Bay of Bengal Large Marine Ecosystem Project

Report of the

Indian Mackerel Working Group Meeting

28-29 May 2012 • Colombo Sri Lanka

BOBLME-2012-Ecology-05

BOBLME contract: PSA-GCP 32/2/2012

For bibliographic purposes, please reference this publication as:

BOBLME (2012) Report of the Indian Mackerel Working Group Meeting, 28-29 May 2012, Colombo, Sri Lanka: BOBLME-2012-Ecology-05

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1. OPENING OF THE MEETING AND ADOPTION OF THE AGENDA

1 A Workshop on assessing the data and assessment potential on Indian Mackerel (*Rastrelliger kanagurta*) was held on 28th and 29th of May, 2012 at Colombo, Sri Lanka. The BOBLME Stock Assessment Coordinator, Dr Rishi Sharma welcomed the participants and wished them well in their work.

2 Dr Sharma reminded the meeting that BOBLME Project is mandated to develop regional fishery assessments for Indian Mackerel, and this meeting was the third one to that end. The focus of the meeting was to develop a region wide stock structure study to understand how Indian Mackerel stocks are distributed in the region.

3 The meeting was opened by Dr Haputhantri (NC for Sri Lanka).In his address he, highlighted the lack of information on the species and stocks which contribute to the fishery in different parts of the bay. Dr Haputhantri also noted that this is an important meeting for the region to assess the transboundary nature of stocks within the region. The rest of the meeting was chaired by Dr Rishi Sharma.

4 The participants of the meeting are listed in Appendix I and the agenda for the Meeting was adopted as presented in Appendix II.

5 Dr Sharma informed the meeting about the scope of the project, and how far the work has proceeded. The agenda was adopted (Appendix II); and the participants were introduced.

6 The list of documents presented to the meeting is given in Appendix III.

2. STOCK STRUCTURE APPROACHES AND CONSEQUENCES ON MANAGEMENT

2.1. Indian Mackerel Fisheries Assessment in Bay of Bengal (Thailand):

7 The distribution of *Rastrelliger brachysoma* and *Rastrelliger kanagurta* overlap in Thailand waters to a greater degree than on the Indian side of the Bay of Bengal. This can lead to difficulties distinguishing the two species in fisheries samples. A third species *Rastrelliger faughni* also overlaps range with the other two species. Studies used 22 morphometic characters and multivariate analysis to separate the species do not work well Instead a simple relationship between head depth (HD) and standard length (SL) provides a better measure for separating *R. brachysoma* and *R. kanagurta*. To separate *R. faughni* from the other two species gill raker counts can be used. This means that sampling *Rastrelliger* spp. for genetic stock structure should require sampling fish over 16 cm if *R. kanagurta* is the target species. It is suggested that the sampling objectives consider initial identification determined from morphometric relationships followed by the use of cytochrome b to determine genetic differences along west coast of Thailand. Ten possible sites have been identified along coastal Thailand coast as well as possibly 50 fish taken north of Phuket and 50 fish taken south of Phuket. Lab capacity allows analysis of RFLP, AFLP, and microsatellites.

2.2. Indian Mackerel Fisheries Assessment in Bay of Bengal (Malaysia):

8 Malaysia has two institutes that would coordinate the study in this region. They are the Fisheries Research Institute (FRI) and Southeast Asian Fisheries Development Center (SEAFDEC).

9 The Fisheries Research Institute (FRI) has national responsibility for management of Indian Mackerel and it is a important food fish commonly used in south and south-east Asian cuisine. It is found in warm shallow waters along the coasts of the Indian and west Pacific Oceans and their surrounding seas. The population structure is largely unknown in the entire BOBLME region. Information on spawning areas and seasons is also lacking. A project to generate new information and knowledge on Indian Mackerel stock structure would

facilitate better management of the fisheries. The plan by FRI is to design a sampling plan, undertake the sampling, and arrange for the genetic testing and report the results. Data will be collected from five sampling sites along the west coast of Peninsular Malaysia: 1) Kuala Perlis (Perlis), 2 Kuala Kedah (Kedah), 3 Banga Panchor (Perak), 4 Hutan Melintang (Perak), and 5 Port Kelang (Selangor). A total of 200 specimens will be collected, 100 from trawlers and 100 from purse seiners. The sampling period is three months. Tissue samples will be preserved in 95% ethanol in individual vials. The whole fish will be preserved in 10% buffered formalin and stored at FRI. Cytochrome b will be amplified using primers and then sequenced for each specimen. Phylogenetic analysis, parsimony and maximum likelihood algorithms will be used to determine stock structure. The sampling work will be carried out by the staff of the Fisheries Resource Section of the Marine Research Center. Genetic analysis will be undertaken at the FRI, Batu Muang, by Dr Masazurah Abdul Rahim. During the sampling period, the sampling team and supervisor(s) will meet at the end of the first quarter to summarize progress and discuss problems and issues. Quarterly reports will be submitted to BOBLME project. The sampling program for Indian Mackerel will record weight, length-frequency data (body, fork and standard lengths in mm), gonad maturity and weight data, analyze the findings and report on the results. A total of 600 individuals will be collected. At least 50 samples collected monthly over a twelve-month period. Samples will be kept on ice, the type of fishing and location of fishing operation recorded. Total weight, length, body length, fork length, and standard length in mm and gonad weight will be recorded for each sample. Laboratory analysis will identify sex, maturity (5 stage scale).

10 The SEAFDEC has a main objective to ascertain if economically important pelagic species in the South China Sea and the Andaman Sea contain sub-populations or are one panmictic population. Depending on the stock unit determined from tagging information, morphometric studies, and genetic data, this would inform fisheries management planning. Life history is important to understand. For small pelagic fish like Indian Mackerel, adults aggregate to spawn and fertilize their eggs. The eggs hatch into pelagic larvae that drift and disperse with the currents to nursery areas where they feed and grow. Juvenile and young fish move through migration routes possibly returning to the same locations to spawn. If this is so then we would expect to see separate populations. There may be more than one spawning area with exchange or overlap between spawning areas. We need to clarify the genetic, biological, and ecological properties of fish populations. We propose sampling 11 South China Sea sites and four in the Andaman Sea. Andaman Sea sample sites would be from Banda Acheh (Indonesia), Pangkor (Malaysia), Yangon (Myanmar) and Ranong (Thailand) taking 35 fish from each site. The molecular marker used would be mtDNA cytochrome b. The amplified products from primers RBCyF and RBCyR would be sent to a private laboratory for DNA sequencing. Haplotype frequency of the 30 samples from each sampling site will be identified and the differences between sampling sites assessed using Fst. Amova using ARLEQUIN would be used to examine the genetic structuring between samples. Results from four Malaysian sample locations for Indian Mackerel shown. Both microsatellite and mtDNA are informative for population studies. However if migration occurs between populations then no stock structure will be detected.

2.3. Indian Mackerel Fisheries Assessment in Bay of Bengal (India):

11 India had three institutes that would coordinate this study in the region. They are the Central Marine Fisheries Research Institute (CMFRI), the National Bureau of Fisheries Genetics Resources (NBFGR) and the Fisheries Survey of India (FSI). They gave an overview of the issues in the following order.

12 CMFRI gave a quick overview of their projects. Marine fisheries are very important to the Indian economy. We have a coastal length of 8,129 km, a continental shelf area of 0.452 million km^2, and an EEZ of 2.02 million km^2 which produced 3.2 million tonnes of fish in 2009. CMFRI has five field centers along the coast so sampling of Indian Mackerel can be directed from these field centers. Sampling design should consider both temporal and spatial effects based on knowledge of migratory patterns, breeding season, and breeding grounds in order to get true representation of breeding populations. Direct sampling from landing centers may not be truly representative of breeding population because of variation of fishing patterns, gear type, and other factors. Scale of sampling could represent the country as a whole (India), individual States, fishing zones, or finally landing sites. Each State may have between 26-352 landing centers representing 158 to 1,076 km of coastline.

