Intergovernmental Oceanographic Commission
Workshop Report No. 71

IOC-FAO Workshop on the Identification of Penaeid Prawn Larvae and Postlarvae

CSIRO Marine Laboratories
Cleveland, Australia, 23-28 September 1990

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1. INTRODUCTION

Dr Burke Hill, Officer-in-charge of the Commonwealth Scientific Industrial Research Organisation (CSIRO) Marine Laboratory in Cleveland, opened the workshop and welcomed the participants from the seven countries. He pointed out that this was the third in a series of the Penaeid Recruitment Programme (PREP) workshops aimed at obtaining a greater understanding of the commercially important Penaeid prawns in the Indo-west Pacific region. The first workshop, held in Cleveland, Australia in 1988, was primarily a planning workshop where existing data on penaeid prawn dynamics were compared across the region and the general objectives and research approaches of the Programme were formulated. This was followed by a workshop in Phuket, Thailand, in 1989, where progress was evaluated and protocols for sampling, data base management and analysis were discussed. The current workshop has resulted from a request by all the countries involved with PREP activities to obtain more training in the taxonomy and identification of Penaeid prawn larvae and postlarvae.

Dr Hill thanked all persons and institutions which had provided facilities and funding for the present workshop. In particular he noted with pleasure the continuing support of the Intergovernmental Oceanographic Commission (IOC) and the Food and Agriculture Organization (FAO). The Australia Marine Science and Technology Project Office (AMSAT) was responsible for all administrative details while the workshop planning and content were organised by Dr D. Staples (Bureau of Rural Resources) and Dr P. Rothlisberg (CSIRO). CSIRO kindly provided workshop facilities including laboratories and equipment.

In concluding his welcome speech, Dr Hill stressed the importance of the Penaeid Recruitment Programme (PREP) for the region and benefits that all participating countries will derive from a collaborative approach to common problems.

2. BACKGROUND AND OBJECTIVES OF PREP

Dr Staples briefly outlined the past history of PREP activities and referred participants to the reports of the two previous workshops (IOC Workshop Report Nos. 56 and 64). He started his talk by showing participants a graph of
banana prawn catches for the Gulf of Carpentaria for the years 1970 to 1990. This time series typifies the type of data on which management decisions have to be made. The data show extreme inter-annual variability with a ten-fold difference between years and an overall downward trend since 1974. The questions raised by these data are:

(i) What causes the wide year-to-year variation in prawn catches and can theses variations be predicted?
(ii) Is the overall downward trend in catches due to overfishing or natural causes?

Because Penaeid prawns are short lived animals, the year-to-year variation in catch is a reflection of the number and size of prawns which recruit into the fishing ground during the same year. This recruitment, in turn, is the end result of chain of events which have affected the survival and growth of the prawns right throughout their life cycle. By understanding these events, scientists are able to provide the type of advice necessary for the rational management of the resource. PREP has been set up to answer these and similar management questions. The overall objectives of PREP are:

(i) Identify the biological and environmental factors which affect recruitment by comparing prawn stock dynamics across the Indo-west Pacific region.

(ii) Separate the effects of fishing from natural variability.

(iii) Provide a basis for rational management.

To achieve these objectives, 10 study sites were set up across the region where all participants are attempting to provide input in the regional study by:

(i) Collecting data on the seasonal patterns of abundance of all life-history stages of three selected species (i.e. Penaeus merguiensis, P. semisulcatus and Metapenaeus ensis.)

(ii) Defining the links between the timing of the different life-history stages to establish the critical time/space windows for quantifying recruitment indices.

(iii) Examining relationships between selected environmental variables and appropriate recruitment indices, both between generations within a year and among years.

(iv) Identifying the causal relationships underlying any significant correlation, including the effects of fishing, climate, ocean and estuarine processes, habitat changes and predation.

(v) Developing environment/stock:recruitment models for predicting changes in recruitment brought about by changes in both fishing pressure and other environmental/biological factors.

(vi) Providing relevant management advice at the national level.

Data collected for the Australian study site were presented to demonstrate why a regional collaborative attempt was necessary to explain the recruitment dynamics of Indo-west Pacific prawns. As pointed out in earlier reports, P. merguiensis in Australia has an apparently complex life history where, although increased spawning activity occurs during the spring and autumn each year, only one of these generations survives to form the basis of the commercial fishery in any given year. On the basis of this seemingly paradoxical result, an hypothesis of two generations and two recruitment events each year in areas closer to the species's origins was generated (Staples and Rothlisberg, 1990). In piecing together the results from other PREP countries, this hypothesis has not been refuted, to date, and has provided an important scheme for understanding the life-history of P. merguiensis in other countries. For example, in the Philippines, it appears that the species mirrors the situation seen in Australia, where one main pulse of recruitment occurs each year despite a bimodal spawning pattern. Closer to the equator in Malaysia, on the other hand, recruitment is much more continuous throughout the year with peaks in spring and autumn, a situation close to the hypothetical life history scheme. In all other countries, an intermediate situation appears to occur, with one or other of the generations dominating.

