

Role of Serotonin in Environmental Stress Induced Sex Determination in *Daphnia*

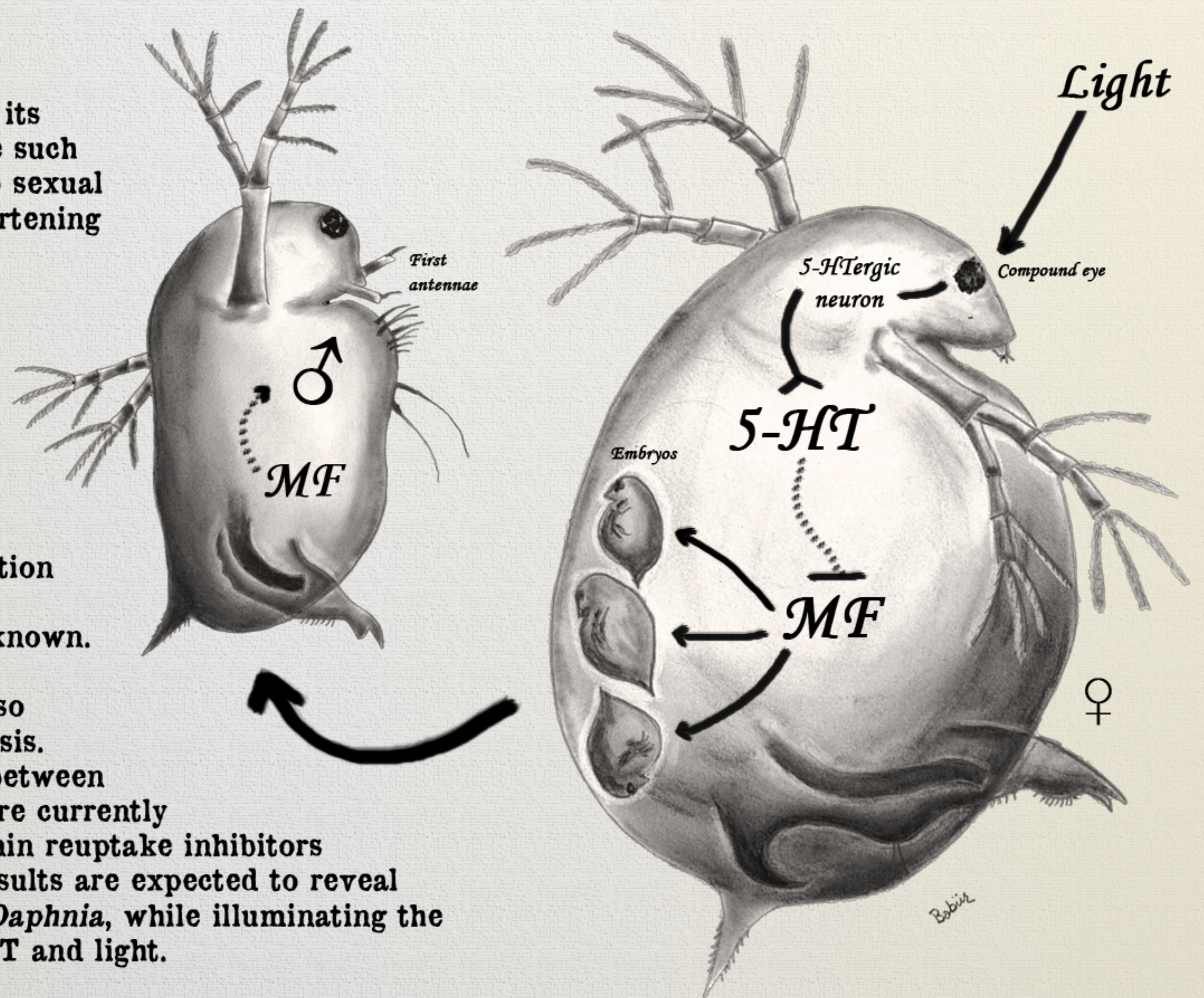


Babür Erdem* and Meral Kence

Department of Biology, Middle East Technical University, Ankara, Turkey

Introduction

Daphnia is a freshwater crustacean well-known for its indicative responses to environmental changes. One such response involves a switch from parthenogenesis to sexual reproduction. Non-optimal conditions including shortening of daylight duration, population crowding or food restriction lead to parthenogenetic generation of males instead of daughters, followed by sexual reproduction. However, the mechanism of environmental change-induced sex determination is not well-understood. Here we investigate how changes in the length of the light period affect sex determination. Although previous studies have suggested methyl farnesoate (MF), a terpenoid hormone, as the causal factor leading to the generation of males, the pathway between detection of light duration and the secretion of MF has remained unknown. Studies in different crustaceans have demonstrated relationships between light and serotonin (5-HT) also indirectly suppressive effect of 5-HT on MF synthesis. We thus hypothesized that 5-HT could be the link between photoreception and MF secretion in *Daphnia*. We are currently testing this hypothesis by applying selective serotonin reuptake inhibitors (SSRI) under short and long light durations. Our results are expected to reveal a novel aspect of the ecoresponsive physiology of *Daphnia*, while illuminating the evolutionary origin of the relationship between 5-HT and light.



