ON THE CELLULAR RESPIRATION OF SOME CULTURE CYPRINIDS EXPOSED TO WINTERING

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Key words: fish, wintering, cellular respiration, gills, muscle

Abstract: The influence of the wintering conditions on the intensity of cellular respiration was followed on three fish (cyprinids) species, namely: common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*). The intensity of the cellular oxygen consumption was determined by the Warburg microrespirometric method, both in gills and muscles, prior to (beginning of December) and after the winter stay (end of March). Certain variations in the intensity of the cellular respiration were recorded, as a function of fish species, type of cells, season and duration of observation. It was noticed that, generally, in December, the intensity of cell oxygen consumption takes higher values in the common carp, comparatively with the other species, as well as in gills, *versus* muscle. At the same time, under the influence of the low temperature and of the other conditions specific to the cold season, some reduction in the intensity of cell respiration may be noticed, in both tissues and in all three fish species, which is nevertheless more intense in the case of common carp so that, by March, it records lower values than the other two Asian species, situation which may be correlated with their different mode of living and their different energetic requirements.

INTRODUCTION

The low winter temperatures influence in a characteristic manner the metabolic processes developed in fish, inducing mainly more reduced energetic requirements, diminution of active feeding and of the movements in water [Ekberg, 1958; Fernandes *et al*., 2007; Misăilă *et al*., 2004, 2005; Perry et Tufts, 1998]. In the winter time, culture cyprinids are usually transferred from their growing basin in wintering basins, which are smaller and deeper, a situation which brings about – apart from the known advantages – the so-called supra-density stress, besides the hypothermic one [Misăilă *et al*., 2004, 2005]. Consequently, in view of reducing such negative effects, adequate measures should be necessarily taken, such as water aeration (by opening holes, in the ice covering the basin) and other specific technological works.

Numerous investigations have been devoted to the influence exercised by the modification of temperature and of other elements (such as inhibiting and toxic agents, pollutants etc.) on respiration processes, permitting to evidence a series of specific effects, manifested of both cellular and global level, under the experimental conditions applied, in other fish species, as well [Brauner et Berenbrink, 2007; Coolidge *et al*., 2008; Ekberg, 1958; Fernandes *et al*., 2007; Giulivi *et al*., 2006; Guderley, 2004; Perry et Tufts, 1998].

For better evidencing the influence of the winter conditions – the low temperature, especially, – on fish respiration, the present paper discusses the intensity of cell respiration in three species of culture cyprinids – an autochtonous and two Asian ones – kept in wintering basins, during the 2006-2007 cold season.

MATERIALS AND METHOD

Three one summer-old (0_+) fish species have been employed for the study: common carp (*Cyprinus carpio*) and two Asian cyprinids - silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), all kept in the wintering basins of the Ţigănaşi Fish Breeding Farm, the district of Iaşi. For each species, two batches, each of 5-7 individuals have been selected for the study, one in the beginning of winter (December 11-12, 2006) and the other in the end of the cold season (March 25-26, 2007), the cellular oxygen consumption being determined for each case in part at the level of gills and striated muscle, by the Warburg micromanometric method [Nuţă et Buşneag, 1977], recording being performed for 60 minutes, at intervals of 15 minutes each.

The values thus obtained, expressed as µL/g fresh tissue, were calculated statistically (Student test) and percentually, the data recorded in March being correlated with those registered in December (taken as 100%).

RESULTS AND DISSCUSION

The results of the investigations show that the intensity of cellular oxygen consumption records different average values, as a function of the fish species, type of cells, season and duration of recordings (Figs. 1 - 3).

The observation is therefore made that, for all fish species considered and for both cells types (gills and muscle), the oxygen consumption takes increasing values for 60 minutes, while the growth ratio from one 15 minutes interval to another is constantly decreasing (Figs. 1 - 3). Such a reduction in the intensity of cell respiration during *in vitro* determinations may be also explained by the gradual decrease of the available amounts of oxygen from the closed respiration vessels, in which the tissue samples occur, to which a diminished metabolic substrate of the cells isolated from the organism, and the accumulation of some cell metabolism residues contributing to the inhibition of aerobe respiration should be added [Hăulică, 2007; Karp, 1996; Lehninger, 1987].

