



Title	Studies on Seed Production and Genetic Markers for Stock Enhancement of Seahorses [an abstract of entire text]
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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
Doc URL	http://hdl.handle.net/2115/61640
Type	theses (doctoral - abstract of entire text)
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主論文の要約

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.

This study primarily aimed to develop the seed production techniques and to establish genetic markers of seahorses for monitoring stock enhancement. Thus, the first component of the present study was the hatchery techniques in seed production of seahorses that have been conducted at SEAFDEC/AQD, while the second component was genetic studies for species identification and population structure analyses including monitoring of wild and released seahorses by using genetic markers.

The first component of this dissertation study (Part A) is about the development of seed production techniques of seahorses. Feeding and water management scheme for seahorse hatchery were developed and resulted in improved reproductive performance and higher survival and growth rates in newborn and juvenile seahorse.

In Chapter 1, the improved reproductive performance of tiger tail seahorse, *Hippocampus comes*, 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. Broodstocks fed with mysid shrimps showed higher brood size and shorter parturition interval.

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 newborns of tiger tail seahorse, *H. comes*. The importance of providing desirable food organisms and maintenance of suitable water quality in order to maintain maximum efficiency in the management of the seahorse hatchery for newborn seahorses was highlighted. Newborn seahorses fed with formalin-treated food organisms and reared in UV-treated seawater had significantly higher survival and daily growth rate.

Chapter 3 discussed the food consumption, growth in stretched height and body, and survival of 1 to 6-month old juveniles tiger tail seahorse, *H. comes*, using single and combined diets comprising of *Artemia*, mysid and frozen *Acetes* sp. Juveniles that were less than 2 months old were sensitive to fluctuation in water temperature and availability of copepods. At this stage, the juveniles could be weaned to mysid. However, they were not able to feed on frozen *Acetes*. Survivals of 3-6 months old juveniles were more stable mainly due to their ability to feed on mysid shrimps and *Acetes*.

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 thus, 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 *cytochrome b* (*cytb*) and *16S* rRNA genes of mitochondrial DNA sequenced in seahorses to reveal phylogenetic relationships among them. Based on the phylogenetic trees, six species of seahorses namely *Hippocampus comes*, *H. barbouri*, *H. kuda*, *H. spinosissimus*, *H. trimaculatus* and *H. abdominalis* could be distinguished by nucleotide sequence of *cytb*. Molecular genetic methods were developed for species identification of seahorses. The novel species-specific primers for *H. spinosissimus* (HiSpiF1) and *H. barbouri* (HiBarF1), which amplify fragments of 5' end region of the

16S rRNA gene, exhibited no-cross amplification when tested to all the other seahorse species examined in this study. To distinguish *H. comes* from the other five species of seahorses, a restriction fragment length polymorphism assay was developed using the restriction enzyme *Bam*HI.

In Chapter 5, the author successfully developed microsatellite (ms) DNA markers for *H. comes* and tested cross amplification to other five species of seahorses in the Indo-Pacific region, *H. barbouri*, *H. kuda*, *H. spinosissimus*, *H. trimaculatus* and *H. abdominalis*. The newly developed ms markers will provide important information for the management and protection of the wild population of seahorse and thus, will be useful in further seahorse restocking, management, and conservation efforts in Philippines as well as in other regions where the six seahorse species studied herein occur or have been introduced.

Information on the genetic diversity for both the wild and hatchery population is important to clarify the population structure of seahorses. Due to very little information of baseline data for seahorse population in the Philippines, the results derived from the present study may be applied to large scale examination of seahorse population in the Philippines.