13 Selection of sampling scale might be quite broad since it is known that pelagic fish tend to exhibit little intraspecific genetic structuring. Marine environment with ocean currents and apparent lack of physical barriers facilitate high levels of gene flow. There is potential for long distance dispersal and the large population size because both Indian Mackerel larval and adult stages contribute little opportunity for genetic subdivision. If subdivision into different populations does occur it could be caused by past sea-level changes or barriers caused by ocean currents. Increasing geographic distance is expected to enhance isolation between populations. Population structuring can also be caused by life history traits such as homing to spawning areas or larval retention. Little is known about migration patterns of Indian Mackerel other than it is widely distributed throughout the Indian Ocean. Understanding genetic stock structure is important for scientific-based resource management and potentially marine stock enhancement program.

14 Four marker types are suggested for use: microsatellites, cytochrome b, control region, and SNPs. So far cytochrome-b and control region have been used for Indian Mackerel species. Microsatellites markers in closely related species could be developed and used. The advantage of microsatellites is that they can be developed for faster mutating nuclear DNA and can be highly polymorphic in nature. SNPs would need to be initiated and developed. Examples of species identification analytical steps using cytochrome b and barcoding Indian tunas using mtDNA COI gene were given.

15 Institutional projects include barcoding, taxonomy and genetic stock structure. Whale shark population structure has been determined using microsatellites and mtDNA control region markers. Other studies include species specific marker developed for bivalves and sardine genetics. A total of twelve scientists and 17 PhD students work in the division.

16 The NBFGR Kochi Unit is on of three NBFGR facilities. The mandate of NBFGR is to collect, classify, and catalogue fish genetic resources in India. Also it is a mandate to maintain and preserve genetic material for the conservation of endangered species as well as the evaluation of introduced species to Indian freshwater and marine ecosystems. So far 23 species, including 11 marine species has stock structure delineated using a combination of microsatellites, mtDNA, allozymes, RAPDs, morphometrics, and life-history characteristics. For example, White fish (*Lactarius lactarius*) and Bombay duck (*Harpadon nehereus*) were both found to form two distinct stocks over the study area. Work on Indian white shrimp (*Fenneropenaeus indicus*) were found to occur as four distinct genetic groups, Arabian Sea, south-east and south-west India, eastern Bay of Bengal, and the Andaman Islands, using 16 microsatellite markers. Another study of black tiger shrimp (*Penaeus monodon*) from southwest, east Bay of Bengal and the Andaman coastal waters indicated surprisingly high degree of stock structure determined by 10 highly polymorphic microsatellite markers. Other species studies include the Sea horse (*Hippocampus trimaculatus* and *Hippocampus kuda*) were found to be genetically distinct while lobsters (*Panulirus homarus* and *Thenus unimaculatus*) and bull's eye (*Priacanthus hamrur*) were found to be genetically identical in

spite occurring over large geographic distances. A total of 81 microsatellite markers have been found by the lab for Indian white shrimp, 21 for lobster and 10 in sea cucumber.

17 The strategy proposed by NBFGR is to sample between 70-80 individuals at landing centers over three sampling intervals. A total of 25 polymorphic microsatellite markers with up to 45 alleles should be developed. Marker development requires cross-priming non-target species and the use of a genomic library. Candidate markers will be run with silver staining to ensure amplification and polymorphism then confirmed through sequencing two or three individuals. Large scale genotyping of samples require M13 tailing (Taqman assay) and multiplexing between 70-80 individuals per population. Next data is processed through Genemapper and allele calling. Once compiled across study area the data can be analyzed using a number of programs such as Genepop, Genetix, Arlequin, Microchecker and Bottleneck. Also 30-35 individuals per population could be run using ATPase 6/8 gene of (827 bp) mtDNA.

18 The NBFGR lab has also initiated Barcoding. A total of 450 teleosts (300 marine species), 12 species of marine lobsters 60 species of deep sea sharks, one species of whale shark, and 30 species of marine crabs and sea stars as an example the lab also conducts annual training programmes on population genetics and molecular markers for capacity building of research scholars and teachers.

19 The FSI is responsible for exploratory surveys and monitoring the Indian EEZ Area which is 2.02 million km^2. It covers this area with seven bases using 12 vessels (4 long liners. 7 stern trawlers, 1 eco-friendly boat). It is responsible for the stock assessment of all demersal fisheries resources and undertakes collaborative projects with CMFRI for Oceanic tuna and squid. We also experiment with eco-friendly fishing gear. Sampling programs use either stratified random sampling or spatio-temporal sampling. Onboard lab facilities provide equipment such as digital scales for weight and verni-calibers for measure length. Survey work has lead to the discovery of first reported occurrences of a number of species in Indian waters such as arrowfin bigeye (*Priacanthus sagittarius*) and the Japanese tsunogashria (*Ostracoberyx dorygenys*).

2.4. Indian Mackerel Fisheries Assessment in Bay of Bengal (Maldives):

20 Small pelagic fish including Indian Mackerel account for around 1% of catch by weight in the Maldives. Maldivian fisheries are heavily dependent on Tuna. Sample for genetic analysis could be collected the fish market in Malé. Fish are landed daily so it should not be a problem getting samples during any season. Another option is to collect samples throughout the Maldives at different locations if there is some thought that there might be local stock structure. As far as running genetic samples, right now the Maldives does not have the capability to analyse genetic samples so would have to cooperate with established laboratories elsewhere for sample processing. As Maldivian fisheries are heavily dependent on tuna, small pelagic fish, including Indian Mackerel, account for only around 1% of catch by weight. For any genetic analyses of Indian mackerel in the Maldives region, sampling is proposed to done at the fish market in Malé, depending on the presence of Indian mackerel in the landings of small pelagics. Another option is to collect samples throughout the Maldives at different locations with the help of yellowfin tuna fishermen, if there is some thought that there might be a local stock structure. However, this would require coordination and reliance on the fishermen to obtain a good sample. As far as analyzing the samples, Maldives currently lacks the capacity for such work and hence, would need to cooperate with laboratories elsewhere for sample processing.

2.5. Indian Mackerel Fisheries Assessment in Bay of Bengal (Myanmar):

21 Myanmar coastline extends along 2832 km. of the north-east side of the Bay of Bengal, bordering Bangladesh on the northern coast and Thailand on the southern coast. Three main fishing areas (< 200 m depth) from north to south are Rakhine with an area of 27,406 km^2 with an estimated 175,000 tonnes (87,500 msy) biomass of pelagic species, Ayeyawady an area of 103,525 km^2 with 505,500 tonnes (252,750 msy) of estimated biomass, and Tanintharyi an area of 94,756 km^2 with 295,000 tonnes (147,500 msy) of estimated biomass. Mackerel species (*Rastrelliger kanagurta* and *Rastrelliger brachysoma*) are caught mostly in purse seines and surrounding gillnets and sometimes bottom trawls both near-shore and off-shore in Myanmar coastal areas. Near-shore areas are defined as less than five nautical miles from shore (Rakhine coastal) and less than 10 nautical miles from shore (Ayeyarwady and Tanintharyi). Drift gillnets, Trammel nets, and small purse seines can be fished in the near-shore areas using boats less than 30 feet in length with not more than 12 h.p. engines. Trawl, purse seine, surrounding net, drift net, and longline can be fished in the off-shore areas out to the EEZ using larger boats. There are two major fishing grounds for *Rastrelliger kanagurta* (Rakhine and Tanintharyi coastal areas) and one minor fishing ground (Ayeyawady coastal area). The fishing season for Indian Mackerel in Rakhine and Ayeyawady occurs between November to February and for Taintharyi occurs between November to April. Total catch of Indian Mackerel was 14,207 tonnes in 2009-2010, 19,357 tonnes in 2010-2011 and 11,853 in 2011-2012 (to December) tonnes.

