WAMSI NODE 5 SUMMARY REPORT 2006-2011

Node Details

Name of Node:	Marine Biodiscovery, Biotechnology & Aquaculture: The Blue Farm
Node Leader:	Howard Shawcross

1. Node KPIs

Brief Description of "**why**" the research in the Node was undertaken. What were the key questions being addressed and were outlined in the original 2006 WAMSI Business Plan? [A listing of the Projects and Project Leaders and their Project staff, including students will form Annexure 1]

To promote the establishment of a WA bioresources library to store marine biodiscovery extracts and to encourage the introduction of WA bioprospecting legislation to improve biodiscovery research investment and exploration projects. Marine biodiscovery and biotechnology within WAMSI is designed to capitalise on the uniqueness and profusion of the State's marine biodiversity. The main hurdle limiting progress in the marine biotechnology sector has been a lack of a legislative framework to underpin security for biodiscovery investment. Since 2006 the WAMSI Board has played a key role in the advocacy for a WA biodiscovery legislative framework. Through previous Chair Bernard Bowen and current chair Peter Rogers correspondence to relevant WA State Ministers and two Premiers calling for legal certainty to provide increased global investment in WA's biological resources. Synergies between the WAMSI Node 5 research areas and some key actions of the WA Biotechnology Industry Development Strategy 2006-2010 were highlighted in several meetings between Bernard Bowen and the State Government executive. The carriage of Node Leaders from Chris Battershill at AIMS through Fiona vom Berg and Jason Froud at Fisheries through to the current Department of Commerce, representative Howard Shawcross has covered all the essential responsibilities of molecular biological research, commercial and scientific regulation and facilitation of and negotiation of future benefit sharing agreements. All previous Node 5 Leaders have been very instrumental in progressing the work undertaken to develop a WA Bioprospecting Act.The Cabinet Submission process for the introduction WA bioprospecting legislation is well underway and it is estimated that the WA Bioprospecting Bill will be introduced into Parliament in 2012.

The initial investment in WAMSI Node 5 was a strategic approach to establish a platform for more targeted research and investment in a WA marine based biotechnology industry. It was envisaged that Node 5 would act as a catalyst to help resolve the uncertainty and investment risk arising from a lack of legislation and to help establish a structure for receiving, curating and making available specimens for research. A third element to the scope of WAMSI Node 5 was the promotion of capacity building within the State's marine research organisations

2. Research Plan/Science Plan

Describe the high level "**Research Plan/ Science Plan**" for the Node in simple language [Note: the approved Science Plan for each Node will form Annexure 2]

The objective of the Node is to provide a platform for integrating multidisciplinary research based on utilising the diverse and unique metabolite chemistry associated with Western Australia's marine biodiversity in a sustainable manner. The Node is based on understanding how marine organisms respond to their environments at microbial and molecular levels and utilising that knowledge together with their bioactive metabolites in applied developmental programs across a range of sectors including medicinal and agrichemical industries.

The original Node 5 Science Plan and Business case built on the broad concept in the original WAMSI Business Plan to implement a suite of complimentary projects as a proof of concept for sustainable marine biotechnology for the State against a backdrop of effort to support the necessary re-definition of biodiscovery policy in Western Australia. Key components of the plan assumed ongoing activities to support a review of relevant legislation within the State needed to provide security for business development, of any opportunity associated with marine biodiscovery, in the current absence of a specific policy system; and provide a robust scientific program to build on existing capability and immediate opportunity in the state. The strategy was put in place to achieve outcomes across a range of relevant sectors including medicine and agriculture. The aim of Node 5 was to identify opportunity; stakeholders and co-investment sources; and provide an achievable strategic pathway relevant to WAMSI. Project Plans were furnished incorporating feedback from the R&D committee providing for prioritisation of work to facilitate as much positive pressure on policy development as is appropriate and to provide achievable research projects for the funding allocated and collaborative support available.