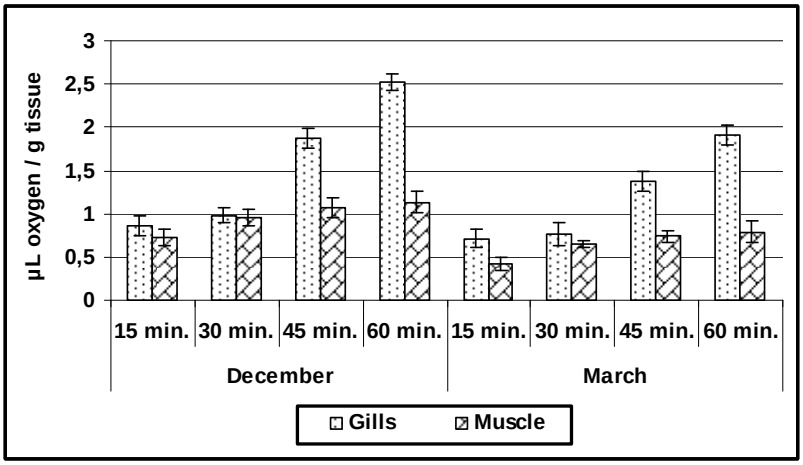

Fig.1. The cellular respiration (µL O_2/ g tissue) in branchial and muscular tissue of common carp, before and after wintering

As to the results recorded in the three species, mention should be made of a different intensity of cell respiration, as a function of the both species and season. A first observation refers to the fact that the values recorded in gills are generally higher than in the muscle, both prior to and after the winter stay (Figs. 1 - 3).

Consequently, prior to wintering, the respiration consumption of oxygen in the branchial cells records, after 60 minutes, the highest values in the common carp (2.51 µL/g fresh tissue), followed by bighead carp (2.31 µL/g fresh tissue) and silver carp (2.11 µL/g fresh tissue). In the muscle, the values are lower, their decreasing order being also different: the highest values are recorded in silver carp (1.21 µL/g fresh tissue), followed by common carp (1.13 µL/g fresh tissue) and bighead carp (1.02 µL/g fresh tissue).

When winter is over, a considerable reduction may be noticed in the intensity of cell oxygen consumption, after 60 minutes, especially, on common carp and silver carp, and in both types of cells (Tables 1 and 2, Figs. 1 - 3). The most pronounced decrease is nevertheless noticed in the common carp, especially in the muscle cells – namely 69.63%, comparatively with the December values, in muscle, and 75.99%, in the gills, respectively, the March values recorded in the silver carp being of 86.22% in muscle and of 95.13%, respectively, in the gills, *versus* the ones found in December.

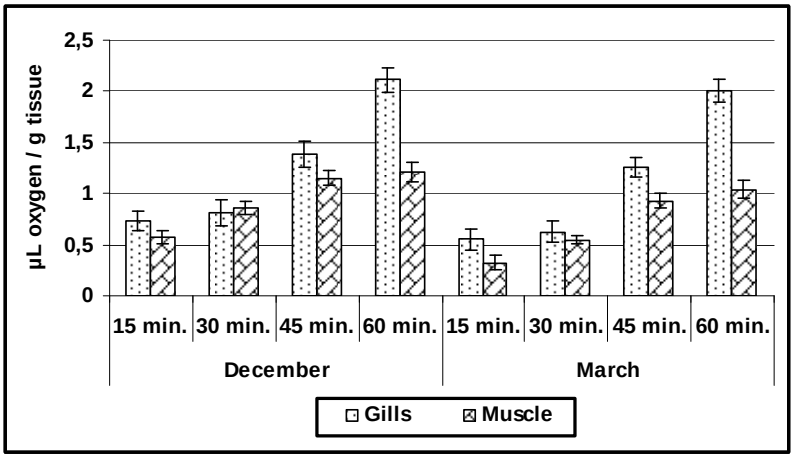

Fig.2. The cellular respiration (µL O_2/ g tissue) in branchial and muscular tissue of silver carp, before and after wintering

In the case of the bighead carp, however, only minor modifications may be observed in the intensity of cell respiration after the winter stay, comparatively with the values recorded in December, in both types of cells (Tables 1 and 2, Figs. 1 - 3). Quite interestingly, after 60 minutes a slight intensification in the intensity of branchial cell respiration may be recorded in the bighead carp, comparatively with December measurements (103.08%) while, in the muscle cells, the values are not modified (100.01%).

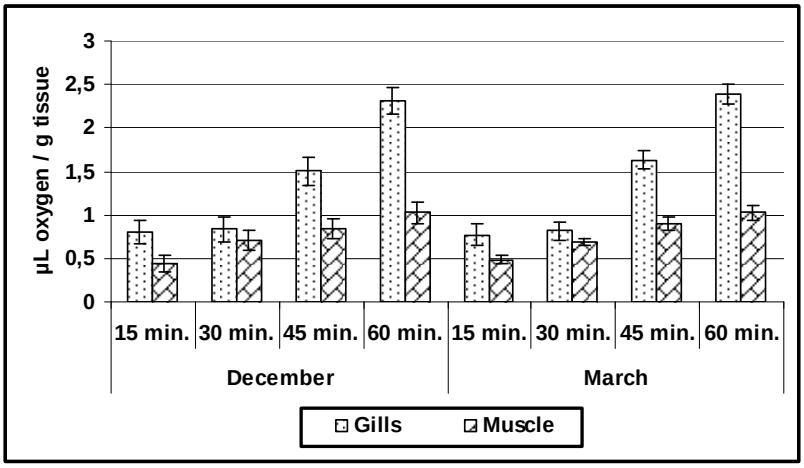

Fig.3. The cellular respiration (µL O_2/ g tissue) in branchial and muscular tissue of bighead carp, before and after wintering

