PROBLEMS IN SPECIES IDENTIFICATION OF THE MUD CRAB GENUS *SCYLLA* (BRACHYURA: PORTUNIDAE)

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ABSTRACT

Mud crabs in the genus *Scylla* inhabit brackish waters such as mangrove areas and estuaries and are widely distributed throughout the Pacific and Indian Oceans, from Tahiti, Hawai'i, New Zealand, Australia and Japan to southern Africa. This species is an important fisheries and aquaculture resource in Australia, Japan, Taiwan, Indonesia, and the Philippines.

The Japan Sea-Farming Association (JASFA) has developed technologies for stock enhancement activities, such as broodstock management, seed production, and experimental release of hatchery-raised juvenile mud crab in Japan. Seed production is performed using three morphologically distinguishable species, *S. serrata*, *S. tranquebarica*, and *S. oceanica*. If these three morphologically distinguishable species are, in fact, genetically different, it is recommended that resource management activities be tailored to each species, individually.

Stephenson and Campbell (1960) regarded four varieties of mud crab as one species using samples collected from Queensland and New South Wales, Australia, and suggested that the morphological differences were produced by environmental differences. Fuseya and Watanabe (1996), however, studied the genetic variability at three loci in the mud crab and reported that the species *S. serrata*, *S. tranquebarica*, and *S. oceanica* are clearly distinguishable from one another.

Distribution and Commercial Value

Mud crabs in the genus *Scylla* inhabit brackish waters, such as mangrove areas and estuaries, throughout the Pacific and Indian Oceans, from Tahiti, Australia, and Japan to southern Africa (Chahpgar 1947; Hill 1975; Sakai 1976; Dai and Yang 1991). This crab is an important fishery resource in Australia, Japan, Taiwan, Indonesia, and the Philippines where it is also targeted for aquaculture (Fukunaga and Fukumoto 1960; Fushimi 1983a; Cowan 1984; Oshiro 1988; Chin and Amandakoon 1992; Cholik and Hanafi 1992; Jamari 1992; Larda and Lin 1992; Rattanachote and Dangwatanakul 1992; Watanabe and Sulistiono 1993; Watanabe et al. 1996). In recent years, this mud crab has been selected as one of the target species for stock enhancement programs in Japan (Fukunaga and Fukumoto 1960; Fushimi 1983b; Oshiro 1988). Stock enhancement activities, such as broodstock management, induced spawning, seed production, and acclimatization for release, have been carried out by the Japan Sea-Farming Association and some Prefectural Fisheries Experiment Stations. Artificial seed production has been successfully achieved for three morphologically distinguishable species, *Scylla serrata*, *Scylla tranquebarica*, and *Scylla oceanica* (Cholik and Hanafi 1992; Larda and Lin 1992). Seed production and release data for *Scylla tranquebarica* from 1984 to 1997 are shown in Fig.1. Seed production of the mud crab has steadily increased year after year and over 4 million individuals were produced in 1996. Annual release is also increasing steadily from an average of 500,000 during the early years to 1 million individuals in 1996. The annual fluctuation in seed production and release of *Scylla oceanica* is summarized in Fig. 2. In contrast to *Scylla tranquebarica*, seed production of *Scylla oceanica* is more difficult. Controlling the environmental conditions, such as salinity, has

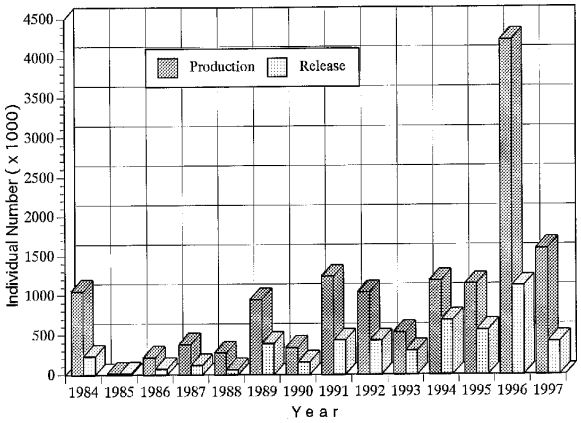

Figure 1. Annual fluctuation of individual number of seed production and release of *Scylla tranquebarica* (Data from JFA and JASFA).

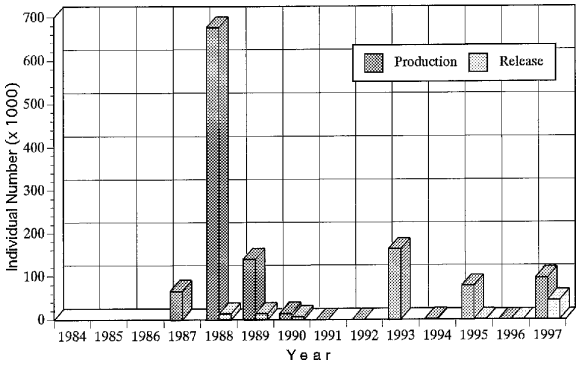

Figure 2. Annual fluctualtion of individual number seed production and release of *Scylla oceanica* (Data from JFA and JASFA).

hampered successful larval rearing past the zoeal stage. Although seed production has at times reached 700,000 individuals, the overall production and release of *Scylla oceanica* remain comparatively low.

