1.43	6.81±0.08	7.11±0.37[a]	6.81±0.38[a]	7.39±0.65
Alkalinity (as mg L^{-1} $CaCO_3$)	65.9±8.27	58.1±11.02	68.3±7.89	62.9±3.80[a]	68.1±4.61[a]	65.7±2.42[a]
Hardness (as mg L^{-1} $CaCO_3$)	98.9±6.12	97.6±9.33	101.3±8.12	105.2±10.1[a]	109.5±1.28[a]	89.6±6.23[a]
Total nitrogen (mg/L)	1.26±0.11	1.14±0.18	1.42±0.08	1.16±0.02	1.18±0.20	1.45±0.15
Total phosphorus (mg/L)	0.017±0.003	0.019±0.005	0.020±0.001	0.022±0.008	0.016±0.003	0.022±0.006

Water quality data are means±SEM of triplicate samples.