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A Molecular Assay for Ecotoxicological Endpoints

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Introduction

Recent environmental tragedies, such as the dams bursting in Brazil and several oil spills, have caused a renewal of interest in the methods used to detect environmental contaminants. The disaster in Brazil alone has been estimated to take up to 30 years for the Doce Basin to be cleaned and the ecosystem to return to something similar to its original state (ABC News, 2015). The dams released toxic mud and acid mine drainage which contains high levels of heavy metals such as zinc, iron, and cadmium. Previous research has already shown that prolonged exposure to heavy metals causes upregulation of expression of several classes of genes and the release of zinc into the environment via the zinc that is stored naturally in the organism (ATSDR, 2003). Most of the methods used require molecular assays in order to determine the presence of a particular gene, in this case one that deals in detoxifying the organism, as well as the quantification and functionality of the gene. Based on aquatic amphipods reactions to heavy metals coupled with their ability to reproduce after exposure they will have a more specific molecular bioassay.

One of the most common genes associated with the process of detoxification is Glutathione S-transferase (GST), which has been heavily studied in insects, but not in aquatic amphipods. Although, GST is currently being heavily studied in other organism as well, such as the copepod *Calanus finmarchicus,* to demonstrate its potential to be used as a reliable biomarker in relation to ecotoxicology and determining the presence of toxins. It has been shown to be upregulated by the presence of naphthalene as well as other toxins such as cadmium and copper when looking at the mRNA expression (Hansen et. al, 2008). While there are not many studies for amphipods, the studies performed in other organisms have provided substantial proof that GST expression is upregulated by heavy metals and is a viable indicator of pollution in the environment.

Parhyale hawaiensis is a marine amphipod that has a sequenced genome, well-established culture methods, and a high rate of reproduction. It is thought that exposing the organism to ecologically applicable concentrations of heavy metals then the GST genes will be upregulated, further allowing *P. hawaiensis* to function as a molecular bioassay as well as to determine the effects on the organism.

Materials and Methods

Parhyale hawaiensis maintenance and collection: The cultures were split into three different tanks, one of them was designated the neonate tank (less than 1-2 days of age). Each tank was cleaned and fed every other day throughout the project. Pipettes were used to remove debris and food particles that had accrued on the bottom of the tank. When cleaning the tanks, the debris was deposited into a 150-mL beaker and examined to ensure no neonates were removed. A thin stemmed pipette was then used to remove any neonates that may have been deposited in the debris container and placed back into the tank; the same type of pipette was used to collect neonates for DNA and RNA extraction. Once all debris was removed, the tank was emptied halfway and fresh saltwater with a refractive index of 1.025 was added to replace the water content. Around 30 pellets of food were then scattered around the bottom of the tank, some of the tanks may have required more pellets depending on the amount of *P. hawaiensis* present.

Locating and Aligning Glutathione S-transferase genes: The NCBI database was primarily used to locate the GST genes present in other crustaceans due to the *P. hawaiensis* genome not yet being annotated. The crustaceans used were: *Gammarus Pulex, Lygus lineolaris, Penaeus monodon, Procambus clarkii, Portunus trituberculatus, and Trigiopus japonicas*. Once the genes were located, the BLAST program in NCBI was used to align the sequences of one organism to another's. The program was then used again to align the individual sequences against the *P. hawaiensis* genome in order to discern if the GST sequence was present.

Results

CAAGGTGGTGTTCTGGGACCCAACCATGACTAT
TGACTTCTACTACGTTATCCGCTCAGCCCCTTGC
CAGGGCCCCATGATGGTGGCCAAGGCTCTTGGT
GTTGAGCTCAACCTCAAGGAAGTGGATCTAACA
AATAAGGAGCAGTTGAAGCCGAGCTTCCTCGCT
CTGAACCCTCAGCACACCGTGCCCACCATGGTG
GACGGGGACTTCTCGTTGTGGGAAAGCAAAGC
CATCATTGGTTACCTGGTTGGAAAGTATGGTAA
AGACGACAGCCTGTACCCGACAGATCCAAAGA
AGAGAGCCATCGTAGACCGCTTCCTGTACTACG
ACCTGAGTCTTCTTGGTGCATTCAGAGACTGGG
CGGTCCCAGCCATGCACCAGAACGCAATTGCCG
ATCCTCAGAAGAAAGAAAAGCTGCACGAAACC
ATCGACAAGCTGGAGCAGCATTTCAAGCGGAC
GGGCAACAAGTTTGTTACTGGCGACACTGTGTG
TGTCGCAGACCACGCCCTGGCTTCCTCCCTGAC
CA

