



Title	Studies on Seed Production and Genetic Markers for Stock Enhancement of Seahorses [an abstract of dissertation and a summary of dissertation review]
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Citation	北海道大学. 博士(水産科学) 乙第6984号
Issue Date	2016-03-24
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# 学位論文内容の要旨

博士の専攻分野の名称：博士（水産科学）

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## 学位論文題目

Studies on Seed Production and Genetic Markers for Stock Enhancement of Seahorses  
(タツノオトシゴ類資源増殖にむけた種苗生産と遺伝マーカーに関する研究)

Seahorse culture has been practiced throughout the world to meet the demand for global trade and to reduce the pressure on wild stocks through overexploitation. Replenishment of the diminishing wild population has been a pressing issue in the Southeast Asian waters where wild seahorses have been heavily fished. However, stock release needs to be carefully managed to prevent risks such as disease transmission, genetic threats and community disruptions. Two components of a hatchery-release program are the availability of the cultured organism and the release of these organisms to the natural environment.

Therefore, the first component of this dissertation study (Part A) is about the development of seed production techniques of tiger tail seahorse *Hippocampus comes*. In Chapter 1, the improved reproductive performance of seahorses was discussed based on the food consumption, brood size, parturition occurrence and parturition interval of seahorses using single and combined diets comprising of *Artemia*, mysid and frozen *Acetes* sp. Chapter 2 discussed the effects of sterilization of ambient water and feed using UV-treated and chlorinated seawater and formalin-treated food organisms on the improved survival and growth of newborn tiger tail seahorse. Chapter 3 discussed the food consumption, growth in stretched height and body, and survival of 1 to 6-month old juvenile seahorses using single and combined diets comprising of *Artemia*, mysid and frozen *Acetes* sp.

Studies using DNA markers are important before embarking on any release program as it is a very useful tool in the long-term monitoring of the released stocks. Genetic structure of population in target locality of seed release should be clarified before any restocking is initiated. Furthermore, species identification of seahorses must be done since genetic information on the seahorse species is sparse and species identification

based on the external appearance, color and other morphological characteristics is difficult. The second component of this dissertation study (Part B) is on the genetic studies for species identification and development of microsatellite DNA markers for monitoring of wild and released seahorses.

Chapter 4 discussed the developed method of molecular species identification of seahorses based on (a) sequences of mtDNA *cytb* and *16S*rRNA genes, (b) novel species-specific primers (SSP) for *H. barbouri* and *H. spinosissimus*, and (c) Restriction Fragment Length Polymorphism (RFLP) assay using the restriction enzyme *Bam*HI on six species of seahorses *H. comes*, *H. barbouri*, *H. kuda*, *H. spinosissimus*, *H. trimaculatus* and *H. abdominalis*. In Chapter 5, the author successfully developed microsatellite DNA markers for *H. comes* and tested cross amplification to other species of seahorses.

Lastly, the author discussed the importance of the improved culture techniques for seed production of seahorses as an effective measure to reduce the pressure on wild stocks. Clarification of seahorse species and population structure of wild seahorses are important considerations for resource management and conservation purposes. Species identification method and the development of microsatellite DNA markers for *H. comes* can also be applied to other species of seahorses, thus the information from the present study will add to the baseline data for large scale examination of seahorse population in the Philippines, as well as other species of seahorses in the Asian region.