3. Research Activities

Describe the "**Research Activities**" for the Node in simple language. Please try and summarise the methodologies utilised as part of each Project in the Node [Note: the methods section of the approved Project Plans will form Annexure 3]

5.1 Western Australia Marine Bioresources Library (WAMBL)

Collections of marine frozen samples have been made in the past during research voyages throughout the State. These have not been processes for any screening activity due to a lack of appropriate regulatory permission. Some of these collections date to the mid 1980's (AIMS collection) and others have been collected in the late 1990's to present (AIMS, Western Australian Museum). These collections are scattered in repositories at UWA, AIMS and the WAM. This project employed a Technical Officer (TO) to gather these collections into a single repository (WAM). This project established a table linked to the Western Australian Museum marine invertebrates collections database. Consequently data is backed up every 24 hours. The table fields were modelled on the AIMS Bioresources Library database and include amount of frozen material and how and where it is stored, as well as tracking material that has been sent for research.

A library of sponge specimens has been established in the WA Museum with 157 frozen Commonwealth collected samples and 50 State collected samples incorporated into WAMBL. 466 frozen samples with extracts, collected from Western Australian waters are virtually incorporated into WAMBL i.e they are held at AIMS in Townsville but have been 'signed over' to WAMBL which is now their only release point for biodiscovery research. The WAMSI project did not identify funds for the AIMS material to be physically incorporated into WAMBL. This aspect is being costed in the business plan currently being drafted that will also identify the costs associated with continuing to operate WAMBL.

The milestone related to the release of samples for biodiscovery research to WAIMR was delayed for over one year, from July 2009 to October 2010, reflecting this significant difficulty. The solution was an agreement between Fisheries, WA and UWA which allowed for samples to be released to research institutions associated with UWA. It is uncertain if a similar agreement would have to be negotiated between Fisheries and a research institution if the latter was not associated with UWA. Currently non UWA affiliates have shown interest in accessing WAMBL samples, the latest being a researcher at the Chem Centre, Curtin University in April 2011, but have not currently finalised the Fisheries reg 179, which is the permit required to handle WAMBL samples. Subsequent release of samples to UWA affiliates has been straightforward.

5.2 Biomolecular Diversity and Partnered Biodiscovery

The project has two general components: one is based on marine natural product lead identification from Western Australian marine biodiversity (macro-organism) in conjunction with one complimentary biomedical partner (WAIMR); the second being an opportunity associated with investigation and elaboration of marine microbes and the secondary metabolites they elicit. The goals are to identify valuable lead compounds from Western Australia's marine biodiversity utilizing a hypothesis driven approach to optimize discovery potential as well as adding to the knowledge about the role of marine secondary metabolites in nature. The key aim of the project was to perform proof-of-principle studies on a carefully selected group of marine samples in order to identify ones that have novel anticancer properties. This is an area of significant interest world-wide, as some marine-derived compounds are now entering early phase clinical trials in patients with cancer. So the precedent is a good one, and as WA has such unique and diverse marine organisms, the project holds great promise.

Fifty compounds isolated from up and down the WA coast were carefully selected by Prof Battershill and Dr Evans-Ilidge for potential anticancer activity based on an intimate knowledge of the marine organisms and Prof Battershill's experience in the mining of other organisms with a collaborator at the NIH in the USA. These samples were provided by AIMS to Dr Jane Fromont from the WA Museum, who then delivered them to the Leedman laboratory in WAIMR (Western Australian Institute for Medical Research) as freeze dried preparations. They were then dissolved predominantly in methanol and used in cell proliferation assays to determine the IC50 of growth inhibition of two different human cancer cell lines (LNCaP, human prostate cancer; MCF-7, human breast cancer).

5.4 Microbial Quorum Sensing

The project examined the biomolecular diversity associated with marine micro-organisms and explored the capacity of microbes to elaborate metabolites through Microbial Quorum Sensing or as influenced in symbioses by micro-environmental variables.
The project was set up to investigate aspects of the diversity, ecology and application of novel marine natural products termed quorum quenching compounds (QQCs). These products have potential pharmacological and commercial uses in the control of bacterial activities (including human and aquaculture disease and biofouling), and also have important ecological roles, including in invertebrate and algal settlement. The following are the key steps that were undertaken in the methodology behind this project.

Bioassay for detection of QQ bacteria:
Natural marine samples were screened for the presence of bacteria with QQ activity using a bioluminescence inhibition assay developed specifically for the purpose.

Bacterial isolation, culture and purification*:*
Bacteria testing positive in the screening assay were isolated, purified and preserved using standard microbiological culturing techniques.

