## <u>In Memoriam</u>

## Karl Erik Zachariassen 1942-2009: Cryobiologist and ecophysiologist

Karl Erik Zachariassen died on 11 December 2009 in Trondheim, Norway at the age of 67. We have lost one of the most innovative scientists within the field of low temperature biology. His studies in insect cold hardiness revealed several basic physiological principles.

Karl Erik Zachariassen graduated from the University of Oslo with an MSc degree in zoophysiology in1972. With a Fulbright Scholarship he subsequently worked for two years with Ted Hammel at the Scripps Institute of Oceanography in California. They discovered that the haemolymph of freeze tolerant tenebrionid beetles contained nucleating agents. For this reason, freezing is extracellular and lethal intracellular freezing is avoided. In contrast, Zachariassen found that freeze avoiding species lacked such nucleating agents.

Following his return from Scripps, Zachariassen became an Associate Professor at a teacher training college, which was later incorporated into the Norwegian University of Science and Technology at Trondheim. Zachariassen obtained his Norwegian Dr. Philos. degree in 1980. He became a full Professor in 1988, and remained in this position until his death.

In his further work on insect cold hardiness, Karl Erik Zachariassen demonstrated the colligative effect of polyols in freeze-tolerant beetles. He was also among the first scientists to demonstrate the presence of antifreeze proteins (AFPs) in freeze susceptible species. AFPs are of fundamental importance for supercooling, and prevent ice formation by adhering to embryo ice crystals. He demonstrated that in the snow scorpion *Boreus westwoodi* inoculative freezing is prevented by AFPs when the insect walks on the snow surface at freezing temperatures. He also showed that the effect of AFPs increase with polyol concentration, and suggested that AHPs stabilize the supercooled insect over its entire supercooled range. More recently, Zachariassen and his co-workers isolated and sequenced antifreeze protein from the haemolymph of larvae of the longhorn beetle *Rhagium inquisitor*. These AFPs are more active than previously known insect AFPs, and their amino acid composition is different.

Cold exposure and freezing affects ionic gradients in physiological solutions. Zachariassen and his collaborators showed that supercooled insects maintain their ionic gradients, while ions are lost from the cells in frozen species but replaced quickly during thawing.

A step from cold to heat may be shorter than expected. Through his expeditions to Kenya, Zachariassen became interested in the water balance of desert beetles. In the tenebrionid *Phrynoculus petrosus*, the main water loss is through the spiracles during respiration, and very little through the cuticle or faeces. Zachariassen characterized the adaptations of the tenebrionids as a "water saving physiological compromise". He also discovered that carabid beetles living in arid habitats have higher rates of metabolism and water loss.

In addition, Zachariassen also led a research group in ecotoxicology. They studied the effects of oil and organic chemicals on sodium and calcium in muscles and tissues of marine mussels. Their results were important for monitoring pollutants in the natural environment. They also found a relation between the presence of metallothionein, a detoxifying protein, in the gills of trout and the concentration of organic metal ions in river water.

Publications from Zachariassen and co-workers included 16 book chapters and reviews and 87 original scientific papers. Some recent references are given below. The research results of Karl Erik Zachariassen were highly original and penetrating and the references below are representative of the scope of his work. His publications contributed to a basic understanding of the adaptation of terrestrial arthropods to cold and desiccating conditions. His results are frequently cited and he was a popular lecturer at international symposia and conferences. The "International Symposium on Insect Cold Hardiness" was founded by him and his co-workers in 1980 and survives to this day as part of ISEPEP (International Symposium on the Environmental Physiology of Ectotherms and Plants). Throughout his life Karl Erik was an active collector of beetles, and President of the Norwegian Entomological Society for 14 years.

## References

Zachariassen KE & Lundheim R 1999 Applications of antifreeze proteins. In Margesin R & Schinner F (eds.) *Biotechnological applications of cold -adapted organisms*, pp. 319-332. Springer, Berlin, Heidelberg.

Zachariassen KE, Kristiansen E, Pedersen SA & Hammel HT 2004 Ice nucleation in solutions and freeze-avoiding insects – homogeneous or heterogeneous. *Cryobiology* **48**, 309-321.

Kristiansen E, Ramløv H, Hagen L, Pedersen SA, Andersen RA & Zachariassen KE 2005 Isolation and characterization of haemolymph antifreeze proteins from larvae of the long horn beetle *Rhagium inquisitor* (L.). *Comparative Biochemistry & Physiology* **142B**, 90-97.

Zachariassen KE, Li NG, Laugsand AE, Kristiansen E & Pedersen SA 2008 Is the strategy of cold hardiness in insects determined by their water balance? A study on two closely related families of beetles: Cerambycidae and Chrysomelidae. *Journal of Comparative Physiology B* **178**, 977-984.

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