Method

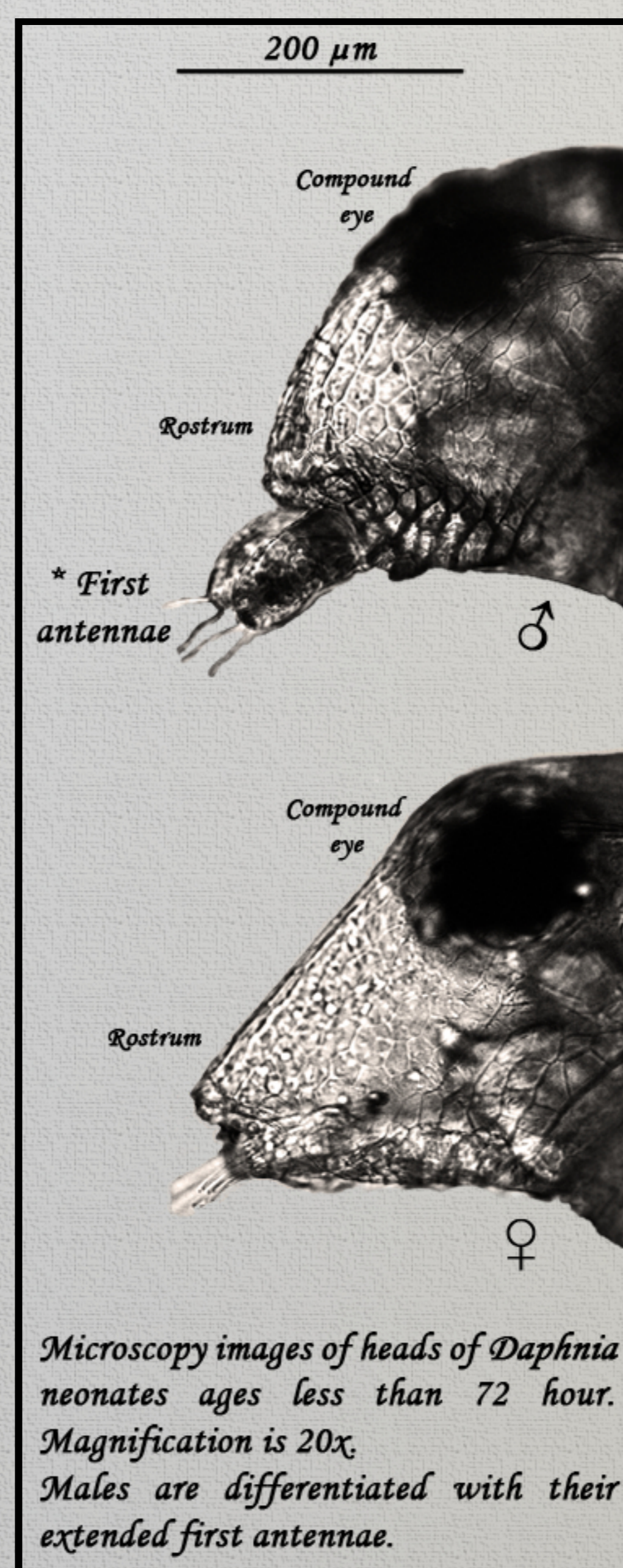
Animals were collected and isolated from small pond in Aydın Province and *Daphnia* culture has been maintained in Artificial *Daphnia* Medium (Klüttgen et al., 1994) more than one year. Neonates ages less than 24 hour transferred 100 ml jars and each jar contains one individual. Experiment has been continued in two incubators was set 20 ± 1 °C and photoperiod 16 h light, 8 h dark (normal condition) and 8 h light, 16 h dark (dark condition) cycles. Each jar was provided with *Scenedesmus obliquus* as food at a concentration of 10^5 cells/ml daily. Animals were exposed to fluoxetine, which is a SSRI also have 98% purity, at 100 and 250 µg/l. Offspring born over the 21 days removed and growth several days in different beakers. Then, they were examined under light microscope for sex differentiation by the length and morphology of the first antennae.