Bacterial identification*:*
Bacterial isolates were identified using exhaustive biochemical testing to establish phenotypic characteristics, volatile fatty acid analysis, and molecular techniques based on 16S RNA analysis of genomic DNA. Test results were compared with published data on bacterial phenotypes (phenotypic characters) and online BLAST sequences (molecular analysis). Known and unknown isolates were grouped using phylogenetic analysis.

Confirmation of QQ activity and qualitative QQ assay*:*
The purified bacterial isolates were assessed for QQ activity in a quantitative assay (based on spectrophotometric methods to detect both growth and bioluminescence, enabling assessment of relative bioluminescence inhibition.

Extraction, purification and identification of QQCs:
Using a bioassay-directed procedure the QQCs from selected bacterial isolates were purified and identified using a combination of chemical procedures including solvent extraction, HPLC, GC, NMR and MS.

***Assay of QQCs against additional quorum sensing processes**:*
Purified QQCs from the selected bacterial isolates were screened for their effects in a range of QQ assays including inhibition of bioluminescence by *Vibrio harvey*i, pigment production by *Serratia marcescens* and *Chromobacterium violaceum*, and biofilm formation by *Pseudomonas aeruginosa.*

4. Research Findings

Describe the "**Research Findings**" for the Node in simple language. Follow the sub-headings:

a. Overall research findings [very high level]

The promotion of the establishment of a WA bioresources library to store marine biodiscovery extracts and development of 'operational procedures' for users of WAMBL to obtain a WA Fisheries Regulation 179 authority to access samples in WAMBL. Both the promotion of WAMBL and development of operational procedures were severely delayed due to the uncertainty and delays in permitting.

b. Specific research findings/ outputs [specific to the individual projects]

5.1 Western Australia Marine Bioresources Library (WAMBL)

Development of 'operational procedures' for users of WAMBL to obtain a WA Fisheries Regulation 179 authority to access samples in WAMBL. WAMSI specimens added in 2010, written prior informed consent to place in WAMBL obtained from AIMS. Pending success for additional resources, produce draft business plan for WAMBL, including a cost analysis to operate WAMBL. The delay of two and a half years for the access to 50 samples held at WAMBL was a serious impediment to progress on the project. The uncertainty of future investment in the repository is a key issue going forward.

5.2 Biomolecular Diversity and Partnered Biodiscovery

The identification of novel compounds from a unique source of samples, purify them by bioguided factionation and then develop lead compounds as potential anti-cancer drugs. The expected outcome would be several purified lead compounds which are anticipated to have compelling anti-cancer properties. Successful collaboration between WAMBL and WAIMR on Regulation 179 process after a significant delay.

5.4 Microbial Quorum Sensing

Successful completion of PhD project to:

Search for bacteria producing quorum quenching compounds (QCC), assess the diversity of QCC effects and mechanisms of action of QCC, and assess the potential applications of QCC.

The major differences from what was originally proposed were (a) the decision to focus on marine bacteria as a source of QQCs, made because the production of these compounds by bacteria greatly simplified (because of the capacity to grow the bacteria in culture) the generation of material for chemical analysis; and (b) less of a focus on objectives (iii) and (iv) in preference to investigating possible mechanisms of action (objective ii). This was in part necessitated by the complexity of the analysis of mechanisms of action.

c. Intra-Nodal scientific outcomes [across the projects]

Collaboration between WAMBL and WAIMR to access samples through the Regulation 179 authority.

d. Inter-Nodal scientific outcomes [between this and another Node[s]

None at present.

e. What wasn't addressed by the Node research, but is likely to be important from a scientific perspective?

Once WA bioprospecting legislation is implemented what effects that will have marine research and what type of research will be a priority as well as the uncertainty of investment and the need for legislation, organised institutional processes for managing specimens and capacity building. For the future fact finding outcomes a WAMSI sponsored marine biodiscovery workshop held in Perth in 2012 would promote impending legislation as well as open up discussion on possible collaborations between research organisations in marine biodiscovery as well as biotechnology industry players.

[Note: the final Project Reports for the Node will form Annexure 4]

5. Implications for Management (address the "So What")

a) What "management objectives" were being addressed by the research in this Node?

- Influencing beneficial review and enactment of new policy governing marine bioprospecting in the State.
- Create a marine bioresources repository
- Develop industrial partnerships for ongoing research
- Develop products for medicine and agriculture
- Revenue streams for R&D and royalty opportunity downstream
- Patents and papers

b) Who are the key management agency "beneficiaries" of the work in this Node?