22 Myanmar does not have the knowledge or technical expertise for genetic marker development and stock structure identification for Indian Mackerel. Myanmar relies on organizations such as FAO, SEAFDEC and BOBLME for information sharing, encouraging research and development of long-term monitoring programs such as assessment of Indian Mackerel fisheries resources, development of appropriate technology, and stock assessment training for institutions and stakeholders. For example, in 2008-2010 the Department of Fisheries Myanmar in cooperation and collaboration with ASEAN-SEAFDEC under Japanese Trust Fund carried out a tagging program for *R. brachysoma* and *R. kanagurta*. Myanmar has a rich Mackerel fishery resource and with sufficient support will be able to maintain it on a sustainable basis.

2.6. Indian Mackerel Fisheries Assessment in Bay of Bengal (Indonesia):

23 Indian Mackerel is highly exploited in the southern part of BOBLME project area in Indonesia waters. Main fishing areas include West Sumatra (Sibolga), Banda Aceh, and North Sumatra (Idi Rayeuh, Tanjung Balai). The objective of the study is to use mitochondrial DNA to determine genetic structure and estimate stock units for Indian Mackerel found in Indonesian. Outputs from the analysis would be size of the restriction fragments, restriction sites, and the distribution of haplotype frequencies. Tissue samples will be taken from the dorsal part of the fish from three landing sites for about 50 individuals. The samples will be stored in tubes containing 70% ethanol, labeled with date of sampling, location, and individual characters of the sample. Sampling period will be directed at the spawning season starting in August. RFLP steps and data analysis are outlined. Similar work has been conducted on Indian Scad (*Decapterus russelli*) and Shortfin Scad (*D. macrosoma*) around Java Sea including Sunda Strait, Makassar Strait, Flores Sea, and Banda Sea; and Mackerel Scad (*D. macarellus*) around Sulawesi including Makassar Strait, Flores Sea, Banda Sea, Tolo Bay, Tomini Bay, Molluca Sea and Sulawesi Sea.

2.7. Indian Mackerel Fisheries Assessment in Bay of Bengal (Bangladesh):

24 Three main species of mackerel were recorded in Bangladesh marine waters: *Rastrelliger kanagurta,Scomberomorous guttatus,* and *Scomberomorus commerson*. While *R. kanagurta* inhabit depths of 50 to 100 m, *S. guttatus* and *S. commerson* tend to be shallower, from 10-

50 m. The actual sizes of Indian Mackerel stocks are not known in Bangladesh but is thought to comprise 70% of the commercial catch in the Bay of Bengal. Catch figures for 2003-2004 indicate the 57.4 tonnes were caught of which 31.8 tonnes from Coz's Bazar and 25.6 tonnes from Chittagong where fisheries where mainly concentrated at the 40-100 m depth range. The trawl survey of *R. kanagurta* standing biomass accounted for 1826 (±42) tonnes indicated that 10.5% was caught in 10-20 m, 10.8% was caught in 20-50 m, 21.6% in 50-80 m and 57.2% in 80-100 m. *R. kanagurta* is mainly caught in drift gillnets (95%), longline (4%), and set bag nets (1%). Poor landings were observed during January-February while peak landings occurred in November and July. Highest observed concentration of Indian Mackerel occurred at 20°15'N and 91°20'E in the Bay of Bengal. Recruitment occurs during two pulses one in March-May and another in September-October. A comprehensive assessment is needed on the stock structure of Indian Mackerel but financial and technical support is needed to conduct this work.

2.8. Indian Mackerel Fisheries Assessment in Bay of Bengal (Sri Lanka):

25 Indian mackerel is not a major target fishery but is taken as minor bycatch. Small mesh gillnets and beach seines are the major gear types used to catch Indian Mackerel. Collections of a total of 200 samples will be made from all around Sri Lanka. Sample sites will be Jaffna, Mannar, Kalpitiya, Chilaw, Negombo, Beruwala, Galle, Hambantota, Batticaloa, and Trincomalee. Tissue collections for genetic analysis will be taken from the muscle and from fin clips. Samples will be stored in 90% ethanol and kept at 4°C. DNA extraction will be done according to standard phenol chloroform methods and molecular markers to be used could include micosatellites, SNPs, and cytochrome b. Data analysis could use the parsimony and maximum likelihood methods. The NARA lab has capability for sample preservation, DNA extraction, PCR analysis, PCR product purification, and basic microsatellite analysis. Studies conducted in the laboratory include stock structure of tiger prawns (*Penaeus monodon*), molecular identification of stranded whales and dolphins, identification of jellyfish species using DNA sequencing, barcoding important marine fish, confirmation of marine sponges identity, and genetic differences between wild-cultured Barramundi (*Lates calcarifer*).

2.9. Information Needs for a Defensible Stock Assessment – Dr Rishi Sharma

26 Dr Rishi Sharma gave a overview on the BOBLME Project and mandate. Crucial to this are the stock assessment components which are essential to the entire project success. This is why the meeting and Indian Mackerel assessment is extremely important in the region, and why its important to understand the stock structure as it accounts for the transboundary nature of the species in the area. In developing the overall stock assessment model for the region once the stock structure is understood, essential elements are CPUE and effort at the resolution that mimics the stock and the life-history of the species. A basic stock assessment was presented using a Surplus Production (SP) Model and how B_{MSY} would be estimated as well as elements of a age-structured assessment were presented. Methods that use different sources of data from different countries and gear-types could be integrated into the overall fitting procedure using Maximum Likelihood Estimation (MLE) technique, and depending on the stock structure could be kept as separate populations or shared amongst countries. Essential in this would be to stratify catch and effort by gear, sector and country. In this manner fleet catchability could be assessed as well so we could compare effort controls, and a desired outcome in fishery yield.

2.10. Stock Structure Studies-Designs, Desirable Attributes, and Case studies (John Candy, DFO, Nanaimo):

27 In order to be successful at stock structure analysis conditions must exist in the species under study that allows the development of stock structure by limiting gene flow. This can be the

result of specific spawning areas for different groups or populations of fish where there is little interchange between different sites over a period of time allowing for differentiation to occur. The sampling must successfully sample each of these groups independently. Finally marker sets must provide sufficient information content to detect these genetic differences. Restricted gene flow between populations can occur for a number of reasons; "isolation by distance" just means that populations further apart are less likely to exchange with one another than populations that are closer together. Geographical barriers, oceanographic features, and temporal effect such as spawn timing can all play a role by inhibiting geneflow.

28 The sampling program can be done in a phased approach where widely spaced geographical locations are sampled first and then locations can be filled in if there is evidence sufficient differentiation between the more distant sites. Spawning individuals should be targeted and multi-year sampling will provide a measure of annual sampling variability. If possible individuals should be sampled separately so corresponding biological data can be matched.

29 A number of marker types are possible for the analysis of genetic stock structure. Previously marker types like allozymes, RFLP's have been used. Recently microsatellites and SNP's have been found to provide the most power at differentiating populations. However finding a set of useful robust markers requires a lot of work. Fortunately, sequencing costs have become cheaper and new technologies are making marker discovery simpler.

30 We are generally looking for a set of 12 to 20 good highly polymorphic microsatellites or between 70 and 100 SNPs to provide sufficient power to detect genetic stock structure if it is present. On option to test the detection power of a set of markers may be to use simulated data where migration and mutation rates can be fixed and different sets markers projected forward through may generations using a program like EASYPOP.

31 Some case studies were presented on Herring and Pacific Salmon by Mr Candy.

32 Herring is a species on the north-west coast of North America that has may similar life-history characteristics to Indian Mackerel, both are near-shore spawners, free larval dispersion, and potentially extensive rearing areas. Tagging studies indicate that Pacific Herring has a relatively low fidelity to spawning location. To determine genetic stock structure of Pacific Herring we use 15 microsatellites ranging from 27 to 65 alleles. Homogenization of allele frequencies are thought to be a result of multiple-year spawning for individuals and low spawning fidelity resulting in between location F_{ST} less than 0.014. However some population structure exists probably due to temporal and spatial isolation. Both Alaska and California out-populations for our study show significant genetic distances between those populations sampled in British Columbia. A few geographically isolated populations occurring at the head of long inlets or at those locations with different spawn time were significantly different as well.