In assessing the current status of the Programme, Dr Staples highlighted several areas where considerable progress has been made. We now have a better understanding of the basic life history of P. merguiensis, but there are still major gaps in our knowledge. In several countries it has not been possible to establish links between the main life-history stages. One of the constraints has been uncertainty in the identification of species, especially earlier life-history stages. Adult penaeids are relatively simple to identify and the taxonomy is well known but the available species descriptions and keys rely heavily on adult characteristics such as the morphology of male and female genitalia. These characters are of little use in earlier life-history stages and different techniques have to be developed. Two techniques are available to facilitate species identification of these stages viz:- Numerical taxonomy and electrophoresis.

Numerical Taxonomy

Numerical taxonomy is the identification of species by the statistical differences in the size and shape of different parts of the body. Although prawn larvae of the different species look the same to a human observer, careful measurements of different body parts reveal subtle differences which can be used to separate the species. In general a combination of these characters is required for positive identification. The optimal combination for separating any one species can be found using standard statistical packages on a computer. This information can then be used to develop a numerical taxonomic key for routine identification by a trained operator.

Electrophoretic identificationn

Although numerical taxonomy is an useful technique for identifying larvae, continuous changes in the body proportions during the rapid growth stage of postlarvae prevents its use during this stage. An alternative technique is to identify identify species on the basis of their different genetic make-up. Each species has its own characteristic genetic code, and one way to detect genetic differences between species is to examine the enzymes present in the body tissues. The different genetic code results in any one type of enzyme carrying a different electric charge in different species. These enzymes can be separated on a gel plate using electrophoresis. By selecting a combination of different enzymes, species identification is possible. The advantages of the technique include the ability to be able to obtain a positive identification for all individuals, including small prawns down to 1 mm carapace length (total length 1 cm).

Dr Staples concluded his introductory lecture by stressing the importance of correct species identification to PREP. Obtaining the scientific information required by managers is a time-consuming and costly process. Governments as well as private industry are presently investing large amounts of money to this research but none of the objectives of PREP will be realized if mistakes in species identification are being made. It is therefore imperative that scientists in all participating countries are confident with their own abilities and have the necessary skills and knowledge to make these important decisions.

3. WORKSHOP OBJECTIVES

The objectives for the larval/postlarval identification workshop were:

(i) Improve the skills and knowledge of PREP scientists in the techniques used to identify penaeid prawn larvae.

(ii) Improve the skills and knowledge of PREP scientists in the techniques of electrophoretic identification of penaeid postlarvae.

(iii) Provide a basis for the formulation of a regional database on:
- a) Numerical taxonomy of larvae
- b) Electrophoretic identification of postlarvae

At present, there is insufficient knowledge to provide PREP scientists with simple keys and recipes that will enable them to routinely identify larvae and postlarvae. However, after the workshop, it is hoped that participants can return to their respective countries and continue their research and thereby contribute to a regional database which will then become available for all PREP scientists to use a routine tool for species identification. This will require dedication and commitment of at least one full-time scientist in each country for approximately one year. The outputs from such research will be a computerized key for larval identification and an electrophoretic key for postlarval identification of the Indo-west Pacific penaeid prawns.

4. WORKSHOP STRUCTURE AND ORGANIZATION

In order to meet the above objectives, participants were asked to provide information on the occurrence of Penaeus and Metapenaeus species in their countries. Results of the questionnaire are given in Table 1. Many species were common to all countries, thereby supporting the concept of a regional taxonomy database. Areas having more than one species which were morphologically similar, would be expected to have more problems with identifications. These included China (although geographic separation of species from north to south could reduce the problem), Malaysia, Brunei and Indonesia. Participants were also asked to provide samples of the species available in their country. Table 2 shows the response to this request with all countries providing some material, but not all species and life-history stages were represented. This collection of penaeids from all over the Indo-west Pacific, however, provided an unique opportunity to examine the taxonomy of a wide range of species of commercial interest.

