

Effect of freezing on the metabolism of L3 larvae *Anisakis simplex* s.s.

Elżbieta Łopieńska-Biernat[1], Robert Stryiński[1], Iwona Polak[1], Bogusław Pawlikowski[2], Joanna Pawlak[2], Magdalena Podolska[2]

1 Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 1A, 10-719 Olsztyn, Poland; 2 Department of Fisheries Resources, National Marine Fisheries Research Institute, Kołłątaja 1, 81-332 Gdynia, Poland

Corresponding author: Elżbieta Łopieńska-Biernat; e-mail: ela.lopienska@uwm.edu.pl

Human fishery product-borne parasitic diseases primarily include those caused by cestodes, trematodes and nematodes. The only parasite in fishery products that is implicated in allergic reaction, except an infection following ingestion of viable parasites, is cosmopolitan nematode *Anisakis simplex*. The hypersensitivity episodes can not only be elicited by infection but also by exposure to allergens remaining in food with no live larvae. The fish-borne parasite, *A. simplex* s. s., triggers a disease called anisakiasis, that is associated with a gastrointestinal infection. The Anisakis is also associated with allergic response which may lead to anaphylactic shock. With the assumption that the *A. simplex* L3 larvae may be freeze tolerant we have generated sets of transcripts expressed in that parasite, when the nematodes have been cooled rapidly to -20°C according to the sanitary authorities of the USA and the EU. The aim of this work was to study metabolic status of *A. simplex* L3 larvae when frozen in terms of viability, expression of genes involved in the nematodes' survival of freezing, as well content of carbohydrates which play a cryoprotective role in thermal stress and are the main source of energy.

For this purpose, qReal-time PCR and HPLC analyzes were done, as well the main factors affecting the survival of larvae during a freezing process, the in homogeneity of temperature in freezers and freezing rate, have been studied. The levels of trehalose were significantly higher after slow freezing treatment ($p < 0.0001$), than the fast freezing ($p < 0.002$). The lower temperatures induce changes, especially in synthesis trehalose gene expression/content (trehalose-6-phosphate synthase (tps), heat shock cognate protein 70 (hsc 70), heat shock protein 90 (hsp 90), genes responsible for oxidative metabolism, superoxide dismutase Cu-Zn (sod), glutathione s-transferase (gst), were up-regulated. When compared to the control treatment (larvae cold acclimated for 24 h at 4°C in 0.9% NaCl), and when compared to the expression levels after slow and/or fast freezing experiment. We cannot state clearly whether these changes occur during freezing, or because they are already prevalent during cold acclimation. The induction of mentioned genes seems to be a common trait of both cold- and dehydration tolerance.

The metabolic status of L3 *A. simplex* s. s larvae is affected by cold-treatment not only in term of desiccation and freezing stresses which appear together, but also because of the composite mechanism by which, parasitic nematodes regulate their development in complex life cycles. Survival strategies of fish-borne parasite, like *A. simplex*, should be studied in more detail to

better aid the efforts towards eradication of human anisakiasis and improvement of the fish product safety.

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