33 Pacific salmon are considerably different than the marine species like Pacific Herring in that they return to fresh water to spawn (anadromous). Different populations are identified by their natal river system and depending on the salmon species freshwater life-history characteristic can vary. The differences in life-history helps explain the degree of genetic stock structure found in different salmon species. The greatest degree of genetic stock structure occurs in sockeye salmon with the most complicated life-history requiring a lake to rear for up to one year or more (F_{ST} ~0.10). Accurate homing is required to successfully locate the natal lake limiting the amount of staying between populations. At the other extreme are pink salmon which tend in gravel near the river mouth so can successfully stray between river systems tending to homogenize allele frequencies (F_{ST} ~0.001).

3. INDIAN MACKEREL (*Rastrelliger kanagurta*) GENETIC STOCK STUDY RECOMMENDATIONS AND WORKPLAN

3.1.1. Capacity Building in Microsatellite Marker technology

34 There is a strong need for capacity building to support the implementation of a standardised set of microsatellite markers for the region. While the status and current knowledge may be sufficient in some countries, a set of standard markers needs to be developed across all labs and adopted regionally. The countries were all supportive of an integrated stock structure plan in the region that would standardise the markers and have a comprehensive plan for data assessment.

Recommend

- ***Provide workshops and trainings on marker identification, standardization of markers across labs, sample design and tools to analyse the data.***
- ***Create a regional and national pool of experts that would facilitate development of stock structure studies in the region.***

3.1.2. Develop a Sampling plan for the region to understand the stock structure of Indian mackerel

35 All countries expressed concerns of unilateral implementation of genetic study that were not standardised in other areas if this were a common shared stock for the region. Using a standardised set of markers that would identify the stocks in the region would be important. In addition systematic spatial and temporal sampling would be important to get representations of all stocks in the region and coordinated with other stocks from out of the basin.

36 The outline of a basic sampling plan was discussed and developed at the meeting (Appendix IV).

Recommend

- ***Marker development along with sampling done by all countries and SEAFDEC.***
- ***Additional samples taken to avoid tissue contamination/damage/for marker development at each location.***
- ***Given the roughly 10,000 km of coastline in the Bay, the WG recommended at least 25 sampling sites with across the Bay at 400 km distance spacing.***
- ***The sites will be developed by each country and then coordinated across the entire region.***

3.1.3. Develop Harmonization procedures for processing tissues across labs

37 Once the markers are developed, include a study that would process tissues across all labs to test for accuracy of the processing of the tissue samples.

Recommend

- ***Train people on the processing markers for the region.***
- ***Implement blind test on marker identification across labs.***

3.1.4. Develop a standardised short and long term program for sampling across the region for stock structure

38 A long discussion occurred on the range and existence of Indian Mackerel in this region, and how dynamic the BOBLME ecosystem and varied the ecosystem is from one year to the next. The systems that need to be covered with one or 2 sampling sites and representative temporal sampling are shown below:

i. India-Gulf of Mannar, Palk Bay, Coromandel Coast, Andaman Islands, and Orrisa
ii. Sri-Lanka-Jaffna Peninsula
iii. Bangladesh-Chittagong and Cox bazaar coastal areas
iv. Myanmar-Rakhine Coast (Thantwe District) and **Tanintharyi** Coast (Kawthaung District).
v. Thailand- Mergui Archipelago, and Andaman Sea.
vi. Malaysia-West coast (Langkawi Island to Port Klang)
vii. Indonesia- Banda Aceh coast (Aceh Province, North Sumatra and West Sumatra)
viii. Maldives islands for partial coverage when the mackerel are seen in the atolls.

Recommend

- ***Study should be implemented with a comprehensive sampling plan for the entire region.***
- ***BOBLME and Genetic expert will help in developing the sampling plan for the region along with the countries help.***

3.1.5. Workplan and timeline for the project

39 Other items were discussed and are shown below:

a. MOU for sharing tissue in the region (Intellectual Property Rights) for BOBLME project required for tissue, data sharing, and marker sets.
b. Myanmar will need to develop a sampling plan with SEAFDEC to cover the coastal areas.
c. India will submit a plan and budget for marker development and sampling along the coast and Andaman Nicobar islands (contact Dr Ayappan, ICAR Director).
d. LOA's will be completed by end of June, 2012 for Malaysia, Indonesia, Sri Lanka and Thailand. If India, Bangladesh and Maldives send something then we will complete that as well.
e. Bangladesh will send us a plan to collect data (contact Mr Kibria).

40 The timeline was discussed (Appendix V) and recognizing the need for marker development several options were discussed:

f. Using a dissertation in Malaysia that may identify the markers on a study done in Australia.
g. Develop the markers in-house.
h. Outsource the development to a US lab for $8,000.

41 All three options will be pursued in this project and will follow the time line shown in Appendix IV.

3.2. Stock status advice for Indian mackerel in BOBLME region

42. The workshop conducted in Sri Lanka did not improve on any information presented in India in December of 2011. As such we considered the range of information available back then, and adopted the same stock status advice for the regional Indian Mackerel fish-stock in the Bay of Bengal from the previous meeting.

The stock status of Indian mackerel (*Rastrelliger kanagurta*) is unknown.

All countries except Maldives catch Indian mackerel (even Maldives do in certain time and areas), but it is uncertain whether this species is one large stock or whether two or more sub-stocks exist. For example, it is possible that Indonesia, Malaysia and Thailand may be fishing one stock and India/Bangladesh/Myanmar or India and Sri Lanka may be fishing another.

In the eastern areas of the Bay of Bengal it may be confused with the short mackerel (*Rastrelliger brachysoma*). The minimum current catch estimate is around 174,570 t in 2009 (India 58097 t, Myanmar14207 t, Thailand 23337 t (Average of 2005-2007 landings), Indonesia 20000 t (based on equal split of data shown in Hariati and Nugroho, 2010 study of 40000 t), Sri Lanka 400 t, Malaysia 56520 t, and unknown number from Bangladesh). FAO data estimates the average landings across India, Thailand and Indonesia in the BOB region to land 47887 t on average between 2004-2009.

No conclusive stock assessments are currently available, though FISAT/Elefan based methods suggest a huge amount of variation in both the length at age methods and exploitation rates observed in the fisheries ($F= 6.78$ in Thailand, $4.93<F<6.13$ in Malaysia, 3.24 in Indonesia in 2009). The huge amount of disparity is primarily a function of length at first capture, and while F of 3.24 maybe sustainable, F of 6.78 with M between 1.1 and 1.5 maybe difficult to sustain.

While the current catch trends show fairly stable catches over the region, this rate may not be sustainable if small changes occur in temperature in the region. Indian Mackerel is a r-selected species with a high fecundity and short life-span (high growth rate) and could possibly sustain these rates currently, but it is not known if this is sustainable in the long run.

ADOPTION OF THE REPORT

The Report of the third meeting of the BOBLME Indian Mackerel Fisheries Assessment Working Group in Colombo, Sri Lanka was adopted by email on July 5th, 2012.