Table 1: **(a) Distribution of Penaeus species across the Indo-west Pacific region.**

Country	**Species**											
	Pm	**Ps**	**Pi**	**Pp**	**Pc**	**Pmo**	**Pla**	**Pj**	**Pe**	**Pch**	**Plo**	**Ppe**
China	+	+	+	+	+	+	+	+		+		
Philippines	+	+	+		?	+	+	+			+	
Thailand	+	+				+	+	+				
Brunei	+	+	+		?	+	?	?				
Malaysia	+	+	+	+		+	+	+				
Indonesia	+	+	+	+	+	+	+	+		?	+	
PNG	+	+	+		+	+	+	+				
Australia	+	+	+			+	+	+	+		+	+

Pm = Penaeus merguiensis, **Ps** = P. semisulcatus, **Pi** = P. indicus, **Pp** = P. penicillatus, **Pc** = P. caniculatus, **Pmo**= P. monodon, **Pla** = P. latisulcatus, **Pj** = P. japonicus, **Pe** = P. esculentus, **Pch**= P. chinensis, **Plo** = P. longistylus, **Ppe** = P. plebejus

(b) Samples provided for the workshop

Country	**Species**											
	Pm	**Ps**	**Pi**	**Pp**	**Pc**	**Pmo**	**Pla**	**Pj**	**Pe**	**Pch**	**Plo**	**Ppe**
China	LPA	J		LPA			PJ			LPJA		
Philippines	A	A	A			P	A					
Thailand	LP					LPJA						
Brunei	A	A	A			PJA						
Malaysia	LJA	A				LPA						
Indonesia	LP					A				J?		
PNG	PJA											
Australia	LPA	LPA	L			LA	L		LPJA		LP	L

A= Adult, J = juvenile, P = postlarvae, L = larvae

Table 2: **(a) Distribution of Metapenaeus species across the Indo-west Pacific region.**

Country	Species									
	Me	**Ma**	**Mm**	**Mj**	**Mb**	**Meb**	**Md**	**Men**	**Ml**	**Mbe**
China	+	+	+	+						
Philippines	+	?	?	?	?	?		+		
Thailand	+	+	+	?					+	
Brunei	+	+		+	+				+	
Malaysia	+	+		+	+				+	
Indonesia	+	+			+			+	+	
PNG	+	?		?		+	+	+		
Australia	+	+				+	+	+		+

Me = Metapenaeus ensis, Ma = M. affinis, Mm = M. moyebi, M; = M. joyneri, Mb = M brevicornis, Meb = M. eboracensis, Md = M. demam, Men = M. endeavouri, Ml = M. Ivsianassa, Mbe = M. bennettae

(b) Samples provided for the workshop

Country	Species									
	Me	**Ma**	**Mm**	**Mj**	**Mb**	**Meb**	**Md**	**Men**	**Ml**	**Mbe**
China	A		A	LJA						
Philippines	JA									
Thailand	LA	A	A							
Brunei	A									
Malaysia		JA								
Indonesia	LJA									
PNG	J									
Australia	LA		J			LA		LA		LA

A = Adult, J = juvenile, P = postlarvae, L = larvae

Because of the very different techniques used to identify larval and postlarval penacid prawns, participants were divided into two groups, one concentrating on larval identification and the other on postlarval/juvenile identification. Training for the larval identification was provided by Mr Chris lackson (CSIRO). Mr Don Heales (CSIRO) and Mr Shane Lavery (University of Queensland) provided training on postlarval/juvenile identification.

5. WORKSHOP CONTENT

The following provides a brief account of the material covered during the workshop. More details are contained in the two manuals produced to cover techniques of both larval and postlarval/juvenile prawn collection and identification.

Development of a Regional larval identification key

Larval reference collection

Most of the workshop was devoted to examining the techniques needed for developing a reference database for use in subsequent numerical taxonomy. The first step is to assemble a collection of larvae of all species for the Indo-Pacific region. These should be obtained either from rearing the larvae in the laboratory or collecting them from aquaculture hatcheries. Protocols for collection to provide sufficient sample numbers to account for individual variability and variability within each substage were presented. A number of spawnings from each species and a number of samples within each substage are required (see manual for details). Larvae should then be fixed in 5%
formaldehyde and then later transfered via ethanol (70%), to Polyvinyl alcohol (PVA) mixed with lactic acid and phenol (see manual).

Measurement of characters

Techniques for obtaining a range of measurements from each specimen (characters such as telson length, length of segments on legs etc) were then demonstrated and practiced. All measurements were carried out under a microscope and because the success of this project will rely on having accurate measurements from all countries, several days of the workshop were devoted to this activity. The necessity to cross check and calibrate the relative efficiency of different operators was emphasised. Participants were provided with a computer package which enabled entry of these measurements along with checking and editing facilities. The Programme is menu driven and easy to use and allows the operator to add and edit data.

