Small project proposal

Genetic relationships and identification of mud crabs, genus Scylla, from the Indo-Pacific

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1 SUMMARY

As a preparatory activity to a proposed ACIAR project that will examine improved culture systems for mud crabs, it is necessary to address the basic identification of the crabs that will be cultured. Conflicting scientific views and a high probability that the crabs from different areas are different species suggests that there is an urgent need to clarify the taxonomic status of these animals prior to commencement of the broader project. Electrophoretic techniques, to be used by this project, provide a simple and direct method of determining the genetic relationships of mud crabs and the extent of differentiation. The results from this project will provide valuable knowledge to the countries that are actively involved in the research, culture and wildstock management of mud crabs. Further, these results could be adopted by the broader project should it proceed.

2 BACKGROUND

This preliminary project was originally included as an important component of ACIAR Collaborative Project Proposal No. 9219 "Development of improved mud crab culture systems in Thailand, the Philippines arid Australia." The broader project has stalled and is facing redefinition because of the withdrawal of one collaborating Agency.

An understanding of the taxonomic details of Scylla populations throughout the Indo-Pacific is central to the development of a more successful aquaculture industry based on the mud crab, as well as improved wild-stock management of the different types found throughout the Indo-Pacific. This preliminary project will provide basic information on the genetic relationships of the animals presently used for culture in each area and thereby ensure that research work is comparable between the countries involved in culture and wildstock management of mud crabs. This research will also provide a starting point for the broader project, which is not confirmed at this point in time. The necessity for the research proposed by this project was recognised at the Regional Seminar on Mud Crab Culture and Trade in the Bay of Bengal Region in Surat Thani, Thailand in November 1991 and the need for the work was also addressed by Brown (in press). This meeting was sponsored by the FAO-supported Bay of Bengal Programme for Fisheries Development in an attempt to improve conditions for small scale fishing communities through mud crab fattening and culture. Interest in this seminar was very high with 35 papers being presented from Australia, Bangladesh, India, Indonesia, Malaysia, Myanmar, Philippines, Sri Lanka and Thailand (Infofish, 1992).

The uncertainty of genetic relationships was recognised as one of the primary constraints to the management of the wild fishery and development of aquaculture. A recommendation of the meeting was:

"The genetic or systematic basis of mud crab populations in south-east Asia needs to be defined. This arises from the experience throughout south-east Asia with different 'races' or species of crabs which grow with different growth rates and appearance - and differential market value."

It is now widely recognised that mud crabs of the Indo-Pacific region belong to more than one group of the genus Scylla. Forskal (1755) first named the single species Cancer serratus from type material collected from Jedah on the Red Sea, which is now missing. Since this time a number of species and varieties have been proposed. Estampador (1949a,b) performed an extensive study of mud crabs in Philippine waters and recognised three species - Scylla serrata (Forskal), S. oceanica (Dana), S. tranquebarica (Fabricus) and one variety (S. serrata var. paramamosain). These groups were distinguishable by colour patterns, relative size, cheeped spination, chromosome 'form' and process of gamete development.
The occurrence of multiple forms have been reported for almost all regions where the mud crab occurs. Morphological features and variation tend to confirm Estampador's (1949a,b) descriptions although the arrangement of species and varieties are questioned. Serene (1952) found four distinct forms in waters of Vietnam and basically agreed with Estampador's groupings although he considered *S. tranquebarica* to be a variety of *S. oceanica*. Ong (1964) distinguished four forms of *Scylla* in Malaysia. Kathirvel (1981) found two types in the Cochin backwater of India and Joel and Raj (1983) reported *S. tranquebarica* and *S. serrata* from Pulicat Lake. Chayarat and Kaew-ridh (1978) determined that three types occurred in waters of Chantaburi in Thailand. In Australia Taylor (1984) described two different forms in Western Australia which he called separate species. To date no genetic orientated studies have been completed.

While justification for the classification of mud crabs into different species and varieties is controversial (Stephenson and Campbell 1960) it is clear that recognisably different forms occur, often together, throughout the crabs' distribution. What is interesting from an aquaculture viewpoint is that certain aspects of their biology appear to be different. Kathirvel (1981) reported a maximum size of approximately 200 mm and size of first maturity for females of 120 mm for *S. oceanica* (Dana). Comparable sizes for the type *S. serrata* (Forskal) were 120 mm and 85 mm, and for the common Australian mangrove crabs 240 mm and 150 mm respectively (Brown, in press). Comparative growth rates have not been determined, but it seems that significant benefits could be gained through selection of faster growing, larger types. Chen (1990) reports that of the three types recognised by crab farmers in Taiwan the ‘white’ crab or *S. oceanica* is considered the most suitable for culture as it grows larger, is less aggressive and more tolerant of a wide range of salinities.