Table 1. The cellular respiration (%) in branchial tissue of fish, before and after wintering

Species	15 minutes		30 minutes		45 minutes		60 minutes	
	Dec.	Mar.	Dec.	Mar.	Dec.	Mar.	Dec.	Mar.
Common carp	100%	82.28%	100%	77.59%	100%	73.48%	100%	75.99%
Silver carp	100%	75.42%	100%	77.24%	100%	90.83%	100%	95.13%
Bighead carp	100%	96.29%	100%	97.91%	100%	108.57%	100%	103.1%

All these data put into evidence a series of clear-cut differences among the three fish species as to the intensity of the respiratory processes, both prior to and after the winter stay, which may be probably explained by the different feeding regime, behavior, metabolic characteristics and morpho-physiological peculiarities of the two types of cells [Brauner et Berenbrink, 2007; Fernandes *et al*., 2007]. Thus, the intensity of respiratory oxygen consumption, more pronounced in the branchial cells than in the muscular ones, may be the results of the branchial morpho-physiological characteristics.

Table 2. The cellular respiration (%) in muscular tissue of fish, before and after wintering

Species	15 minutes		30 minutes		45 minutes		60 minutes	
	Dec.	Mar.	Dec.	Mar.	Dec.	Mar.	Dec.	Mar.
Common carp	100%	52.12%	100%	67.93%	100%	69.38%	100%	69.63%
Silver carp	100%	56.01%	100%	63.63%	100%	80.56%	100%	86.22%
Bighead carp	100%	109.9%	100%	97.25%	100%	107.73%	100%	100.01%

As specialized organs assuring the exchange of respiratory gases (O_2 and CO_2) in aquatic organisms the gills have a morphological structure adapted to such functions. Consequently, their cells appear as lamellae arranged on bony arches, which assure a large surface contact between the external aqueous environment and the intracellular one, by means of cellular membranes, known as highly permeable to gases. At the same time, the branchiae are characterized by a continuous activity, and have an extended vascularization, all these aspects assuring an intense cell respiration [Brauner et Berenbrink, 2007; Ekberg, 1958; Fernandes *et al*., 2007; Perry et Tufts, 1998].

As to the differences to three cyprinids species, regarding the intensity of cell respiration and the respiratory response to the low temperature of the winter season, one should consider the different biological characteristics of these fish species, which – as known – belong to the two different categories. Thus, the common carp is an autochtonous species with an omnivorous feeding regime, more attached to the bottom area of the aquatic basin, which restricts its movements to some extent and assures a physiological equilibrium specific to this life regime, as

also reflected in the respiratory processes, firstly in a more intense reduction of cell respiration during wintering.

The silver carp and the bighead carp are worm water species, originating from South-East Asia. They feed themselves with phyto- and zooplankton, for the filtering of which they make more active movements in water, even in winter, which requires a higher energetic level and, consequently, relatively more intense oxygen consumption – as actually evidenced by the results of the present investigation, especially in the intensity of branchial cell respiration.

The cell oxygen consumption represents the aerobe cellular respiration involving a series of processes developed at mitochondrial level, representing the Krebs cycle, correlated with oxidative phosphorylation, ATP production and cellular energogenesis [Alberts *et al*., 1998; Ekberg, 1958; Karp, 1996; Lehninger, 1987; Perry and Tufts, 1998]. The various studies approaching the influence of temperature on the respiration processes of fish showed that a lower temperature causes a series of morpho-functional changes in the mitochondria, such as: modifications in the number and shape of the crystae from the internal membrane, and in the membranary polynonsaturated phospholipids, the occurrence of some reactive oxygen species (ROS), as a response to thermal stress, modification of some membrane proteins (thermal stress proteins) and the production of nitric oxide (NO) by means of the NO-syntase enzyme, all these leading to the inhibition of the mitochondrial respiratory processes at low temperatures, which has been also put into evidence by the results here presented.

CONCLUSIONS

The wintering conditions influenced the respiratory processes of the three fish species, determining certain variations in the intensity of cellular oxygen consumption, as a function of fish species, type of cells, season and duration of observation.

Generally, in December the intensity of cell oxygen consumption is higher in common carp, comparatively with the Asian carp, as well as in gills *versus* muscle.

Under the influence of the low temperature, some reduction in the intensity of cell respiration take placed in both tissue and in all three fish species, which is nevertheless more intense in the case of common carp, so that by March, it records lower values than the two Asian species.

The observed modifications are neither extremely ample nor irreversible, their character being rather an adaptive one.

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