Development of the larval key

The use of discriminant analysis to define which combination of measurements best separates the different species of larvae was discussed using the statistical package SYSTAT as an example. It was agreed at the workshop that the CSIRO Marine Laboratory in Cleveland should carry out the statistical analysis required to produce a working computer key from the reference data base.

Sampling and Identification of larvae

Sampling and sample handling

In order to collect penaeid larvae quantitatively in the field, normal zooplankton sampling techniques must be modified. The sampling equipment and protocols must take into account: the very low abundances of larvae, their very small size, and their diurnal migratory behaviour. To obtain accurate quantitative estimates of larval abundance sampling protocols must: minimise net clogging, use calibrated flow meters and depth recorders to measure the depth of the plankton tow. These sampling techniques must be coupled with through net washing and immediate on-board sample fixation. In the laboratory, methods for sample splitting prior to larval sorting, curation and biomass were discussed. Details for all these processes are provided in the manual.

Larval identification

Both protozoeal and mysis stages of Indo-west Pacific penaeid prawn larvae can be identified to genus using newly published keys (Jackson et al., 1989; Dall et al., 1990). Species identification, however, can only be carried out using the numerical taxonomy. It is envisaged that all countries will be supplied with a computer identification kit which will enable species identification for PREP species. The system works by an operator being prompted for a particular set of measurements.

If this is sufficient for species identification, the computer returns an identification. Otherwise, the operator is prompted for further measurements until an identification can be made. By this process, up to 100 individual larvae can be identified each day by an experienced operator.

Development of a regional electrophoretic key for postlarvae/juveniles

Collection and storage of postlarvae

The need for correct methods of collecting and storing postlarvae for electrophoretic work were stressed right throughout the workshop. Specimens must be frozen immediately on collection and stored in freezers (preferably at ultra-low temperature -70°C) until analysis. They must not be thawed out at any stage. Storage times should also be kept to a minimum.

Sample preparation and analysis

Participants were shown how to prepare tissue extracts from adult prawns and squash whole postlarvae prior to electrophoresis (see manual). Preparation of the chemicals e.g. buffers and the techniques of running gels were explained. The differential movement of the enzymes were then examined using histological staining and incubation.

Workshop results

The main activity of the week was hands-on training using firstly Australian specimens and subsequently material brought by participants from their different countries. Further work was also carried out by Associate Professor J.H. Xiang and Shane Lavery in the two weeks following the workshop. Tissue extracts were prepared from the adult specimens which were then screened for genetic markers. Seventeen enzyme stains were used to try to discriminate between all 13 species of penaeid prawns. The preliminary results indicated that by examining a combination of 2 or 3 enzymes, all species could be positively identified. An interesting side result is that the electrophoretic identification indicated that some participants had incorrectly identified adult specimens on the basis of their morphology.

Postlarvae were available from 6 species. Unfortunately, many of the specimens which had been brought in from overseas had deteriorated in quality and staining of these was poor. Never the less, most species could be identified using the strongest enzymes.

Sampling and Identification of postlarvae and juveniles

Sampling of postlarvae and juveniles

Sampling of postlarvae and juvenile penaeid prawns requires information on both sampling gear and the prawn's ecology. Standard sampling gear includes beam trawls, beach seines, push nets and various traps such as fish corrals. All these catch prawns but to make the results quantitative, detailed information is needed on the variability of catches in both space and time. This is usually gained from pilot studies which must be carried out for each species and locality. For example, P. merguiensis postlarvae enter estuaries as pelagic animals, settle out in the upper reaches of the estuary (small creeks) and then slowly move back down towards the estuary mouth before beginning their main off-shore emigration. Sampling in different areas in the estuary, therefore, will result in the collection of markedly different number of postlarvae. Postlarvae must be sampled well upstream in the estuary, at the place of settlement. Smaller prawns also tend to live in shallower water such that postlarvae are typically found concentrated in water less than 5 metres from the water's edge.

All species of prawns exhibit distinct cycles of catchability to different sampling gears. Highest catches of P. merguiensis postlarvae in beam trawls occur on the last stage of the ebb tide, just before low tide and sampling must be synchronised to this cycle to provide quantitative results (see details in manual).

Sample sorting

Samples of postlarvae and juveniles require two levels of sorting. At the first level, samples are sorted by hand and all penaeid prawns visible to the naked eye removed (Eye sort). Samples are then subdivided and sorted under a dissecting microscope for postlarval prawns (Microscope sort).