A knowledge of the morphology and distribution of any genetically distinct species and populations is an important prerequisite to the development and management of fisheries management aquacultural practices. Electrophoretic techniques, to be used by this project, provide a simple and direct method of determining the genetic relationships of mud crabs the extent of differentiation. The advantage of electrophoretic genetic methods over traditional morphological taxonomy is that breeding relationships and the absence of gene flow can be quantified. Therefore conclusions as to specific status and the ability of isolated populations to interbreed in nature are much stronger than those based on morphology. In addition such conclusions can be used to produce morphological keys to clearly identify the different types.

### 3 PARTNER COUNTRY PRIORITY

This project was one of four key recommendations from the Regional Seminar on Mud Crab Culture and Trade in the Bay of Bengal Region in Surat Thani, Thailand in November 1991. Interest in this seminar was very high and all counties that are currently involved in mud crab culture considered this research a priority. The development of improved culture systems was seen to be constrained by a lack of adequate information on the taxonomic status of the animals presently under culture.

### 4 OBJECTIVES

**i To determine the genetic relatedness of mud crab stocks from the Philippines, Australia, the Pacific Islands and South East Asia.**

Collaborators will collect live mud crabs from their local area, from each of the different types that they recognise. Samples will be required from the Red Sea (type material), Australia, Indonesia, Malaysia, the Philippines, Thailand, Vietnam, India, Papua New Guinea and other Pacific Island countries. Ideally, other locations within south-east Asia
will also be requested to provide collections to obtain as broad a coverage as possible. Using gel electrophoresis, genetic screening will provide information on the relatedness of mud crab types thought most suitable for culture in each country.

**ii To discuss with the participating countries the influence these results have to the ongoing national research programs and to the objectives of the proposed broader project.**

These discussions, working with the countries most interested in mud crab culture, will integrate this preliminary project's results into the selection of the species most appropriate for further research and aquaculture production.

**iii To establish morphological procedures for use in the identification of the different mud crab types.**

Morphometric data will be collected from each specimen. Using the genetic data for grouping of specimens, analysis of this morphometric data will produce identifiable characteristics for each type. These characters will be used to produce a morphological key for the field identification of these types.

## 5 METHODOLOGY

Samples of ten individuals from the different morphological types available from throughout the Indo-Pacific range of the genus will be obtained from widespread locations, including eastern and western Australia, the Philippines, south-western and central Indonesia, Malaysia, east and west Thailand, Vietnam, India, Papua New Guinea and other Pacific Island countries. Six specimens is a minimum requirement to determine genetic relationships between closely related species (Keenan 1991). An additional sample of mud crabs from Jedah in the Red Sea (to use as type material) will also be obtained. Crabs can readily be obtained throughout the year. QDPI has already made many contacts with overseas countries concerning this project and material has been received from Singapore, Phuket, Bangkok and Karachi, and also collected from within Australia. Collaborators from each area will be requested to send samples that they identify as different types. They will also be requested to provide information about the biology, and distribution of their local types. It is expected that approximately 400 crabs from throughout the species range will be obtained. Preliminary discussions with AGES regarding the importation of material suggest that the crabs can be easily imported in a frozen and dead state, being treated as a normal seafood item.

All specimens will be initially screened for general proteins using polyacrylamide gel electrophoresis to identify closely related specimens (Keenan and Shaklee 1985, Shaklee and Keenan 1986). Buffers suitable for starch gel electrophoretic determination of enzyme loci will then be determined for approximately 40 loci. These loci-buffer combinations will be used to gather genetic data for individual loci and to determine the presence of polymorphism. Genetic data will then be analysed for major taxonomic groupings (species and sub-species) by cluster and parsimony analysis (Keenan, 1988, 1991). Using the data collected from this project, a follow-up within-species population analysis could then be conducted. However, such a study is not within the scope of this project. Such a study would entail a more detailed analysis of approximately 100 specimens per locality, using identified polymorphic loci.

Full documentation will be kept of all specimens received and typical representatives of both sexes will be indexed and stored at the Queensland Museum. Mr Peter Davie, Curator of Crustacea at the Queensland Museum will be involved with the collection of data from all samples for relevant morphometric characters (Stephenson and Campbell 1960). Using the genetic data for grouping of specimens, the morphometric data will be analysed using descriminant function analysis to produce identifiable characteristics for
each species. These characters will be used to produce a morphological key for the genus and to describe new species should the need arise.