Department of Fisheries, West Australian Museum, WAIMR, UWA, Curtin University, Murdoch University, Edith Cowan University, ChemCentreWA, Department of Commerce, Department of Environment and Conservation, SEWPaC, AIMS

c) What "management strategies" were being addressed by the research in this Node?

Scoping of critical risks including ability to influence legislative policy, the reduction of the gap between research and commercialisation through building on experience and developing secure industry partnerships.

d) What "strategies and actions" are likely to be changed as a result of the research in this Node?

The main refocusing of the Node 5 strategies has been affected by the still current lack of legislation. The previous Node 5 Leader Jason Froud worked tirelessly and eventually successfully to find an alternative strategy for access to marine samples based around the use of Regulation 179 of the *Fish Resources Management Regulations 1995.*

Regulation 179 of the *Fish Resources Management Regulations 1995* prohibits the taking or handling of fish for the purposes of genetic or chemical extraction or analysis unless approved or authorised to do so by the CEO of the Department of Fisheries. The Department of Fisheries had been considering an application from the University of Western Australia for a permit under Regulation 179 on behalf of WAIMR and linked directly to the research undertaken by Project 5.2 Leader Peter Leedman. If approved, the permit would enable WAIMR to assess/search for bioactive metabolites in 50 extracts from sponges and ascidians collected in Western Australian waters that were held at the Western Australian Marine Bioresources Library. With regard to the issue of benefit sharing, the Department of Fisheries did not enter into an explicit benefit sharing agreement with UWA at the time. Instead, the Department of Fisheries developed a Heads of Agreement with the UWA which would require the university to enter into a benefit sharing agreement with the Western Australian Government in the event the university wishes to commercialise any of its findings from this project. The proposed Regulation 179 permit is conditioned so that it is a offence for UWA to breach its obligations under the Heads of Agreement.

The CEO of the Department of Fisheries indicated his intention to approve the Heads of Agreement and issue the Regulation 179 permit to UWA in October 2010. A full two and a half years after WAIMR's initial application to WAMBL.

Operating procedures to access material for biodiscovery purposes from WAMBL were established in December 2009. These procedures and the Material Transfer Agreement have been used on the two occasions extracts and samples were released for biodiscovery, to WAIMR and to the Pharmacology Unit, School of Medicine & Pharmacology, UWA. Further, these operational procedures and MTA were drafted such that they can continue to be used when Biodiscovery Legislation is introduced in Western Australia.

e) Has the research in this Node improved "management effectiveness"?

Yes through the establishment of operating procedures for WAMBL and the "case study" previously mentioned of the use of a Heads of Agreement for future collaborations.

f) Are the research findings "easily accessible" and in a format that allows for ease of interpretation and "take-up" by management agency staff? How has this "knowledge transfer" been facilitated to ensure the maximum value has been gained from the research?

The Project Reports in Annexure 4 clearly state the findings, the need for improvement in processes and major impediments due to a lack of legislation.

g) Has the research in this Node improved the overall management of the marine/ coastal environment in WA? How and give examples.

Not directly but once legislation is implemented the findings of this node will be an invaluable tool for the administration of a standardized approach to the management of marine resources.

h) What are the longer-term likely impacts of the research for the State?

All three projects have facilitated work towards an understanding of the access and benefit sharing agreement process and progress towards State biodiscovery legislation as well as an understanding of the value of collections of marine samples both in terms of increased knowledge of the State's biodiversity as well as the potential for the commercial development of synthesized marine samples.

6. Capacity Building

Detail the additional "capacity building" this Node has provided to WA. Considerations include Masters and PhD students, postdocs, technical support staff, research staff, equipment, facilities, building expertise and associated "knock-on" opportunities and income streams.

Jamie Summerfield (PhD candidate).
Jamie carried out the Microbial Quorum Sensing study under the supervision of the other participants. There was establishment of key collaborations with AIMS and the US National Cancer Institute for Project 5.2. All three projects have had presentations at past WAMSI symposia. .

Infrastructure established specifically for the Western Australian Marine Bioresources Library **(WAMBL)** includes marine invertebrates collections database. The collections database contains all details on provenance of the specimens, including all collecting data including georeferencing of locations and depths.

Guidelines for access to WAMBL: these guidelines provide advice on how to fill out a Fisheries reg 179

permit to access samples from WAMBL. They explain reporting requirements and what to do with material remaining post project. They also contain the Material Transfer Agreement between WAMBL and the recipient of the samples which must be signed before samples are released from WAMBL, and all terms and conditions associated with the agreement.

Library Inventory: this project established a table linked to the Western Australian Museum marine invertebrates collections database. Consequently data is backed up every 24 hours. The table fields were modelled on the AIMS Bioresources Library database and include amount of frozen material and how and where it is stored, as well as tracking material that has been sent for research (list of fields below). The collections database contains all details on provenance of the specimens, including all collecting data including georeferencing of locations and depths (list of fields below).

Guidelines for access to WAMBL: these guidelines provide advice on how to fill out a Fisheries reg 179 permit to access samples from WAMBL. They explain reporting requirements and what to do with material remaining post project. They also contain the Material Transfer Agreement between WAMBL and the recipient of the samples which must be signed before samples are released from WAMBL, and all terms and conditions associated with the agreement.

Permissions: The WA Museum and other WA institutions hold frozen samples collected for biodiversity purposes, but of suitable provenance for WAMBL. WAMBL established a standard letter requesting formal lodgement of these samples into WAMBL. Permissions were achieved for collections of both Commonwealth and State samples, which are now held in WAMBL.

Storage: There are three 700 litre freezers holding bulk sample in WAMBL. 2g aliquots are held in a -80°C upright freezer in small storage boxes, and 10g aliquots for rapid replacement of 2g aliquots are held in a dedicated chest freezer. All freezers are on the Museum back up electrical system (generators) so power cannot fail to these collections. Provenance is excellent.

7. Data Management

Summarise the “Data and Information products” that have been generated by this Node.

Project 5.1
WAMBL database fields including registration number, number of samples, sample size, storage location
MIZ (marine invertebrate zoology) database fields including registration number, species and geographic data

Project 5.2
Information applies to dataset
Project 5.4
Information applies to dataset

8. Modelling

Describe [where applicable] the modeling tools that have been created by this Node. What are their strengths and limitations?

The key modelling tools in Node 5 revolve around the access to the Western Australian Marine Bioresources Library (WAMBL). The WAMBL Project Leader scoped four main objectives for the establishment of the re.pository.

1 Establish a marine bioresources library to facilitate sustainable access to characterised Western Australian marine biodiversity for biodiscovery. These guidelines provide advice on how to fill out a Fisheries Reg 179 permit to access samples from WAMBL. They explain reporting requirements and what to do with material remaining post project. They also contain the Material Transfer Agreement between WAMBL and the recipient of the samples which must be signed before samples are released from WAMBL, and all terms and conditions associated with the agreement.

2 Establish standard operating procedures to access samples in the library in compliance with all access and benefit sharing provisions required by WA State government agencies and WA Museum data capture requirements.

3 Maximise the capture of knowledge regarding Western Australian marine biodiversity that is made available for biodiscovery, and consolidate it at the Western Australian Museum in a format that is accessible to the State's natural resource managers.

4 Identify ways that the Western Australia Marine Bioresources Library could be maintained and expanded after the completion of WAMSI. A collaboration between stakeholders in Node 5 will result in a business plan not only assessing the future funding requirements of WAMBL but also WA marine biodiscovery industry needs in general.

The WAMBL model certainly provides a clear process for the access, transfer and analysis of marine resources collected in WA. Unfortunately as has been documented, the lack of relevant legislation and the uncertainty of current regulatory provisions has interrupted the process. Once legislation is in place it is hoped the WAMBL model will operate to its full capability

The Node has pointed the way through a proof of concept approach to the potential cross collaborations between State Government, academia and biodiscovery industry players for value added marine research focussed on the commercial applications of biodiscovery.

9. Societal Benefits

Summarise the societal benefits of the research in the Node [suggest you summarise section 7 [*Benefits*] from the individual Project Reports]. Give a snapshot of where we were at the beginning of 2006 and where we are in mid 2011?

5.1 Western Australia Marine Bioresources Library (WAMBL)

Environmental: The Western Australian Museum marine invertebrates collections database contains all details on provenance of the specimens in WAMBL, including species identification, and all collecting data including georeferencing of locations and depths. Therefore this database provides information on where the species occur.

Technical: The project has delivered a Material Transfer Agreement that can be used as a model or template for transfer of specimens or extracts for biodiscovery research and a standardised prior informed consent letter to signover specimens for biodiscovery research as per international biodiscovery agreements.

The procedures in this Project have been gradually developed from 2006 through 2011. Most of the projected outcomes have been achieved. The transfer of samples process has been delayed resulting in less amounts of data recorded.

5.2 Biomolecular Diversity and Partnered Biodiscovery

In 2006 there was a lot of optimism for this project's expected outcomes. Delays in the regulatory applications for the transfer of samples have meant that by 2011, the project's potential social benefits were severely jeopardised.

5.4 Microbial Quorum Sensing

This project comprised an initial survey of the production of a unique class of chemical compounds (QQCs) by marine bacteria, and a preliminary assessment of the mechanisms and scope of activity of any compounds identified. Consequently, it was not expected that commercially useful products would be generated, although this was a possibility. However, the project has clearly demonstrated that there is the potential to discover and develop novel QQCs from marine bacteria, and suggests that this is an area worth pursuing. Although still in a developmental stage, the research has focussed on alternative ways of controlling harmful and detrimental microbial activities, and indicates the potential for new natural products to be found from marine bacteria to address important industrial and medical issues, including antibiotic resistance.

10. Future Research

Further work recommended. Outline what the next stage of the Node research should look like [if it was to continue] and where are the priority regions? Outline your hopes for the future as it relates to the ongoing uptake and maintenance of this work

As is stated by many researchers across Australia, bioresources are the “bio” in biodiscovery and likewise biodiscovery is definitely the “bio” in biotechnology. Approximately 50% of all drugs in use come from natural products or synthetic copies of natural products. Bioprospecting and biodiscovery typically requires high levels of investment, over long lead times and involves high financial risk. Within this scenario comes the added complication of previously unavailable standardised access benefits and sharing arrangements. To facilitate a growing biotechnology and biodiscovery industry government, academia and industry need to collaborate to promote a future legislation in order to enhance global investment. The importance of a value added WAMBL with not only greater capacity to accept more samples for research as well as the ability to transfer data can not be emphasised enough. A future funding model for WAMBL may be focussed on the need for a National Compound Library initiative. The biomedical applications from WA’s marine samples have been well documented but there are many other potential sector applications such as environmental, agricultural, alternative energies and industrial. The opportunities exist for further research into microalgae applications for biofuels, bioremediation, bio-mining and as a source of omega 3 antioxidants.

The three main objectives for a better WA marine biodiscovery future will definitely focus on a funding model for WAMBL, implementation of the WA Bioprospecting Act in 2012 and subsequent downstream research activities.

Over 100 delegates from all states and territories of Australia came together to share key developments in research and policy at the second National Biodiscovery Forum, held at Flinders University 4th-5th August 2011. At the forum it was recognised that Australia was one of the world’s first nations to implement benefit-sharing agreements and has set world standards. These themes were continued on Day 2, with detailed discussions on the 2010 Nagoya Protocol on access and benefit sharing of global genetic resources. Other take-home messages from the Forum included the need to value add to marine resources by the use of taxonomic and cross referenced collections so as to facilitate investment in the research and development pipelines; the importance of communicating the science of biodiscovery not only to academia and government but most importantly to industry. There was only one representative from industry at the conference which was an indication that this sector needs to be better engaged. The other harsh reality of biodiscovery research is that not one commercial product has so far resulted from the three pieces of legislation surrounding Australian biodiscovery. This is an indication of the long lead times and large investment required for such activities.
The Forum was a great example of the intensive research undertaken nationally in sponges, actinobacteria, bioinformatics, ex-situ repositories, microalgae, fungi, food production, seagrasses and traditional knowledge and demonstrated the potential for Western Australia to be a key player in these research disciplines.

Sign off the Node report in your capacity as Node Leader.

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Annexures

Annexure 1: A listing of the Projects and Project Leaders and their Project staff, including students

Annexure 2: The approved *Science Plan* for the Node [2006 or 2007]

Annexure 3: The methods section of the approved *Project Plans*

Annexure 4: The final *Project Reports* for the Node

Annexure 5: Any other relevant information