Appendix I LIST OF PARTICIPANTS

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Sri Lanka-NC	BOBLME RCU
Dr S S K Haputhantri Research Officer/ Marine Biological Resources Division, NPC/BOBLME National Aquatic Resources Research and Development Agency (NARA) Crow Island Mattakkuliya Colombo 15 Sri Lanka sisirahaputhantri@yahoo.com Tel: +941 125 210 00 Mob: +947 182 465 35 Fax: +941 125 219 32	**Dr Rishi Sharma** Stock Assessment Coordinator Bay of Bengal Large Marine Ecosystem Project (BOBLME) C/- Andaman Sea Fisheries Research Development Center 77 Moo 7, Sakdidej Rd. Makham Bay Amphur Muang, Phuket 83000 Thailand rishi.sharma@boblme.org Tel: +66 76 391 861 Mob: +66 844 395 209 Fax: +66 76 391 864

Appendix II Agenda

BOBLME INDIAN MACKEREL FISHERIES ASSESSMENT WORKING GROUP

Colombo, Sri Lanka May 28th and 29th, 2012.

Programme- Day 1, May 28th.

9.00	Registration
9.05	Ice breaking/Self introduction
9.10	· Welcome Address &Introduction to Assessment Workshop on Indian Mackerel Fisheries Working Group from BOBLME- Dr Rishi Sharma · Address by NC/ (Dignitary) Sri Lanka to the meeting
9.30-10:30	John Candy, Senior Scientist, Fisheries and Oceans Canada, Pacific Biological Station Genetics Lab, Nanaimo, BC, Canada. Requirement for Successful Stock Structure analysis (20 min) Case studies from British Columbia (20 min)
10:30	Country Report on Genetic study planned and Lab capability for undertaking study: Thailand
11.00	Tea break
11.15	Country Report on Genetic study planned and Lab capability for undertaking study: Malaysia
11.45	Country Report on Genetic study planned and Lab capability for undertaking study: India
12.15	Country Report on Genetic study planned and Lab capability for undertaking study: Maldives
12:45	Lunch
13:45	Country Report on Genetic study planned and Lab capability for undertaking study: Myanmar
14:15	Country Report on Genetic study planned and Lab capability for undertaking study: Indonesia
14:45	Tea Break
15:00	Country Report on Genetic study planned and Lab capability for undertaking study: Bangladesh
15:30	Country Report on Genetic study planned and Lab capability for undertaking study: Sri Lanka

16:00	Overview of Laboratories and Coordination for such a study Examples from learned experiences: John Candy (Status of Genetic Labs west coast North America)
16:30	Casual Discussion Poster Session

Programme- Day 2, May 29th

9.00	Day 1 Discussion- Rishi Sharma/John Candy
9.30	Laboratory Standardization Issues: J. Candy -Marker types and marker ascertainment
10.00	Sampling: J. Candy-Field Sampling for Stock Structure
10.30	Tea Break
11.00	Discussion on BOBLME stock Structure study for Indian Mackerel: Data needs, Sampling and Analysis.
12.30	Lunch
13.30	Rishi Sharma/ Stock Assessment model Framework following stock structure studies.
14.00	Databases John Candy: Common databases and potential GSI applications (20 min)
14.30	Next Steps/Follow Up
15.00	Tea Break
15:30	Wrap Up- Concluding Remarks
16.00	End

Appendix III LIST OF DOCUMENTS PRESENTED TO THE MEETING

Presenter	Title
Dr Haputhantri	Introduction and welcome to Indian Mackerel Workshop in Colombo
Dr Rishi Sharma	Indian Mackerel Working Group: Data Needs and Alternative Approaches to Assessment
Mr John Candy	i) Needs for successful stock structure studies with some case studies ii) Marker Types and Marker discoveries iii) Overview of integration across genetic labs in North America. iv) Sampling for Genetic stock structure studies v) Standardization, Databases and GSI.
Indonesia-Country Report	Country Report on Genetics Study Planned and Lab. Capability
Sri Lanka- Country Report	Country Report on Genetic study planned and Lab capability for undertaking study
Dr A. Gopalakrishnan	*Overview of National Bureau of Fisheries Genetics Resources*
Dr K.K. Vijayan	Overview of Central Marine Fisheries Research Institute (India) and Genetics.
Dr S. Ramachandran	Overview of Fisheries Survey of India (FS) Sampling capabilities.
Khin Maung Tun & Soe Win	Assessment evaluation of India mackerel fishery in Myanmar.
M. Ahusan, F. Islam & S. Rasheed	Indian Mackerel Fishery in Maldives
R. Rumpet, A. H. Arshad and M.A. Rahim	Malaysia Country Report on Genetic Study Planned and Laboratory Capability For Indian mackerel (*Rastrelligerkanagurta*)
Dr J. Kettratad	Thai Country Report on the *Rastrelliger* Genetic Study Plan and Lab Capability
BFRI-Bangladesh	*Country Report on Indian Mackerel in Bangladesh*

Appendix IV Genetic Sampling Plan Indian Mackerel

A standardised sampling procedure is required to ensure all tissue collections throughout the study area provide sufficiently high quality DNA from Indian Mackerel (*Rastrelliger kanagurta*) specimens for genetic analysis. The agreed upon sample sites selected are listed in Table 1 and a map in Figure 1 shows the sample locations. In addition to the Bay of Bengal sample locations, samples will be collected from populations outside the study area. These will be from possibly Australia, South China Sea, east-coast Africa, and Arabia. A total of 3,100 samples have been identified as a maximum number of samples collected from Bay of Bengal study area and the out of study area.

General Considerations

- Take tissues from 100 individuals from each sampling location will be taken unless otherwise stated.
- Where Short Mackerel (*R. brachysoma*) are common caught with Indian Mackerel (*R. kanagurta*) select fish over 16 cm in size to ensure Indian Mackerel are being sampled.
- Preferred sample tissue can be either fin clip or muscle tissue.
- Each tissue sample should be placed in individual vials, approximately 20mg of tissue for 1.5 ml of ethanol.
- Fish should be wiped off to avoid cross-contamination with other fish in the catch. Muscle tissue can be taken below the skin.
- Sampling implements must be cleaned between taking samples. Preferably wiped with ethanol or passed through flame.
- Use non-denatured (molecular biology grade ethanol), exchange with fresh ethanol after 24 hrs.
- 90% ethanol is better than 70% ethanol
- No more than ¼ tissue to ¾ ethanol by volume, overloading the vials causes the tissue to be poorly preserved.
- Vials should be labelled with a non-dissolving ethanol resistant marker or printed labels.
- Where possible collect biological data for each sample such as weight, length, sex, and gonad development stage.

Sampling at Sea

- Sampling at sea provides the best opportunity to get fresh tissues preserved very shortly after catching.
- Latitude and longitude of fishing location should be recorded.

Sampling at ports

- Fish samples must be taken as shortly as possible after being caught and brought to the landing sites to ensure fresh samples. The samples must be taken from the catch to be representative of the catch and preferably from spawning fish.
- Ice or dry ice should be used to keep the fish fresh until tissue preservation is done. Once packed on ice sampling can occur at the landing site or back at the laboratory. Protect the tissue from freezing before sampling which could be a problem if using dry ice.
- Try and get fishing locations for samples.
- Cross-contamination can be a problem when fish are sampled from totes or baskets at landing sites. Ensure fish are properly wiped clean of slim around sample site before

sampling or muscle tissue taken below the skin. See attached SEAFDEC "*Standard Operating Procedure for Tissue Sample Collection and Preservation*" manual for more details.