Species Identification of the Mud Crab

The species identification of mud crab has been controversial, and for many years, only one species was recognized in the genus *Scylla* (Fuseya 1998). Recently, however, researchers have reported that the genus *Scylla* includes several species (Estampador 1949; Serene 1952; Stephenson and Campbell 1960; Ong 1964; Fushimi 1983a; Joel and Raj 1983; Oshiro 1988; Kathirval and Srinivasagam 1992; Fuseya and Watanabe 1995, 1996; Watanabe and Fuseya 1997; Fuseya 1998). As summarized in Fig.1 and 2, seed production of mud crabs in Japan is performed using three morphologically distinguishable species. Estampador (1949) classified the mud crab into three species and one variety, *S. serrata*, *S. oceanica*, *S. tranquebarica* and *S. serrata* var. *paramamosain* using specimens collected in the Philippines, based on their external morphology (e.g., color of carapace and legs, anterolateral teeth of carapace, and outer spines of cheliped carpus) and gametogenesis. Serene (1952) also recognized the existence of four forms in Vietnam, in accordance with the finding of Estampador (1949). However, Stephenson and Campbell (1960) regarded the four forms as only one species and based their conclusions on samples collected from Queensland and New South Wales, Australia. They suggested that the morphological differences were produced by environmental differences and it is their recommendation that: "Therefore, and tentatively, the four forms of Estampador and of Serene are fused into synonymy." Subsequently, Ong (1964) in Malaysia and Joel and Raj (1983) from India also noted differences in forms between specimens.

Fushimi (1983b) pointed out the presence of three forms of mud crabs in Hamana Lake, and Oshiro (1988) recognized at least three species based on specimens from Japan. Fuseya and Watanabe (1996) carried out a study on genetic variability at three loci in the mud crab and determined that three species, *S. serrata*, *S. tranquebarica*, and *S. oceanica*, could be clearly distinguished. Overton et al. (1997) carried out a multi-variate analysis of mud crabs from four locations in Southeast Asia. Although they could distinguish three distinct morphological forms, their conclusion was the same as that of Stephenson and Campbell (1960), that the morphological differences were produced by environmental variations. They did not, however, specify what environmental condition(s) would produce the three morphological forms. Keenan et al. (1998) made a revision of the genus *Scylla* using specimens collected from the Red Sea and from locations throughout the Indo-Pacific region. Two independent genetic methods, allozyme electrophoresis and sequencing of two mitochondrial DNA genes (cytochrome oxidase I and 16s RNA) were employed in an attempt to differentiate species. They recognized up to four

species using morphological criteria but there were differences in nomenclature.

Fuseya (1998) carried out morphometric analyses of specimens within the genus *Scylla* collected from throughout the geographic distribution of the mud crab. She also examined morphological characteristics of the first and second pleopod of the male sex. Based on her analysis, the species *S. serrata*, *S. tranquebarica*, and *S. oceanica* were clearly distinguishable. Morphological characteristics of these three species were found to correspond to those described by Estampador(1949). The dorsal and ventral views of the carapace are compared in Fig. 3. The morphological characteristics of the rostrum and antero-lateral teeth and cheliped are summarized as follows:

S. serrata: Front cut into four lobes or blunt teeth of about equal size and prominence. Antero-lateral border cut into nine sharply acuminate teeth of about equal size.

S. tranquebarica: Front cut into four sharply acuminate teeth with spine. Antero-lateral border cut into nine sharply acuminate teeth with spine.

S. oceanica: Front cut into four acuminate teeth of about equal size. Antero-lateral border cut into nine sharply acuminate teeth.

There are also differences in the numbers of spines of the cheliped carpus and behind the finger joint The first pleopod tip of each of the three species was examined using scanning electron microscopy and found to be different from each other (Fuseya 1998).

