PROGRESS REPORT (as of January 2021)
Genetic Assessment of Blue Swimming Crabs in the Philippines

1. Summary of activities

Table 1. Number of blue swimming crab specimens collected and analyzed in the lab.

Collection Sites (as of December 03, 2020)		Specimens collected	DNA extracts obtained	Cytochrome c oxidase I gene sequences generated	Control region sequences generated	Cytochrome b sequences generated	Microsatellite Loci		
							pPp9	pPp10	Pp100028
LUZON	Dagupan, Pangasinan	30	30	26	9	32	16	16	5
	Ragay Gulf, Camarines Sur *	30	30	17	0	23	10	10	4
	San Miguel Bay, Camarines Sur *	30	30	19	0	27	9	9	0
	Tayabas Bay, Quezon*	30	20	19	4	23	9	9	2
VISAYAS	Dumaguete City, Negros Oriental	30	30	15	8	10	9	9	5
	Visayan Sea, Iloilo*	30	30	21	0	0	0	0	0
	Guimaras Strait, Guimaras*	30	30	15	0	0	10	10	8
MINDANAO	Surigao City, Surigao del Norte	30	30	16	12	14	10	10	2
	Hinatuan, Surigao del Sur	30	30	17	7	10	10	10	7
	Sarangani Bay, General Santos	30	16	5	0	0	0	0	0
	Panguil Bay, Lanao del Norte*	30	30	19	4	13	9	9	7
TOTAL		330	306	189	44	152	92	92	40

*** Major Crab fishing grounds included in Ingles 2004**
Highlighted are items added/updated from progress report submitted last July

Blue swimming crab samples were collected from eleven (11) localities. Out of these six (6) are listed by Ingles (2004) as major fishing grounds for the blue swimming crab.

Sampling has not resumed due to quarantine conditions. If second tranche will be released, specimens from at least five (5) more sampling areas can still be processed.

Table 2. Completion of activities listed in the proposed workplan.

Objectives	Expected Output *	Activities or Workplan	Proposed schedule of completion				Target completion after 2 quarters	Actual completion using 1st tranche funds
			Q1	Q2	Q3	Q4		
		Procurement of supplies, reagents and equipment					73%	73%
		Sample Collection					69%	69%
		DNA Extraction					69%	69%
Genetically characterize *P. pelagicus* populations in the Philippines based on mtDNA profiles	*P. pelagicus* populations ranked based on genetic diversity parameters	Amplification, sequencing, and sequence analysis of mtDNA genes					50%	50%
Amplify microsatellite loci in *P. pelagicus* using markers developed from other *Portunus* species	5 microsatellites successsfully cross-amplified	Cross-amplification of microsatellites					0%	100%
		Characterization based on microsatellite markers					0%	50%
	Published paper that can inform policy	Manuscript writing for publication					0%	

2. Preliminary findings

Mitochondrial marker sequences

Problems were encountered in mitochondrial DNA sequencing as explained further in the subsequent paragraphs. Hence, we produced more COI sequences, and we are redoing optimization for cytochrome b amplification to rectify the situation. To cover for this unexpected activity, we requested and were provided funds by the university, so as not to affect the project objectives.

We previously reported that based on preliminary analysis, the mitochondrial control region is highly variable. Although this is indicative of high genetic diversity, these will not be informative for comparative analyses. We temporarily discontinued sequencing the mitochondrial control region.

We sequenced cytochrome b gene as an alternative. Although, amplification rate was high, closer examination of the region of the cytochrome b gene that was amplified did not show much variation. We are currently checking if this is true only in the region that was sequenced or within the entire gene (which would be quite concerning). We are currently optimizing the amplification of a larger portion of the cytochrome b gene.

Cytochrome c oxidase I (COI) sequencing was continued to further assess the two lineages observed. Moreover, genetic diversity analysis of the blue crab populations will also be conducted for this gene in addition to cytochrome b to make the results more informative.

