# Environmental influences on reproduction in Antarctic krill, *Euphausia superba*

by Toshihiro Yoshida BE, ME Environmental Engineering for Symbiosis Soka University, Tokyo, Japan

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Institute of Antarctic and Southern Ocean Studies University of Tasmania

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## Declaration

This thesis contains no material which has been accepted for a degree or diploma by any institution. To the best of my knowledge the thesis does not contain any material written or published by another person, except where due reference is made.

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#### Abstract

Antarctic krill (*Euphausia superba*) is a keystone species in the Antarctic marine ecosystem, being a major food source for most Antarctic predators. Understanding krill reproduction is important since reproductive output is one of the key determinants of population size. Environmental parameters (food, light and temperature) are generally thought to be responsible for regulating the seasonal maturity cycle of organisms. This study investigates the effect of these environmental parameters on reproduction of krill from several aspects including maturation of female krill, development and hatching of eggs and larval survival.

Female krill were incubated for 10 months under controlled conditions to determine the influence of light and diet on sexual maturity and lipid and fatty acid (FA) compositions. Results from this investigation indicated that endogenous rhythm governed krill sexual maturation. The external sexual characteristics of krill were found to have undergone regression and re-maturation regardless of whether they had been kept under the natural photoperiod (emulating the field environment), or complete darkness. However, photoperiod significantly influenced the ovarian maturation cycle. Diet, either excess or limiting, did not affect this maturation and regression process. There was no significant effect of the addition of carnivorous diet (clam meat) on krill maturity. During five months of low food treatment, lipid was mainly utilized from digestive gland stores in the first two months, and later from other body fractions. Various feeding treatments did not induce any difference in FA compositions in krill. The FA composition of krill was however influenced by sexual maturity status.

After hatching, the larvae of *E. superba* undergo three non-feeding larval stages. This development is dependent on maternal fuel stores laid down in the eggs, which is in turn a function of the dietary conditions prior to spawning. To investigate the effect of maternal diet on embryogenesis, eggs were collected from both laboratory reared krill incubated under different feeding conditions and gravid krill caught in the field. Hatching success, and lipid and FA content and composition differed among the treatments. Positive correlations were found between hatching success and the levels of some specific fatty acids, namely docosahexaenoic acid, linoleic acid and arachidonic acid. Maternal diet to some degree influences the FA composition of eggs, and specific FA may play an important role in embryogenesis of krill eggs.

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Krill larvae cope with extremely low phytoplankton concentrations during their first winter, a period thought to be most critical. To investigate the starvation tolerance of larvae, the ultrastructure of digestive gland cells was investigated. The digestive gland is the major site of absorption and storage of nutrients. The effect of starvation on the ultrastructure of the digestive gland of krill larvae was observed as early as 5 days, and these effects increased with duration of starvation. Lipid droplets were rarely found in the digestive cells regardless of whether the larvae were fed or starved. These observations indicate that krill larvae utilize all available nutrients for growing rather than laying down storage. This process indicates that krill larvae are adapted to an environment where the food supply is continuous.

Increasing our understanding of Antarctic krill population is imperative to improve management model for the krill fishery. An understanding of factors influencing fecundity and annual recruitment of krill is important in determining population size. The results of this research helped elucidate the influence of environmental parameters on reproduction of krill. This research also facilitated a better understanding of how krill adapt to the extreme seasonal conditions, which makes them one of the most successful species in Southern Ocean ecosystem.

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Appendix. 2. Hagen W, Yoshida T, Virtue P, Kawaguchi S, Swadling K, Nicol S, Nichols	s P

(2007) Effect of a carnivorous diet on the lipids, fatty acids and condition of Antarctic krill, Euphausia superba. Antarct Sci 19: 183-188

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## **Co-Authorship**

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- So Kawaguchi, Patti Virtue and Peter Nichols assisted with the general supervision of all aspects of this thesis. These included experimental design, interpretation of data and proof reading manuscripts.
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We the undersigned agree with the above stated "proportion of work undertaken" for the above published peer-reviewed manuscripts contribution to this thesis.

Supervisor:
Dr. So Kawaguchi

Supervisor: Dr. Patti Virtue Supervisor Dr. Peter Nchols