Standardized Sampling Labelling

- Standardized labelling protocol should provide all relevant information needed for determining sample location.
- County/Lab Code: Bangledesh (BGD), India (CMR, NBR, FSI), Indonesia (IND), Malaysia (FRI, SEA), Maldives (MDV), Myanmar (MYA), Sri Lanka (SRI), Thailand (THA)
- See Table 1 for landing code.
- Format: Year/CountryLabcode/LocationCode/SampleNumber
- Example: Bangladesh: 12/BDG/CG/001
  SEAFDEC: 12/SEA/KT/020

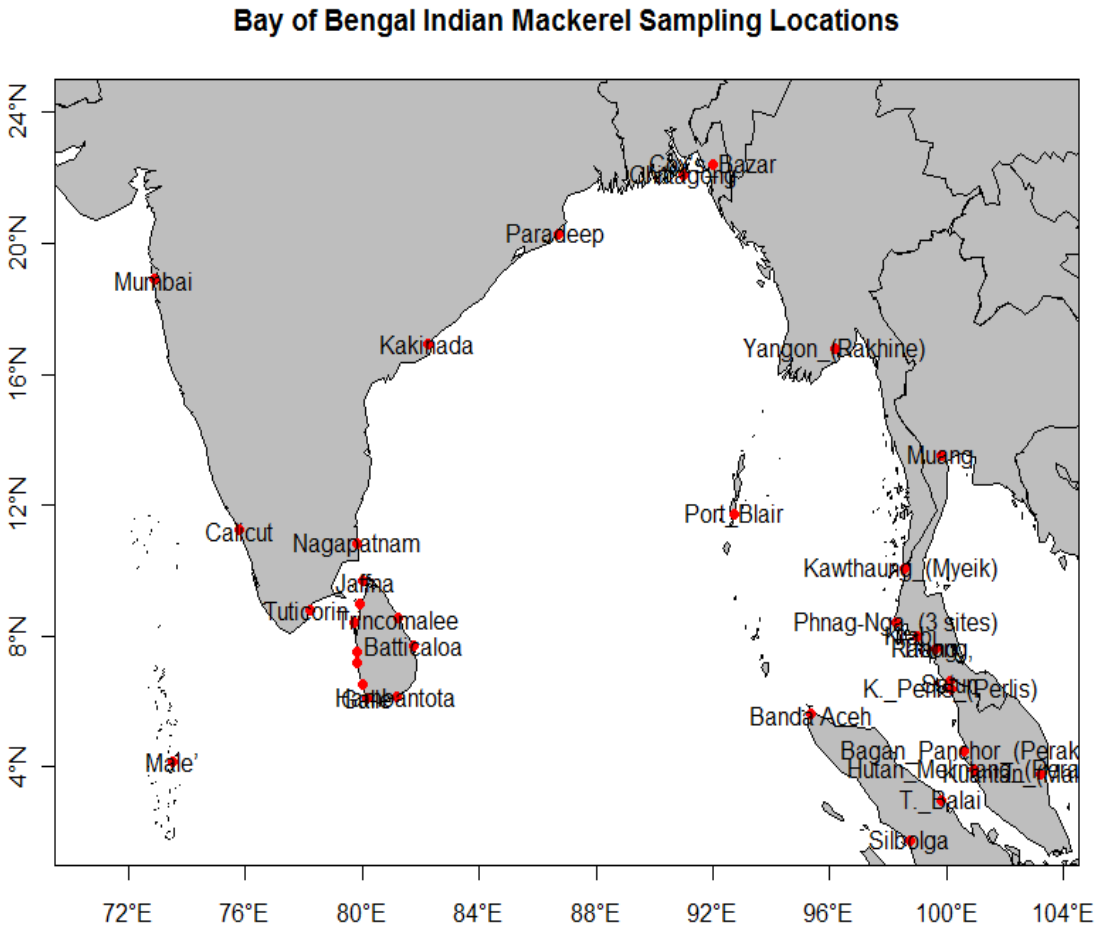
Shipping and Storage of Samples

- Shipping samples may require draining ethanol before shipping, or alternatively it can be replaced with non-combustible DMSO solution.
- Samples should be placed in sealed plastic bags and a suitable shipping box before shipping.
- A technical officer may be required to send samples by courier service.
- Notify receiving laboratory before shipping and receiving laboratory should notify shipping laboratory once samples arrive.
- Once preserved in ethanol samples can be stored for many years. Ethanol should be checked periodically for evaporation
- Storage in fridge or freezer will reduce ethanol evaporation.

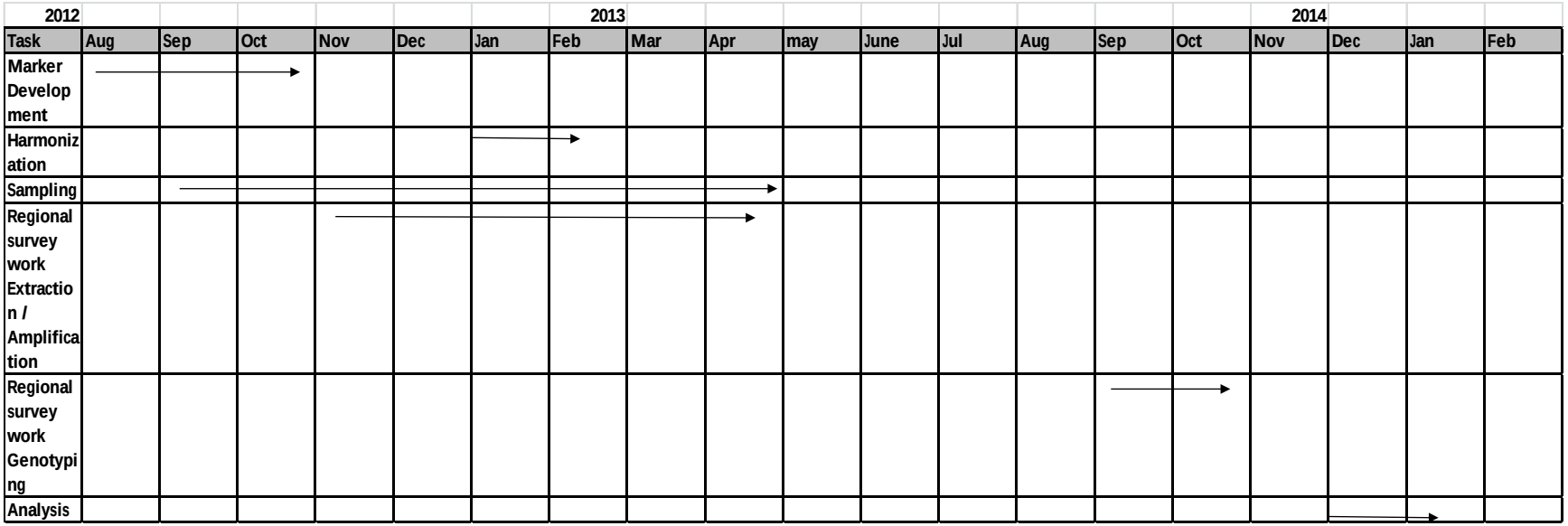
Table 1. Proposed sample locations, sample code, sample size and latitude and longitude by country

Country	Landing/Fishing Site	Code	Sample size	Latitude	Longitude
Bangladesh	Chittagong	CG	100	22° 05'N	91° 00'E
Bangladesh	Cox's Bazar	CB	100	21°26'N	91° 59'E
India	Mumbai	MB	100	18° 55'N	72° 54'E
India	Calicut	CT	100	11° 15'N	75° 49'E
India	Tuticorin	TC	100	08° 48'N	78° 11'E
India	Nagapatnam	NP	100	10° 49'N	79° 50'E
India	Kakinada	KK	100	16° 55'N	82° 13'E
India	Paradeep	PD	100	20° 18'N	86° 42'E
India	Port Blair	PB	100	11° 40'N	92° 43'E
Indonesia	Banda Aceh	BA	100	05° 35'N	95° 20'E
Indonesia	Silbolga	SB	100	01° 44'N	98° 46'E
Indonesia	T. Balai	TB	100	02° 58'N	99° 47'E
Malaysia	K. Perlis (Perlis)	KP	100	06° 23'N	100° 08'E
Malaysia	Bagan Panchor (Perak)	BP	100	04° 31'N	100° 37'E
Malaysia	Hutan Melintang (Perak)	HM	100	03° 53'N	100° 56'E