Microsatellite marker analysis

Originally, the objective of this study involving microsatellites was limited to optimizing conditions for PCR amplification. Considering the completion of this objective ahead of time, we decided to proceed with the genetic characterization of *P. pelagicus* populations using microsatellite DNA to augment to the results of mtDNA analysis. So far, we have substantial results using three loci. Preliminary results are shown in the subsequent text.

Currently, there are 92 individuals from nine locations (Pangasinan, Tayabas, San Miguel Bay, Ragay Gulf, Dumaguete, Guimaras, Hinatuan, Surigao City, and Panguil Bay) that were included in the analysis. Based on available COI sequences, 61 of these belong to one putative lineage (“Lineage A”) and another 31 to a second lineage (“Lineage B”). Three loci of the five loci previously amplified for this study were chosen based on available literature (Table 3) (Chai et al. 2017; Wu et al. 2018).

Table 3. Loci chosen for assessment in this study.

Locus	Primer Sequences	Annealing Temperature (°C)	Repeat Motif	Expected Product Size (bp)	Source
pPp9	F: GACTTGAGCGATGCTGAAAG R: ATGGATAGATGGAATGCAAAAT	52	$(TG)_{19}$	133-187	Chai et al. 2017
pPp10	F: CCTGTATTGTCATGTGTTTGATTTT R: CTACGACCAACTTTACCGCC	52	$(TG)_{34}$	91-155	Chai et al. 2017
Pp100028	F: TGGTTCTCCTGAAATACTGTTG R: CTCCCCTCTCTCAATTAGTTCC	53	$(AGG)_{8}$	277	Wu et al. 2018

All of the 92 individuals were successfully amplified for loci pPp9 and pPp10. Due to poor amplification for some individuals, only 40 were successfully amplified for locus Pp100028. The population data is analyzed using two data sets: a first data set containing all 92 individuals for two loci and a second data set containing 40 individuals for three loci (Table 4). F_{ST} values suggest that there is considerable population differentiation based on loci pPp9 and pPp10 (Table 5).

Table 4. Observed and expected heterozygosity and F-value for each lineage in each data set.

Data Set	Lineage	Number of Individuals (N)	Average Expected Heterozygosity (H_e)	Average Observed Heterozygosity (H_o)
92 Individuals (for loci pPp9 and pPp10)	A	61	0.940	0.918
	B	31	0.913	0.855
	Overall	92	0.941	0.896
40 Individuals (for loci pPp9, pPp10, and Pp100028)	A	28	0.937 ± 0.003	0.750 ± 0.125
	B	12	0.890 ± 0.001	0.778 ± 0.028
	Overall	40	0.947	0.575

Table 5. Overall F-statistics for Philippine Blue Swimming crab populations.

Data Set	F-Statistics	Value	P(rand >= data)*
92 Individuals (for loci pPp9 and pPp10)	**F_{ST}**	0.010	0.003
	F_{IS}	0.048	0.006
	F_{IT}	0.057	0.005
40 Individuals (for loci pPp9, pPp10, and Pp100028)	**F_{ST}**	0.006	0.135
	F_{IS}	0.203	0.001
	F_{IT}	0.208	0.001

3. Implication of the results

At the moment, we cannot provide inferences on individual populations (as values may change as we add more samples). But we can already have a preliminary assessment of the genetic diversity of the Philippine *P. pelagicus* population as a whole and compare it with other systems. Most of the loci analyzed so far show high genetic diversity in Philippine *P. pelagicus* populations. These loci include control region, COI, and the three microsatellite loci used in this study. The only contrasting results come from cytochrome b sequences, but we are still verifying this result by sequencing a larger portion of the gene. Likewise, diversity indices are comparable to those reported in other similar studies (see Appendix A), meaning we find no evidence yet of a significant reduction in genetic diversity in response to intense fishing pressure (we are analyzing this further by comparing populations collected from major fishing areas as compared to those from other localities). There is an indication though of genetic differentiation between populations based on microsatellite data (lower observed heterozygosity value compared to expected and significant F_{ST} values at least for two of the loci). It is too early to conclude though and results may still be affected by the addition of other loci and increase in samples.

4. Other notes

The funds released via the first tranche were fully liquidated (see attached xlsx file with revised LIB). The objective was revised to include microsatellite analysis in characterizing the populations. The workplan was also revised. Activities proposed for the first two quarters was spread from Oct 2019 to Jan 2021. This is due to the complete stoppage of activities during ECQ and MECQ and a limited work schedule during GCQ.

From Feb 2021 onwards, even if GCQ persists, the staff will be allowed to work in the facility five days a week. As such, if the remaining funds will be released, we expect to complete all remaining research activities in six months (until July 2021).

The only limitation would be the difficulty in sample collection. The best option for us is still to have the specimens shipped, if possible. If so, we could analyze at least five more populations.

5. References:

Chai, C. J., Esa, Y. B., Ismail, S., & Kamarudin, M. S. (2017). Population structure of the blue swimmer crab *Portunus pelagicus* in coastal areas of Malaysia inferred from microsatellites. Zoological Studies, 56.

Hara M, Sekino M (2007) Genetic Differences Between Hatchery Stocks and Natural Populations in Pacific Abalone (Haliotis discus) Estimated Using Microsatellite DNA Markers. Mar Biotechnol (NY) 9:74–81. https://doi.org/10.1007/s10126-006-6060-2

Ingles, J. A. 2004. Status of the blue crab fisheries in the Philippines. DA-BFAR (Department of Agriculture-Bureau of Fisheries and Aquatic Resources). In turbulent seas: The status of Philippine marine fisheries. Coastal Resource Management Project, Cebu City, Philippines, 47-52.

Lazar Jr I, Lazar Sr I. 2019. GelAnalyzer 19.1. Available at: http://www.gelanalyzer.com/index.html

Liu YG, Guo YH, Hao J, Liu LX (2012) Genetic diversity of swimming crab (Portunus trituberculatus) populations from Shandong peninsula as assessed by microsatellite markers. Biochem Syst Ecol 41:91–97. https://doi.org/10.1016/j.bse.2011.12.024

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28:2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Shaw PW, Turan C, Wright JM, et al (1999) Microsatellite DNA analysis of population structure in Atlantic herring (Clupea harengus), with direct comparison to allozyme and mtDNA RFLP analyses. Heredity (Edinb) 83:490–499. https://doi.org/10.1038/sj.hdy.6885860

Sienes, P. M. Q., Willette, D. A., Romena, L. R., Alvior, C. G., & Estacion, J. S. 2014. *Portunus pelagicus* (Linnaeus 1758), in the Philippines. Philippine Science Letters.

Wu Q, Miao G, Li X, et al (2018) De novo assembly of genome and development of polymorphic microsatellite loci in the blue swimming crab (*Portunus pelagicus*) using

RAD approach. Mol Biol Rep 45:1913–1918. https://doi.org/10.1007/s11033-018-4339-9

Wu Q, Miao G, Li X, et al (2018) De novo assembly of genome and development of polymorphic microsatellite loci in the blue swimming crab (Portunus pelagicus) using RAD approach. Mol Biol Rep 45:1913–1918. https://doi.org/10.1007/s11033-018-4339-9

Xu Q, Liu R (2011) Development and characterization of microsatellite markers for genetic analysis of the swimming crab, Portunus trituberculatus. Biochem Genet 49:202–212. https://doi.org/10.1007/s10528-010-9399-z

Yap ES, Sezmis E, Chaplin JA, et al (2002) Isolation and characterization of microsatellite loci in Portunus pelagicus (Crustacea: Portunidae). Mol Ecol Notes 2:30–32. https://doi.org/10.1046/j.1471-8286.2002.00136.x

Appendix A. Comparison of genetic diversity measures among different studies.

	Species	Locus	Number of specimens	Number of alleles	Expected heterozygosity	Observed heterozygosity	Reference
1	*Portunus pelagicus* (sensu stricto)	pPp2	87	34	N/A	N/A	Chai et al. 2017
		pPp9	87	23	N/A	N/A	
		pPp10	87	32	N/A	N/A	
		Ptri2*	87	14	N/A	N/A	
2	*Portunus pelagicus* (sensu stricto)	Pp100028	33	5	0.6797	0.6333	Wu et al. 2018
		Pp100117	33	4	0.6840	1.0000	
		Pp100149	33	4	0.5675	1.0000	
		Pp10024-1	33	4	0.4896	0.5938	
		Pp10012	33	3	0.1766	0.1875	
		Pp240746	33	6	0.7433	0.5714	
		Pp116160	33	4	0.5432	0.6562	
		Pp10359	33	4	0.6277	0.7742	
		Pp16648	33	7	0.8189	0.875	
		Pp100223	33	6	0.6373	0.6333	
		Pp100226	33	2	0.4762	0.5625	
		Pp159887	33	11	0.8770	0.875	
		Pp22143	33	4	0.6642	0.4194	
		Pp1659	33	2	0.3902	0.5172	
		Pp179093	33	7	0.8080	1.0000	
		Pp16422	33	2	0.0615	0.0625	
3	*Portunus pelagicus* (sensu stricto)	pPp9	92	26	0.941	0.902	This study
		pPp10	92	24	0.941	0.891	
		Pp100028	40	26	0.947	0.575	
4	*Portunus pelagicus*(see remarks)	pPp2	851	35	0.90	0.76	Yap et al. 2002
		pPp4	864	34	0.91	0.77	
		pPp5	85	26	0.91	0.32	
		pPp8	847	15	0.84	0.64	
		pPp9	841	23	0.85	0.74	
		pPp10	96	27	0.93	0.78	
		pPp18	858	31	0.88	0.75	
		pPp19	779	5	0.65	0.45	

	Portunus sp. (see remarks)	pPp2	28	13	0.89	0.96	
		pPp4	47	12	0.79	0.75	
		pPp5	7	Poor amplification	Poor amplification	Poor amplification	
		pPp8	29	17	0.92	0.72	
		pPp9	50	12	0.81	0.80	
		pPp10	37	16	0.82	0.87	
		pPp18	47	10	0.78	0.79	
		pPp19	48	2	0.04	0.04	
5	*Portunus trituberculatus*	Ptri1	38	25	0.9373	0.6324	Xu and Liu, 2011
		Ptri2	38	26	0.9532	0.806	
		Ptri3	38	22	0.9348	0.8676	
		Ptri4	38	26	0.9547	0.9403	
		Ptri5	38	22	0.9347	0.8824	
		Ptri6	38	26	0.9538	0.8923	
		Ptri7	38	18	0.9244	0.7727	
		Ptri8	38	20	0.9237	0.8333	
		Ptri9	38	18	0.9257	0.8676	
		Ptri10	38	22	0.9411	0.9	
		Ptri11	38	17	0.8998	0.7391	
	P. pelagicus(see remarks)	Ptri7	14	10	0.8571	0.6429	
		Ptri8	15	11	0.8489	0.6667	
		Ptri10	16	11	0.5625	0.8262	
	P. sanguinolentus	Ptri6	18	16	0.9105	0.8889	
		Ptri10	17	10	0.8304	0.4118	
6	*Portunus trituberculatus*	Ptri2	180	5.5*	0.77	0.83	Liu et al. 2012
		Ptri3	180	2.5*	0.69	0.77	
		Ptri4	180	3.0*	0.69	0.78	
		Ptri5	180	6.5*	0.75	0.79	
		Ptri7	180	5.5*	0.66	0.71	
		Ptri8	180	2.7*	0.73	0.82	
		Ptri9	180	2.8*	0.67	0.73	
		Ptri10	180	6.5*	0.67	0.76	