Identification

Any adult prawns in the sample can be identified to species using published keys. Most other specimens can be identified to genera using an extension of the adult characters which do not include sexual characteristics. Newly settled postlarvae, can also be identified to genera using available keys (Jackson _ al. 1989; Dall et al.,1990). Small juveniles between these stages are the most difficult but it is usually possible to distinguish at least genera (see manual). In the genus Penaeus it is also possible to place specimens into species groups e.g. king, tiger and banana prawn groups.

After the electrophoretic keys become available, identification of postlarvae and juveniles of all Indo-west Pacific penaeid prawns should be possible and must be used to resolve the identification down to the species level within these groups.

6 WORKSHOP OUTPUTS AND FUTURE RESEARCH

The main outputs from the workshop will consist of two manuals, one devoted to larval identification and the other to postlarval identification. These will be published by the IOC and available to all interested Institutions. The manuals will cover the techniques necessary to develop the regional databases required for the routine identification of larvae and postlarvae of the Indo-west Pacific penaeid prawns.

Future Tasks

Larvae

In a discussion concerning tasks arising from the workshop, it was agreed that each country should attempt to provide information into a regional data base. Several species groups were assigned the following priorities:

Priority Group 1: Penaeus merguiensis and P monodon

Priority Group 2: P semisulcatus

Priority Group 3: M. ensis

Each country was given the task of measuring all 19-20 characters from specimens sampled as described above and entering data on the computer package supplied. The results should then be forwarded to:

Mr Chris Jackson
CSIRO Division of Fisheries, PO Box 120
Cleveland Qld 4163, Australia

who will be responsible for collating the measurements from the different countries and carrying out the necessary statistical analyses for the development of the regional larval computer key.

Postlarvae and juveniles

It was agreed that each country should:

(i) Set up access to electrophoretic equipment, either by collaborating with other laboratories already set up as an electrophoretic laboratory or purchasing the necessary equipment and chemicals

(ii) Carry out electrophoretic comparisons between local adult and postlarval specimens of:

Priority Group 1: P. merguiensis and P. indicus
Priority Group 2: P. penicillatus, P. caniculatus and P. chinensis
Priority Group 3: P. Iatisulcatus, P. japonicus and P. monondon
Priority Group 4: Species of the genus Metapenaeus.

(iii) Send results of the comparisons to

Dr John Benzie
Australian Institute of Marine Science.,
PMB No 3
Townsville Qld 4810
Australia

The results should be examples of typical gels. They may be in the form of (in order of preference): (a) photographs (b) photocopies or (c) drawings.

They should be annotated to provide the following details: (a) location of sample application; (b) species and developmental stage (adult/juvenile/postlarvae); (c) enzyme; and (d) electrophoretic conditions (gel type, buffer, run-time and voltage).

Additional useful information to be supplied could include: number of individuals of each species used, average relative mobilities for each species for each enzyme locus (relative to _ merouiensis = 100) and relative mobilities and frequency of occurrence of any rare alleles detected at any enzyme locus for a species.

(iv) Frozen samples of all species tested should also be sent to Dr John Benzie at the Australian Institute of Marine Sciences. This material will be used to standardise between countries.

As stated in the workshop objectives, the most important outcome of the workshop will be the development of regional databases which can then be used by all other PREP scientists in the routine identification of larvae and postlarvae. This will be coordinated by the PREP Technical Coordinator, Dr Derek Staples. Each country has been requested to contribute to this process by supplying data (character measurements in the case of larvae and electrophoretic results in the case of postlarvae/juveniles). These will be collated in Australia and made available to all PREP countries as they develop. Computer software will be provided by Australia. It is envisaged that this process will take approximately one year and the discussion of the results and their interpretation will be included as part of next year's PREP workshop which will be cover techniques for analysis of recruitment data.

ANNEX I

PROGRAMME OF THE WORKSHOP

Sunday 23 September:

1800 Reception

Monday 24 September

Welcome Speech - Dr B. Hill (O.I.C. Cleveland)
General Introduction - Objectives of Workshop
Derek Staples
Identification of larvae - An overview of methods
Chris Jackson

Identification of postlarvae - An overview
Shane Lavery

Concurrent Sessions
A. Larval identification
B. Postlarval identification

Tuesday 25 September

Concurrent Sessions
A. Larval identification
B. Postlarval identification

Wednesday 26 September

Concurrent Sessions
A. Larval identification
B. Postlarval identification

Workshop Dinner

Thursday 27 September

Concurrent Sessions
A. Larval identification
B. Postlarval identification

Friday 28 September

Concurrent Sessions
A. Larval identification
B. Postlarval identification

General Conclusions and Discussion
Report and recommendations
Derek Staples/Chris Jackson/Shane Lavery

ANNEX II

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