Maldives	Malé	MA	100	04° 10'N	73° 31'E
Myanmar	Yangon (Rakhine)	RK	100	16° 48'N	96° 09'E
Myanmar	Kawthaung (Myeik)	MY	100	10° 03'N	98° 32'E
Sri Lanka	Jaffna	JF	20	09° 40'N	80° 02'E
Sri Lanka	Hambantota	HB	20	06° 10'N	81° 10'E
Sri Lanka	Trincomalee	TM	20	08° 34'N	81° 14'E
Sri Lanka	Batticaloa	BC	20	07° 43'N	81° 45'E
Sri Lanka	Galle,	GL	20	06° 05'N	80° 10'E
Sri Lanka	Beruwala,	BW	20	06° 30'N	80° 00'E
Sri Lanka	Negombo,	NM	20	07° 12'N	79° 50'E
Sri Lanka	Chilaw,	CL	20	07° 30'N	79° 50'E
Sri Lanka	Kalpitiya	KP	20	08° 23'N	79° 44'E
Sri Lanka	Mannar	MN	20	09° 01'N	79° 54'E
Thailand	Munag	MG	100	13° 31'N	99° 49'E
Thailand	Ranong,	RG	100	07° 35'N	99° 38'E
Thailand	Phnag-Nga (3 sites)	PN	100	08° 23'N	98° 15'E
Thailand	Krabi,	KB	100	08° 00'N	99° 01'E
Thailand	Trang,	TR	100	07° 35'N	99° 38'E
Thailand	Satun	ST	100	06° 37'N	100° 04'E
Out pop	Kuantan (Malaysia)	KT	100	03° 47'N	103° 13'E
Outpop	Kudat (Malaysia)	KD	100	06° 55'N	116° 50'E
Out pop	Australia	AU	100		
Out pop	Africa	AF	100		
Out pop	Arabia	AR	100		
Total			3,100		


Bay of Bengal Indian Mackerel Sampling Locations
24°N
20°N
16°N
12°N
8°N
4°N
72°E
76°E
80°E
84°E
88°E
92°E
96°E
100°E
104°E
Mumbai
Paradeep
Kakinada
Yangon_(Rakhine)
Muang
Port_Blair
Calicut
Nagapatnam
Jaffna
Tuticorin
Trincomalee
Batticaloa
Galle
Hambantota
Male'
Kawthaung_(Myeik)
Phnag-Nga_(3 sites)
K._Perlis_(Perlis)
Banda_Aceh
Bagan_Panchor_(Perak
T._Balai
Silbolga

Appendix V Timeline for the Indian Mackerel Stock Structure Genetic study



Marker development (Aug-Sept 2012) will be done jointly with the two Indian laboratories by cross-priming non-target species. About 60 potential microsatellite markers have been identified for cross-priming. In addition, a set of microsatellite markers will be developed from paired-end shotgun sequencing at the University of Georgia, Savannah River Ecology Laboratory, Aiken, South Carolina. Tissues from Indian Mackerel for this development work in the USA (n=26) will be provide by India and Malaysian laboratories. Also, recently developed microsatellite markers for Indian Mackerel from graduate students in Thailand and Malaysia could be evaluated if available.

Harmonization (Jan 2013 – March 2013) period begins once candidate loci have been determined. During the harmonization period, each lab will attempt running candidate markers on a small number of standard fish to determine cross-laboratory suitability for each marker. If necessary participants will convene at a central location during the harmonization period to discuss marker selection, PCR conditions, standardization, and multiplexing. This could occur at the Indian lab at Kochi for training.

Sampling(July 2012- Aug 2013) will occur over the follow year. This should allow enough time to obtain high quality tissue samples across the study area and allow sampling of out-populations following the sampling plan. Spawning individuals will be preferentially collected.

Regional Survey work (Nov 2012-Aug 2013) begins once markers have been approved by all labs. Each laboratory will extract and amplify the selected set of markers with their respective samples**. Genotyping (Sept-Dec 2013)** of PCR products from all samples will occur at a central location using the same platform (TBD, possibly India).

Analysis (Dec-Feb 2014) by individual laboratories of their country samples and then all the samples combined (~3100). Participants will convene to discuss results at a workshop in Feb 2014.

Appendix VI Laboratory visits and work planning for Indian Mackerel Stock Structure Analysis (John Candy Report)

Laboratory visits were conducted from May 30, to June 8, 2012. A total of six laboratories were visited in Sri Lanka, India, Thailand, and Malaysia. All of these laboratories will participate in determining the genetic stock structure for Indian Mackerel in the Bay of Bengal. The Sri Lankan laboratory is located at the National Aquatic Resources Research and Development agency (NARA), Colombo. The two Indian laboratories are both located at the Central Marine Fisheries Research Institute (CMFRI) campus in Kochi, India. The Thailand lab is located at the Chulalongkorn University, Bangkok. The Malaysian laboratories are located in the Southeast Asia Fisheries Development Center (SEAFDEC) campus in Kuala Terengganu and the Fisheries Research Institute (FRI) in Penang. Most laboratories with the exception of the NBFGR lab in Kochi, have limited experience running microsatellite markers for genetic stock structure work.

For each laboratory the principle collaborating investigator(s) has been identified for the Indian Mackerel work. There is a short summary of the work presently conducted at each of the laboratories and a list of Indian Mackerel sample collections required from each of the laboratories. Collections sites correspond to those listed in the sampling plan. A summary table shows the capacity and involvement by laboratory at the different stages from sampling and marker development to final analysis of the combined regional genetic data. Finally, a timeline showing the stages of the Indian Mackerel genetic work and a description of each stage corresponding with an anticipated completion date of March 2014.

Sri Lanka- NARA

Dr D. R. Herath, is the principal investigator at National Aquatic Resources and Development agency (NARA), Colombo

The NARA lab has experience using mtDNA and cytochrome-b. Marine species studied include identification of jellyfish species using mtDNA, determination of the difference between wild and cultured barramundi (*Lates calcarifer*), development of genetic markers for identification of stranded blue whales, and identifying shark in fisheries landings using DNA barcodes. Sequencing work is contracted out to a local university. Microsatellites have not been run at this laboratory to-date.

Sample collection sites: Jaffna, Hambantota, Trincomalee, Batticaloa, Galle, Beruwala, Negambo, Chilaw,Kalpitiya, Mannar

India - NBFGR

Dr A. Gopalakrishnan is the Principal Scientist and Head of the National Bureau of Fish Genetic Resources (NBFGR), Kochi Unit, Government of India.

NBFGR lab has extensive experience developing microsatellite markers by cross-priming non-target species. For initial screening, non-target loci PCR products are run out on acrylamide gels. Promising markers are then sent out to a local facility for sequencing. NBFGR recently screened 396 potential markers to find 81 that cross-amplified in the target species of Indian white shrimp *Fenneropenaeus indicus.* From the successfully cross-amplified loci, 16 were found to be useful for determining genetic stock structure. The laboratory has the expertise to cross-priming non-target species to find a useful set of markers for Indian Mackerel. Approximately 50-60 candidate markers for Indian Mackerel have been identified.

Sample collection sites: Mumbai and Calicut (west coast, 200 samples), Tuticorin, Nagapatnam, Kakinada, Paradeep, Port Blair (split with CMFRI)

India - CMFRI

Dr K.K Vijayan is the Principal Scientist and Head of the Marine Biotechnology Division, Central Marine Fisheries Research Institute (CMFRI), Government of India.

The CMFRI Marine Biotechnology lab has an extensive range of activities including work on studying fish and shellfish physiology, nutrition and pathology. They are involved in the development of molecular tools for diagnostic purposes. They are also involved in bioprospecting, which requires developing new products which could be economically significant. The Genetics and Genomics Group has the capacity to develop microsatellite markers and will share the development work with the NBFGR lab. Close cooperation exists between the two labs which are both located at the CMFRI site. Sharing the work load for marker development, sample collection and laboratory analysis of the larger collection appears to be the best approach.