6 BENEFITS / OUTPUTS

In order to maximise the chances of establishing reliable hatchery techniques, the genetic relatedness of mud crab 'types' most favoured for culture in each country will be determined. This is seen as an important requirement for the broader project for it will enable the identification of the species most valuable for culture and standardisation of the crab type cultured. The taxonomic relationships of the different crab types needs to be resolved before culture activities can be done with any confidence. Then research, activities can be targetted on crabs known to be successful in culture facilities.

The data generated this project will have extended benefits beyond the broader project as it will provide fisheries managers with information on the range of the different genotypes of *Scylla*. Because of the high interest in mud crab culture this project will impact on countries from the region with capture and/or culture fisheries, including Australia, the Philippines, Vietnam, Indonesia, Malaysia, Papua New Guineas Sri Lanka, India and the Pacific Island countries.

7 ECONOMIC, ENVIRONMENTAL AND SOCIAL IMPACT

The major impact of this preliminary project will be in clarifying the question concerning relationships between the mud crabs found in each of the participating countries. As discussed in the introduction, there exists considerable confusion concerning the taxonomy and relationships of crabs of the genus *Scylla*. However, these results will extend beyond this field of taxonomic research. The primary benefit of this preliminary project is that each country involved will learn the identity and relationships of the type they are presently using for culture. The results will be used by the broader project to standardise nomenclature thereby enabling information transfer between on the, essential biological comparisons generated by that project. The results are therefore important in the long term economic development of the aquaculture potential of mud crabs in terms of identifying forms and genotypes best suited for further development.

8 ADOPTION OF RESEARCH

The research results will provide assistance to the ongoing research effort in individual countries, with direct application to research programs involving mud crab culture, in addition to wild-stock management programs. The results of this preliminary project could also be adopted immediately should the broader project proceed because of the close integration between them. It is expected that once samples are available this project will have genetically based results that could be adopted by the broader project within three months. When the full extent of the research is completed the results will also be communicated to the wider scientific community by publication in scientific journals and workshop proceedings. Taxonomic publications usually require very thorough literature searches which may take some time to complete to the satisfaction of Referees. Therefore it is expected that the final morphological taxonomic publications will take longer to finalise than publications based on the genetic relationships.
9 PERSONNEL

In Australia the QDPI personnel involved in this preliminary project will be Dr Clive Keenan and Mr David Mann. Dr Keenan is the Senior Fisheries Biologist (Genetics) for QDPI and is based at the Southern Fisheries Centre, Deception Bay which is a 30 minute drive from the Bribie Island Aquaculture Centre. He has been involved in genetic studies on *Lates calcarifer* and native Australian freshwater and marine fish, as well as scallops in the past twelve years. With the assistance of Mrs Raewyn Street, an experienced Fisheries Technician, Dr Keenan will undertake the definition of mud crabs stocks in the collaborating countries.

Mr Mann is a Graduate Fisheries Technician and has worked with Dr Gillespie since 1990 and has previously been engaged in research on the culture of crabs since 1987. He has an excellent knowledge of larval crab culture technique issues and problems and is preparing a paper on mud crab maturation techniques. Apart from his activities associated with the broader project, Mr Mann will assist with the collection of morphometric data for this project.

Mr Peter Davie, Curator of Crustacea at the Queensland Museum, will assist the project with advice and guidance with taxonomy and morphology. He will also be responsible for the cataloguing of specimens into the Museum's collection and assist with the preparation of publications arising from this project.

The role of overseas collaborators from cooperating countries is important to the success of this project. It is envisaged that they will identify local types of mud crabs and provide samples of recognised morphs/species for the genetic analysis, to be undertaken in Australia. The participation of these countries will advance the development of a network of active research groups through direct communication between the researchers involved.

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<th>Personnel</th>
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<td>Dr Clive Keenan</td>
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<td>Mr David Mann</td>
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<td>QDPI</td>
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<tr>
<td>Mr Peter Davie</td>
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<td>Queensland Museum</td>
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<td>Mrs Raewyn Street</td>
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10 DURATION OF PROJECT AND BUDGET

The project will begin in October 1993 and run for 9 months before completion in June 1994. Because the project is dependent on obtaining samples from throughout the Indo-Pacific, the first priority is to obtain these samples shortly after the project commences. It is envisaged that some work, to finalise aspects of this project, will be required within the broader project's budget because of the uncertain nature of the results and their potential application to reseeding programs and natural stock management.
11 REFERENCES:


