

# SC/68B/RP/16

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## IA - Mixed-stock analysis and population assignment of North Pacific humpback whales to assist in allocation of catches

IWC



INTERNATIONAL  
WHALING COMMISSION

## PROJECT PROPOSAL REQUEST

### 1 . PROPOSAL TITLE

Mixed-stock analysis and population assignment of North Pacific humpback whales to assist in allocation of catches

### 2 . BRIEF OVERVIEW OF THE PROPOSAL AND ITS EXPECTED OUTCOME

Give a very brief overview (max 150 words) on your proposal and its expected outcomes. Use bullet point to list outcomes. Be succinct and clear as this may be used to summarise your project for the report.

Work towards a Comprehensive Assessment of North Pacific humpback whales began in 2016, and included an intersessional workshop held in April 2017. Included in the work plan from the workshop and subsequent reports of the intersessional working group is the recommendation to “Initiate and document genetics-based mixed-stock analysis in the feeding grounds, and apply genetic assignments to breeding areas from feeding grounds”. The intent of the mixed-stock analysis and population assignment is to inform the allocation of catches for the assessment model in light of population structure hypotheses. Here, we propose to undertake the recommended analyses using available DNA profiles held in an updated ‘DNA register’ developed for the SPLASH program (Baker et al. 2013). This register currently includes the mtDNA haplotype, sex and microsatellite genotypes (10 loci) for 3,389 individual whales, many of which have associated photo-identification records.

### 3 . RELEVANT IWC SCIENTIFIC COMMITTEE GROUPS OR SUB-GROUPS

List all the IWC Scientific Committee groups or sub-groups that the outcomes of this work would be relevant to and provide a brief (1-2 lines) explanation of how it would contribute more widely to their ongoing programmes of work. Where possible, do not simply list only the sub-committee within which or for which the project proposal was generated.

The assignment of individuals to breeding stocks and characterization of mixed stocks on feeding grounds using genetic methods is widely applicable in population assessments by the IWC.

Specifically, the IA subcommittee is now conducting a Comprehensive Assessment of the North Pacific humpback whale. This requires testing of appropriate hypotheses for population structure and the allocation of catches from feeding grounds to breeding grounds.

The SD/DNA subcommittee considers the genetic evidence for stock structure and methods for assigning individuals to management units.

### 4 . TYPE OF PROJECT (PLEASE TICK)

Research project	XX
Modelling	XX
Workshop/meeting	
Database creation/maintenance	
Compilation work/editing (e.g. on whalewatching regulations, SOCER, etc.)	
Other (please specify below)	

5. BRIEF DESCRIPTION OF THE PROPOSAL AND ITS CONNECTION WITH SCIENTIFIC COMMITTEE RECOMMENDATIONS (DO NOT EXCEED 1500 WORDS)

(A) BACKGROUND, RATIONALE, AND RELEVANCE TO THE PRIORITIES IDENTIFIED BY THE IWC SCIENTIFIC COMMITTEE:

Provide a clear explanation of the background and rationale for the proposal and its relevance to Scientific Committee identified priorities. Clearly identify the most relevant and recent Scientific Committee recommendations.

A large proportion of humpback whales killed during commercial whaling in the North Pacific were taken on the feeding grounds (Figure 1). The ongoing Comprehensive Assessment requires a method to allocate these catches from feeding grounds to breeding grounds (IWC/67b/ Annex F 25/05/2018). Recently, both mixed-stock analysis and genotype assignment procedures have been used to apportion migratory populations and assign individuals to source population (e.g., Albertson et al. 2018; Cypriano-Souza et al. 2017; Schmitt et al. 2014). These methods take advantage of the difference in frequencies of mtDNA haplotypes of ‘pure stocks’ to find the best solution for mixing on feeding grounds, or differences in allele frequencies of breeding stocks for identifying the likely reproductive origin of an individual sampled on the feeding grounds (Manel et al. 2005).

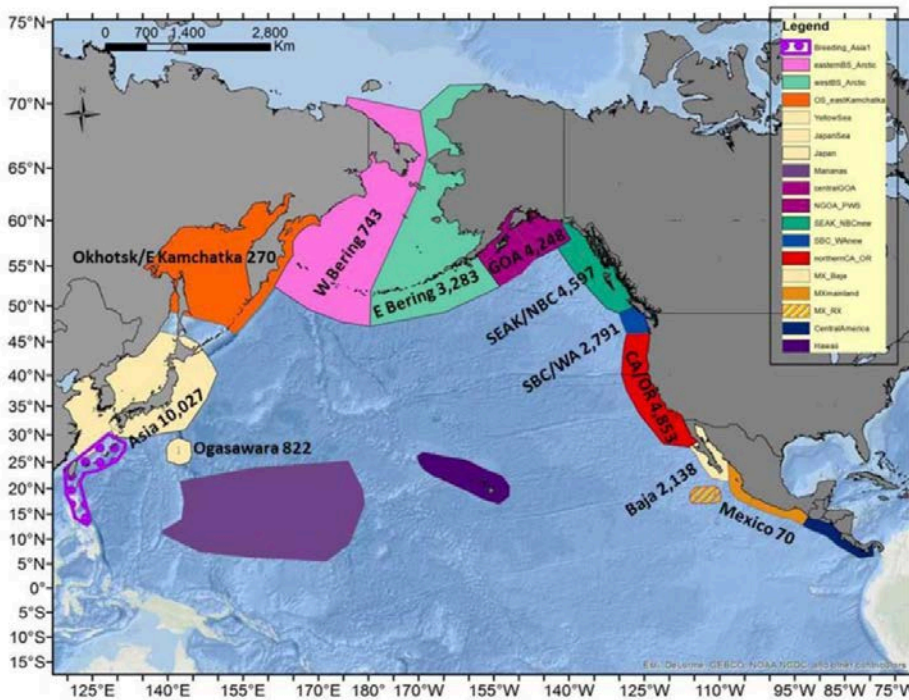


Fig. 1. The distribution of catches in relationship to a stock structure hypothesis that includes 7 feeding areas and 6 breeding grounds (including the ‘unknown’ breeding grounds, which includes the Mariana Islands). Note that Japan, Ogasawara (in the west) and Baja California (in the east) are considered migratory corridors. Catch numbers are shown in the boxes (from IWC/67b/Rep01(2018), Annex F 25/05/2018).

(B) SPECIFIC OBJECTIVES OR TOR AND DELIVERABLES/OUTCOMES:

Provide the specific objectives and the expected deliverables. In the case of workshops and meetings, include the Terms of Reference (ToR) and expected outcomes.

- 1) Review and revise DNA register initially developed for the SPLASH program, as described by Baker et al. (2013), including mtDNA haplotypes, sex and microsatellite genotypes (10 loci), to identify individuals and genotype recaptures;
- 2) Communicate with working group to agree on appropriate strata for feeding grounds and identity of breeding stocks;

- 3) Use mtDNA haplotypes for mixed-stock analysis, as implemented in the program *BAYES* (Pella and Masuda 2001), to apportion feeding ground samples to breeding grounds;
- 4) Use microsatellite genotypes for population assignment, as implemented in the program *GeneClassII* (Piry et al. 2004), to estimate the probability of an individual from the feeding ground originating from a defined breeding ground; and,
- 5) Provide mixed-stock apportionments and individual assignment probabilities to the working group as proxies for catch allocation in assessment models.

### (C) METHODOLOGICAL APPROACH/WORK PLAN/ADMINISTRATIVE DETAILS

Specify the methods to be applied (novel methods require more explanation than standard ones) and the broad workplan – the detailed timetable appears under Item 5 below.

#### *DNA profiles*

We will take advantage of a large database of standard DNA profiles for humpback whales from the North Pacific developed for the SPLASH program (Baker et al. 2013). These DNA profiles include mtDNA control region sequences (haplotypes), sex identification and individual identification by microsatellite genotypes (10 loci). At the time of initial publication of results from the SPLASH analysis of population structure, this 'register' included DNA profiles from 1,805 individuals (Figure 2). With incremental additions, the total DNA register now includes 3,389 individuals sampled between 1987 and 2019 (Figure 3). The majority of these DNA profile originated from samples collected during the SPLASH program (2004-2006) and are publicly available. The inclusion of DNA profiles from some more recent samples will require agreement with regional collaborators. The current register will be reviewed for quality control and stratification of feeding areas and breeding grounds will be revised in light of stock hypotheses agreed by the working group.

#### *Mixed-stocks analysis*

For purposes of mixed-stock analyses, samples from the breeding grounds will be considered 'pure stocks' and samples from feeding areas or migratory corridors will be considered 'mixed stocks'. Current stock structure hypotheses under consideration by the working group include 6 grounds and 7 feeding areas (Figure 1). The apportionment of each breeding grounds to feeding areas will be estimated with mtDNA haplotype frequencies using the program *BAYES* (Pella and Masuda 2001). The Bayesian approach implemented in this program incorporates uncertainty where haplotypes may have a very low frequency, i.e., it accommodates the possibility of rare haplotypes that are actually present but not detected in a small sample. The program also allows for the inclusion of prior probabilities of mixing apportionments based on other lines of evidence, e.g., interchange indices from photo-identification matches. In the absence of other evidence, the program is run with uniform priors. The output will provide the estimated apportionment (and standard errors) of each breeding ground to each feeding area. This mixed-stock apportionment can be interpreted as an index for catch apportionment, e.g., a 75% apportionment of Hawaii to southeast Alaska would reflect a 75% apportionment of southeast Alaska catches to Hawaii.

#### *Population assignment*

For population assignment, the DNA profiles from each breeding stock will be considered a 'reference' database for assignment of 'unknown' individuals sampled on the feeding ground. The likelihood of an individual originating from each of the identified breeding stocks will be calculated using the Bayesian criteria (Rannala and Mountain option) implemented in the program *GeneClassII* (Piry et al. 2014). This can be expressed as an assignment score reflecting the relative likelihood of the unknown individual originating from each of the breeding stocks. These likelihood scores can be considered individually or summed by feeding area to provide an index of relative catch apportionment to the breeding stocks. The program will be run with and without mtDNA haplotypes (see Schmitt et al. 2014) and performance will be evaluated by self-assignment of the reference dataset, using the leave-one-out procedure (Piry et al. 2014).

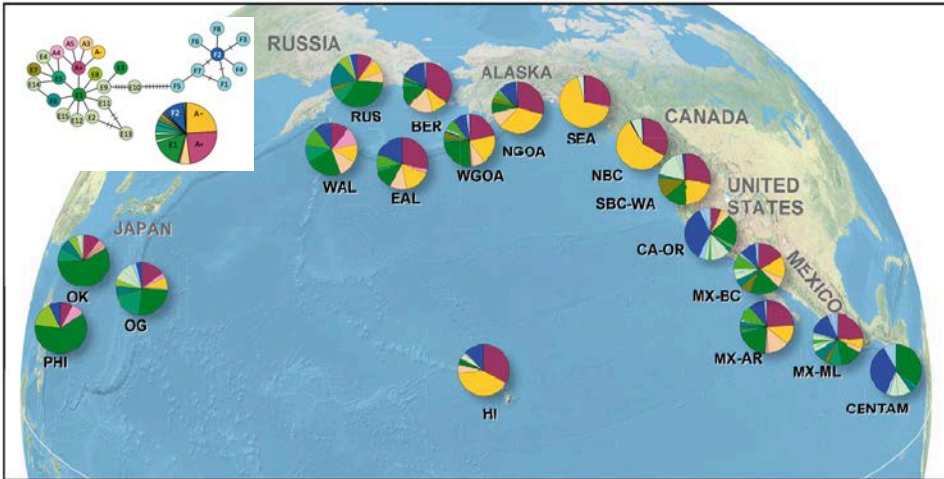


Figure 2: The regional frequencies of mtDNA haplotypes, as represented in pie charts, for humpback whales in the North Pacific based on 1,805 individuals identified by DNA profiles, as surveyed during the SPLASH program (Baker et al. 2013). The inset shows the evolutionary relationship and overall frequencies of the 28 haplotypes.

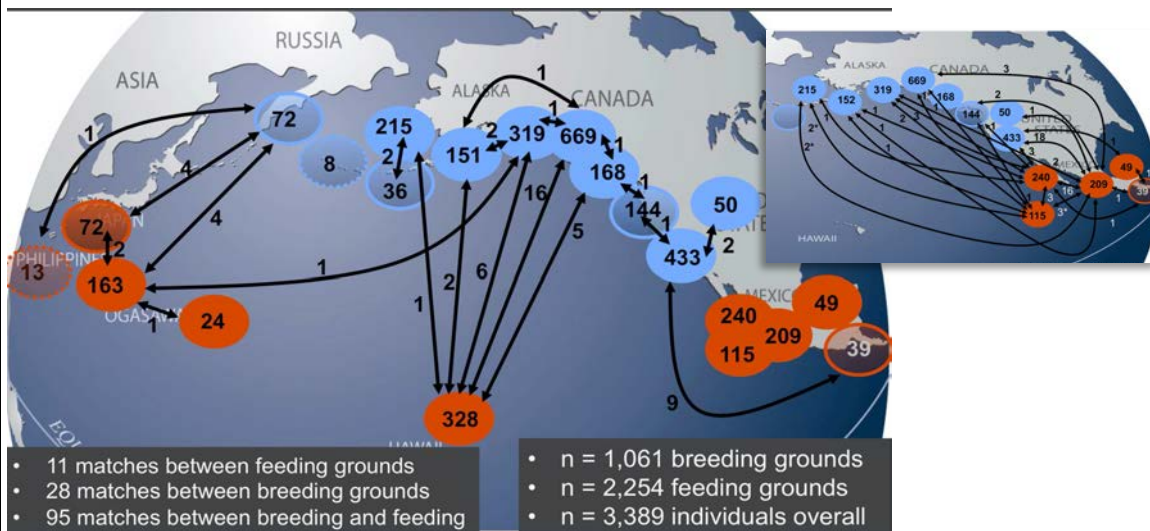


Figure 3: The current status of the DNA register for humpback whales in the North Pacific, with updates from the regional collaborators. This register includes mtDNA haplotypes, sex and microsatellite genotypes (10 loci) for 3,389 individual whales. The number of individuals identified by DNA profiling is summarized for the regional strata used in SPLASH, with some modifications. Lines show regions connected by genotype recaptures. Feeding grounds are shown in blue and breeding grounds in red. The migratory connections with Mexico are shown as an inset due to the complexity of this interchange.

#### (D) SUGGESTIONS FOR OUTREACH

Please, note that successful proponents will be requested to produce ad hoc material that will be used by the IWC Secretariat for dissemination and outreach.

The PI maintains an active presence in the media and will work through the Marine Mammal Institute, Oregon State University, to include updates and press releases related to this research,

<https://mmi.oregonstate.edu/>

#### 6. TIMETABLE FOR ACTIVITIES AND OUTPUTS

Specify the timetable for project activities and expected outputs separately. For projects with multiple distinct elements please indicate interim goals and timeframes. Add as many rows as you need to the tables below. If publications are an expected output please note whether you will submit the manuscript to the IWC's Journal of Cetacean Research and Management.

Activity to be undertaken	Key person(s)	Start(mm/yy)	Finish (mm/yy)
Revise current DNA register for North Pacific humpback	D. Steel	06/2020	09/2020
Communicate with working group to agree on strata for feeding grounds and identify of breeding grounds	D.Steel and C.S. Baker	08/2020	09/2020
Run mixed-stock and assignment procedures for agreed stock structure scenarios	D. Steel	10/2020	12/2020

Expected outputs	Completion date (mm/yy)
Provide summary of output for working group and modellers	December, 2020
Final report for IWC, 68C and subsequent publication	May, 2021

## 7 . RESEARCHERS' (OR STEERING GROUP) NAME(S) AND AFFILIATION

Please, also specify if the project team has any direct connection (e.g. same research group or institute, collaborator on common project) with people involved or likely to be involved in taking the funding decision (e.g. IWC SC heads of delegations, SC convenors, etc.). Add as many rows as you need to the table below.

Name	Affiliation	Connection with decision
C. Scott Baker	Oregon State University	Scientific Delegate and member of the working group on the Comprehensive Assessment
Debbie Steel	Oregon State University	None

## 8 . TOTAL BUDGET

Breakdown into: (1) salaries/wages (include name/position of each individual and breakdown of time and duties i; (2) travel/subsistence expenses (breakdown by person and justification) unless for IPs for workshops where a total estimate based on an average for the total number of IPs is acceptable; (3) services (e.g. aircraft/vessel time, consultancy fees, ARGOS fees, etc.); (4) reusable capital equipment (e.g. reusable equipment such as a hydrophone, cameras, etc. Note that this equipment will have to be registered at the IWC Secretariat and will remain property of the IWC at the end of the project), (5) expendable capital equipment (e.g. consumables, tags, stationery), (6) shipping costs, (7) insurance costs, (8) in kind co-funding (specify whether other funding is available for personnel/name, equipment, venues, etc.). Note that "Overheads" are not admissible. Add as many rows as you need to the table below.

Type	Detailed description	Cost in GB pounds
(1) Salaries (by person)	Debbie Steel, Senior Faculty Research Assistant, 2 months salary and benefits	£13,200
(2) Travel/subsistence (by person or est. total for IPs)		
(3) Services (by item)		
(4) Reusable equipment		
(5) Consumables		
(6) Shipping (by Item)		
(7) Insurance (by item)		
(8) Co-funding		
(9) Other		
<b>Total</b>		<b>£13,200</b>

## 9 . DATA ARCHIVING/SHARING

Please state your plans for data archiving and sharing. Note that data collected primarily under IWC grants are considered publicly available after an agreed period of time for publication of papers, usually about two years. The work of the IWC depends on the voluntary contribution of data to the various databases and catalogues IWC supports. Please consult the Secretariat ([secretariat@iwc.int](mailto:secretariat@iwc.int)).

The PI will follow the principles of the Joint Data Archiving Policy, now adopted by many leading journals (Whitlock et al. 2010) and submit the primary data to the Dryad Archive on condition of publication, <https://datadryad.org/stash/>

## 10 . PERMITS (PLEASE TICK)

Do you have the necessary permits to carry out the field work and have animal welfare considerations been appropriately considered?	None needed
Do you have the appropriate permits (e.g. CITES) for the import/export of any samples?	None needed

If 'Yes' please provide further details and enclose copies where appropriate:

## References

- Albertson, G. R., A. S. Friedlaender, D. J. Steel, A. Aguayo-Lobo, S. L. Bonatto, S. Caballero, R. Constantine, A. L. Cypriano-Souza, M. H. Engel, C. Garrigue, L. Florez-Gonzalez, D. W. Johnston, D. P. Nowacek, C. Olavarria, M. M. Poole, A. J. Read, J. Robbins, A. L. Sremba, and C. S. Baker. 2018. Temporal stability and mixed-stock analyses of humpback whales (*Megaptera novaeangliae*) in the nearshore waters of the Western Antarctic Peninsula. *Polar Biology* **41**:323-340.
- Cypriano-Souza, A. L., M. H. Engel, S. Caballero, C. Olavarría, L. Flórez-González, J. Capella, D. Steel, A. Sremba, A. Aguayo, D. Thiele, C. S. Baker, and S. L. Bonatto. 2017. Genetic differentiation between humpback whales (*Megaptera novaeangliae*) from Atlantic and Pacific breeding grounds of South America. *Marine Mammal Science* **33**:457-479.
- IWC/67b/Rep01(2018), Annex F 25/05/2018. Report on the Sub-Committee for In Depth Assessments.
- Manel, S., O. E. Gaggiotti, and R. S. Waples. 2005. Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology and Evolution* **20**:136-142.
- Pella, J., and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. *Fisheries Bulletin* **99**:151-167.
- Piry, S., A. Alapetite, J. M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**:536-539.
- Schmitt, N., M. C. Double, S. Baker, N. Gales, S. Childerhouse, A. M. Polanowski, D. Steel, R. Albertson, C. Olavarria, C. Garrigue, M. Poole, N. Hauser, R. Constantine, D. Paton, K. C. S. Jenner, S. N. Jarman, and R. Peakall. 2014. Mixed-stock analysis of humpback whales (*Megaptera novaeangliae*) on Antarctic feeding grounds. *Journal of Cetacean Research and Management* **14**:141-157.
- Whitlock, M. C., M. A. McPeck, M. D. Rausher, L. Rieseberg, and A. J. Moore. 2010. Data Archiving. *The American Naturalist* **175**:145-146.



## Appendix 2 – DRAFT SCORING SHEET

If a project presents multiple primary objectives which are achieved using sub-projects, a sheet should be used to evaluate each single sub-project. Note that not all criteria are equally applicable depending on the nature of the project (e.g. field work versus workshops).

IWC SCIENTIFIC COMMITTEE PROPOSALS FOR FUNDING - REVIEW CRITERIA - TEST			
TITLE OF THE PROJECT/sub-projects:			
PRINCIPAL INVESTIGATOR:			
Key criteria	Explanation of scoring	Score	Supporting Remarks
<i>Relevance to Scientific Committee priorities</i>			
1	How well aligned are the scientific outcomes of the project/activity with the current SC priority areas?	1 - Not aligned/poorly aligned (e.g. too vague or generic reference to general SC priorities) 2 - Reasonably aligned (e.g. some aspects may be vague or links are not clear) 3 - Well aligned (e.g. outcomes clearly deliver in the most part on priority areas, may also address longer term or potential future issues). 4 - Closely aligned (e.g. of interest for multiple sub-groups or delivers on specific SC high priority topics/recommendations in the immediate or short term).	
2	To what extent will the outcomes of the project/activity contribute to improvements in the conservation and management of cetaceans?	1 - Not at all 2 - Poorly 3 - Reasonably or over the longer term 4 - Well or over the medium term 5 - Excellently or to almost immediate effect	
<b>Note:</b> if in each of the two above key criteria under this section the project does not score singularly at least 2 points, do not proceed in further evaluation. Of course, proposals within a sub-group would only be developed if in their estimation scores were of 4 or above.			
<i>Approach and methodology</i>			
3	What degree of scientific merit/value is there in carrying out the work?	1 - Not demonstrated or of low scientific value 2 - Useful/basic scientific value 3 - Very good scientific value 4 - Excellent/innovative scientific value	
4	Is the proposed methodology scientifically sound and feasible in terms of field and analytical methods?	1 - Feasibility unrealistic & poor methodology or not properly addressed 2 - Feasibility & methodology acceptable but would benefit from some substantial amendments	

		3 - Feasibility & methodology good, some small changes beneficial 4 - Feasibility & methodology excellent or a highly promising innovative approach to an important question facing the Committee		
5	What is the likelihood of success based on the proposed overall approach and methodology?	1 - No chance of success 2 - Low chance of success/better approaches available 3 - Medium chance of success/some changes to the approach necessary 4 - High chance of success/little or no changes to the approach necessary		
5a	Are objectives of the research likely to be achieved within the proposed time-frame?	1 - No or unlikely 2 - Partially or potentially ambitious 3 - Yes with some minor suggestions 4 - Yes		
5b	Are any proposed intermediary targets timely and achievable?	1 - No or unlikely 2 - Partially 3 - Probably 4 - Yes		
5c	Is the proposed time-frame/work necessary (e.g. can the project produce results in a shorter time period)?	1 - No or unlikely 2 - Partially 3 - Probably 4 - Yes		
5d	Is the sample size adequate to achieve the stated objectives?	1 - Not demonstrated/not properly addressed 2 - No or unlikely (too low/too high) 3 - Probably (additional analysis needed) 4 - Yes		
6	Is the project likely to affect adversely the population(s) involved?	1 - Not properly addressed/ unknown 2 - Yes severely 3 - Possibly at a low level 4 - No		
6a	IF YES, are analyses provided on simulations of the effects using different time-frames for the project if applicable?	1 - No 2 - Partially 3 - Yes		
<p><u>Note:</u> if in each of the above key criteria under this section the project does not score singularly at least 2 points, do not proceed in further evaluation. Of course, proposals within a sub-group would only be developed if in their estimation scores were of 3 or above.</p>				
Project team and Project management				

7	To what extent does the team have the relevant expertise, experience, and balance?	1 – Poor or not demonstrated 2 – Sufficient 3 - Very good 4 - Excellent		
8	Contingency plan: To what extent have potential problems/risks been considered and appropriate mitigation proposed?	1 – Poor or not demonstrated 2 – Sufficient but could be improved 3 - Fully or requiring only minor suggestions or not applicable		
<i>Value for Money</i>				
10	Does the project represent good value for money?	1 – No or significant amendments would be needed 2 – Yes but with some minor amendments 3 – Yes		
11	Have sufficient links been made to the wider research community/other organisations/capacity building.	1 – No 2 – Some but significant amendments needed 3 – Yes but with some minor additions 4 – Yes or not applicable		