Sample collection sites: Mumbai and Calicut (west coast, 200 samples), Tuticorin, Nagapatnam, Kakinada, Paradeep, Port Blair (split with NBFGR)

Thailand – Chulalongkorn University (CU)

Dr Jes Kettratad is Faculty in the Department of Marine Science, Faculty of Science Chulalongkorn University, Bangkok, Thailand.

Dr Sanit Piyapattanakorn is Faculty in the Department of Marine Science, Faculty of Science Chulalongkorn University, Bangkok, Thailand.

Our laboratory belongs to Marine Science Department at Chulalongkorn University. We have two faculties (Dr SanitPiyapattanakorn and Dr Jes Kettratad) supervising the student workers in the lab. Dr SanitPiyapattanakorn is a population geneticist. He is interested in population structure and genetic diversity of marine organisms. He has been working with a wide variety of taxa e.g. short mackerel (*Rastrelligerbrachysoma*), Irrawaddy dolphin *(Orcaellabrevirostris),* and spotted babylon(*Babylonia areolata*). He is supervising one Ph.D. student, one master degree student and 2 undergraduated projects. Dr Jes Kettratad is an ichthyolgist working on phylogeography of freshwater and saltwater fishes. He also interested on taxonomy and systematics of fishes. He is currently working on the revision of the fishes in the genus *Rastrelliger* in the Thailand. His research based on the morphological characters and molecular character (cytochrome b gene). He is supervising 1 Ph.D. student and 4 undergraduated projects. The techniques used in our laboratory are PCR-based, such as ISSR, RAPD, and DNA sequencing. We also have a collaborative research on the investigation of evolutionary and population genetics of some amphibians, with the department of biology.

Sample collection sites: Munag, Ranong, Phnag-Nga (3 sites), Krabi, Trang, Satun, Kuantan

Malaysia- SEAFDEC

Mr Abu Talib Ahmad is the Special Departmental coordinator (SEAFDEC)

Ms. Wahidah binti MohrArcade is the Senior Research Officer, Fisheries Resource and Management. Department of Fisheries Malaysia.

Ms. Noorul Azliana binti Jamaludin is the Research Officer, Fisheries Resource and Management. Department of Fisheries Malaysia.

The SEAFEC lab has worked extensively on Indian mackerel (*Rastrelliger kanagurta*) and Japanese scad (*Decapterus maruadsi*) using cytochrome-b markers. Sampling have been analyzed from South China Sea and Andaman Sea, 14 sites (n=35) ranging from Vietnam (north) to Indonesia (south), Philippines (east) and Myanmar (west). This lab also has used mitochondrial DNA for analyzing genetic stock structure of Green and Hawksbill sea-turtles. Two of the research officers in this lab have had experience running microsatellites during studies but microsatellites have not been run at this lab to-date.

Sample collection sites: Maldives -Malé, Myanmar -Yangon (Rakhine), Kawthaung (Myeik), South China Sea - Kuantan, Kudat

Malaysia - FRI

Dr Masazurah A. Rahim is the Research Officer, Fisheries Research Institute of Malaysia, Penang (masarahim@gmail.com)

The Biotechnology Lab in FRI has the capability to do analysis using mtDNA marker such as cytochrome b, CO1 and other markers like D-loop and NADH. The lab also experienced in doing AFLP and RFLP works. At the moment there are two projects going on. 1) Population analysis of Xenopterus naritus in Sarawak Water using Cytochrome b, and 2) Barcode of puffer fish.

Sample collection sites: Malaysia- K. Perlis (Perlis), Bragan Panchor (Perak), Hutan Melintang (Perak)

Attribute	Sri Lanka (NARA)	India (NBFGR Kochi unit)	India (CMFRI)	Thailand-CU	Malaysia-SEAFDEC	Malaysia-FRI
Marker development	No	Yes	Yes	No	No	No
Phase I: Extraction and Amplification	Yes	Yes	Yes	Yes	Yes	Yes
Phase 2: Genotyping/Sizing	Contracted	Contracted	Contracted	Contracted	Contracted	Contract
Phase 3: Analysis and reporting	Yes	Yes	Yes	Yes	Yes	
Sample Size	200	350	350	600	200 + 100 +200	300
No locations	10	3.5	3.5	6	2 + 1 + 2	3
Equipment and Techniques for study	Extraction (pro mega kit) One PCR machine (Esco health care-swift max-96 well)	Extraction DNAeasy kit, Four PCR machines ABI, Biorad and MJ Research (96 well)	Extraction DNAeasy kit Two PCR machines Biorad (96 well)	Extraction BioScience and Qiagen Two PCR machines Eppendrof, Biorad (96 /48 well	Extraction Masterpure, two Techgene PCR 25 well, Planning a 96 well PCR machine by March 2013	Extraction DNAeasy, Maxwell Extraction machine, Qiagen, 2 Eppendorf 96 well pcr and 1 Eppendorf 25 well and 1 Kyretech 96 well to come
Analysis Software	New for the lab, and will explore programs (GENEPOP and STRUCTURE etc). TBD as a new analytical requirement for the lab.	GENPOP, Genetix, Arlequin, Bioedit, MEGA, TCS, Primer Three, POPGENE, planning to use MFA, STRUCTURE.	GENPOP Genetix, Arlequin, Bioedit, MEGA, TCS, POPGENE	Arlequin, GENPOP, Mega, Paup, phylip	MEGA, Arlequin, Bioedit, Chromaslite, Paup	MEGA, Arlequin, Paup and GENEPOP

2012				2013										2014					
Task	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**
Marker Development	→	→	→	→	→														
Harmonization						→	→	→											
Sampling	→	→	→	→	→	→	→	→	→	→	→								
Regional survey work Extraction / Amplification				→	→	→	→	→	→	→	→	→	→						
Regional survey work Genotyping														→	→	→			
Analysis																	→	→	→

Marker development (Aug-Sept 2012) will be done jointly with the two Indian laboratories by cross-priming non-target species. About 60 potential microsatellite markers have been identified for cross-priming. In addition, a set of microsatellite markers will be developed from paired-end shotgun sequencing at the University of Georgia, Savannah River Ecology Laboratory, Aiken, South Carolina. Tissues from Indian Mackerel for this development work in the USA (n=26) will be provide by India and Malaysian laboratories. Also, recently developed microsatellite markers for Indian Mackerel from graduate students in Thailand and Malaysia could be evaluated if available.

Harmonization (Jan 2013 – March 2013) period begins once candidate loci have been determined. During the harmonization period, each lab will attempt running candidate markers on a small number of standard fish to determine cross-laboratory suitability for each marker. If necessary participants will convene at a central location during the harmonization period to discuss marker selection, PCR conditions, standardization, and multiplexing. **This could occur at the NBFGR lab at Kochi, India for training.**

Sampling (July 2012- Aug 2013) will occur over the follow year. This should allow enough time to obtain high quality tissue samples across the study area and allow sampling of out-populations following the sampling plan. Spawning individuals will be preferentially collected.

Regional Survey work (Nov 2012-Aug 2013) begins once markers have been approved by all labs. Each laboratory will extract and amplify the selected set of markers with their respective samples**. Genotyping (Sept-Dec 2013)** of PCR products from all samples will occur at a central location using the same platform (TBD, possibly India).

Analysis (Dec-Feb 2014) by individual laboratories of their country samples and then all the samples combined (~3100). Participants will convene to discuss results at a workshop in Feb 2014.

Bangladesh, India, Indonesia, Malaysia, Maldives, Myanmar, Sri Lanka and Thailand are working together through the Bay of Bengal Large Marine Ecosystem (BOBLME) Project and to lay the foundations for a coordinated programme of action designed to improve the lives of the coastal populations through improved regional management of the Bay of Bengal environment and its fisheries.

The Food and Agriculture Organization (FAO) is the implementing agency for the BOBLME Project.

The Project is funded principally by the Global Environment Facility (GEF), Norway, the Swedish International Development Cooperation Agency, the FAO, and the National Oceanic and Atmospheric Administration of the USA.

For more information, please visit www.boblme.org