Genetic information is essential for the identification of the three species of the mud crabs in the genus *Scylla*. From June 1994 to May 1995, Fuseya and Watanabe (1996) collected and identified 342 mud crabs from seven locations (Lake Hamana and Okinawa in Japan, Bali and Cilacap in Indonesia, Chantaburi and Surat Thani in Thailand, and Madagascar). They initially classified the crabs into three species, *S. serrata*, *S. tranquebarica* and *S. oceanica*, according to Estampador (1949). Horizontal starch gel electrophoresis was used to analyze muscle tissue

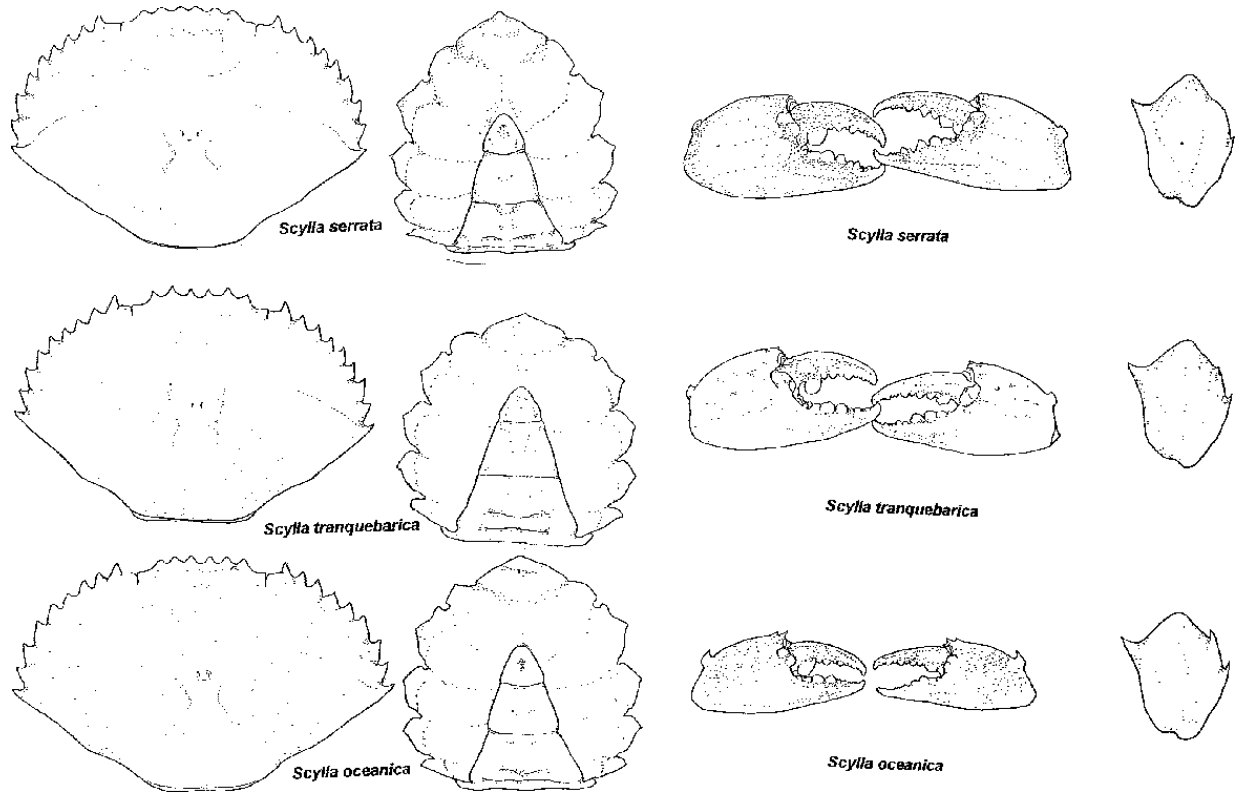

Figure 3. Comparison of carapace (dorsal and ventral view) and cheliped in three species of genus *Scylla* (male). (After Fuseya 1998).

for variation in 11 enzymes at 17 allozymic loci in the three proposed species. Seven of the 17 loci were found to be polymorphic, and fixed differences were detected at three loci (EST, LAP-2, and SOD). The fixed differences found at 3 of the 17 loci sampled and the relatively large genetic distances calculated between the species verify the existence of at least three species of mud crabs in the genus *Scylla*. The three species classified by Estampador (1949) based on morphological traits coincide with the genetic results obtained in this study, however, the genetic analysis shows that *S. serrata* and *S. tranquebarica* are more closely related than *S. oceanica*. The mean heterozygosity of the genus *Scylla* (the whole population) is 0.108. This value is high compared with those reported for other crustacean species. For example, values of mean heterozygosity in other crustacean species are 0.007-0.014 in the swimming crab *Portunus trituberculatus,* 0.0004-0.02 in the snow crab *Chinonoecetes opilio,* 0.072-0.077 in the spider crab *C. japonicus,* and 0.023-0.032 in the hair crab *Erimarcus isenbeckii*. The mean heterozygosity found in this study for each of the mud crab species within the genus *Scylla* is 0.049 for *S. serrata,* 0.014 for *S. tranquebarica,* and 0.004 for *S. oceanica*. The genetic variability of *S. serrata* is higher than that of *S. oceanica.* The information obtained in the current investigation establishes that the three species of mud crabs are clearly distinct from each other and implies that the stocks of each species should be managed separately.

We would recommend that the morphological description of Estampador (1949) is sufficient for species identification of the genus *Scylla,* and it is necessary to confirm the identification of the three species for further study on the crabs in the genus *Scylla*.

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