Preliminary Results & Conclusion

Preliminary results shows that, affect of 5-HT on MF synthesis and sex determination is possible.

- In 16 h light environment, there is no male found in 0, 100 and 250 µg/l fluoxetine treated groups.
- In 8 h light environment, male ratio is found as 19,5 % in control group. At 100 µg/l fluoxetine treated group, there is only 1 male appear through 58 infant taken from 8 mothers. Also, there is not found any male in 250 µg/l fluoxetine treated group.

Actually, our finding is 19.5 % male ratio in control group under 8 h light duration is lower than previous researches, Hobaek and Larsson (1990) found 46.7 % as male ratio in dark condition. Another finding is developmental abnormalities likewise curved shell spine observed on some of the neonates in 100 µg/l fluoxetine treated group in dark environment. Mu and Leblanc (2004) demonstrate developmental abnormalities on juvenoids with MF treatment. It brings to mind; 5-HT could be disrupt MF secretion in dark environment and inappropriate level of MF may causes developmental abnormalities. Also, under the dark condition, mothers have remarkably lower number of infant in the control group, however 100 and 250 µg/l fluoxetine treated groups have close infant number to groups in normal condition. These results parallel to much of researches on crustaceans including *Daphnia*, which are illustrate positive correlation between 5-HT and fecundity. Further researches, we have planned expand the sample size also applying specific serotonin receptor agonists and antagonists.



Acknowledgement

The project supported by Scientific and Technological Research Council of Turkey (TÜBİTAK), under the 2209/A Undergraduate Students Domestic Research Projects Support Program. I express all my gratitude to Assoc. Prof. Dr. Mesut Muyan, he always gave me support. I also thanks to my friends Özgün Kırdar, Enre İlpars, Meriç Öztürk and Damla Bekar because of their assistance. And also special thanks to Prof. Dr. Meryem Beklioglu, she has allowed me to meet with *Daphnia*.

References

- Campos, B., Piña, B., & Barata C, C. (2012). Mechanisms of action of selective serotonin reuptake inhibitors in *Daphnia magna*. *Environmental Science & Technology*, 46(5), 2943-2950.
- Fingerman, M. (1997). Roles of neurotransmitters in regulating reproductive hormone release and gonadal maturation in decapod crustaceans. *Invertebrate Reproduction & Development*, 31(1-3), 47-54.
- Hobaek, A., & Larsson, P. (1990). Sex determination in *Daphnia magna*. *Ecology*, 71(6), 2255-2268.
- Klüttgen, B., Dülmer, U., Engels, M., & Ratte, H. T. (1994). ADA-M, an artificial freshwater for the culture of zooplankton. *Water Res.*, 28(3), 743-746.
- Landau, M., Laufer, H., & Homola, E. (1989). Control of methyl farnesoate synthesis in the mandibular organ of the crayfish *Procambarus clarkii*: Evidence for peptide neurohormones with dual functions. *Invertebrate Reproduction & Development*, 16(1-3), 165-168.
- Makkapan, W., Maikao, L., Miyazaki, T., & Chotigeat, W. (2011). Molecular mechanism of serotonin via methyl farnesoate in ovarian development of white shrimp: *Fenneropenaeus merguensis* de Man. *Aquaculture*, 321(1-2), 101-107.
- Mu, X., & Leblanc, G. A. (2004). Cross communication between signaling pathways: juvenoid hormones modulate ecdysteroid activity in a crustacean. *Journal of Experimental Zoology. Part A, Comparative Experimental Biology*, 301(10), 793-801.
- Rider, C. V., Gorr, T. A., Olmstead, A. W., Wasilak, B. A., & LeBlanc, G. A. (2005). Stress signaling: coregulation of hemoglobin and male sex determination through a terpenoid signaling pathway in a crustacean. *The Journal of Experimental Biology*, 208(1), 15-23.
- Sathyanandam, S., Vasudevan, S., & Natesan, M. (2008). Serotonin modulation of hemolymph glucose and crustacean hyperglycemic hormone titers in *Fenneropenaeus indicus*. *Aquaculture*, 281(1-4), 106-112.
- Strauss, J., Zhang, Q., Verleyen, P., Huybrechts, J., Neupert, S., Predel, R., ... Dirksen, H. (2011). Pigment-dispersing hormone in *Daphnia* interneurons, one type homologous to insect clock neurons displaying circadian rhythmicity. *Cellular and Molecular Life Sciences*, 68(20), 3403-3423.