Figure 1: Primer PhGST1F (green) and Primer PhGST1R (yellow) in *P. hawaiensis* GST gene

Name	Sequence
Ph18SF	CGT GAG CAC CCT ATC TAA CG
Ph18SR	TTC AGC TTT GCA ACC AGA CT
PhGST1F	GCC GAT CCT CAG AAG AAA GAA A
PhGST1R	CCG TCC GCT TGA AAT GCT

Figure 2: Primers used with *P. hawaeinsis* GST sequence

```
OLIGO            start  len     tm     gc%   any    3'  seq
LEFT PRIMER        136   20  60.12  55.00  4.00  1.00 CCCACTACGCCCTTTAGTCA
RIGHT PRIMER       452   20  59.76  45.00  4.00  0.00 TGCTTCCCAACCAAATATCC
SEQUENCE SIZE: 633
INCLUDED REGION SIZE: 633

PRODUCT SIZE: 317, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 0.00

OLIGO            start  len     tm     gc%   any    3'  seq
LEFT PRIMER        256   20  60.05  40.00  2.00  0.00 GGCACGAATGAAAAGGAAAA
RIGHT PRIMER       625   20  59.97  45.00  4.00  0.00 GATTGTTTAATGGGCGTGCT
SEQUENCE SIZE: 645
INCLUDED REGION SIZE: 645

PRODUCT SIZE: 370, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 1.00

OLIGO            start  len     tm     gc%   any    3'  seq
LEFT PRIMER        171   20  60.04  45.00  6.00  2.00 AAGCCCGAATTCCTCAAGAT
RIGHT PRIMER       549   20  59.97  55.00  4.00  2.00 TAGAGGCAGAGGCCATCAGT
SEQUENCE SIZE: 900
INCLUDED REGION SIZE: 900

PRODUCT SIZE: 379, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 1.00
```

Figure 3: Sample of Original primers

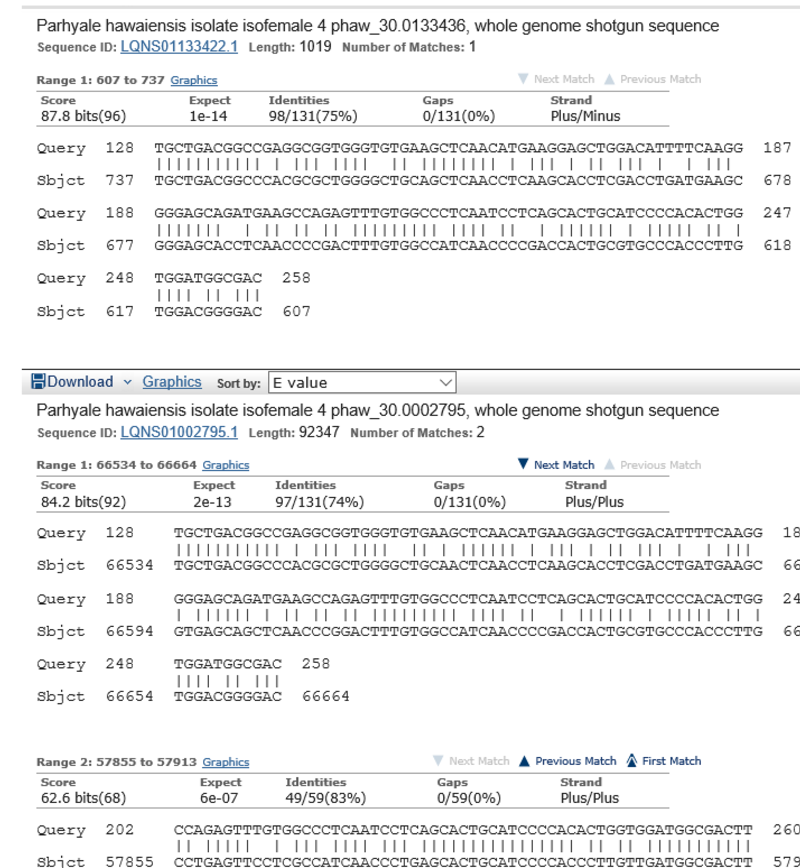

Figure 4: Sample BLAST result >80% (*P. trituberculatus* aligned with *P. hawaiensis)*

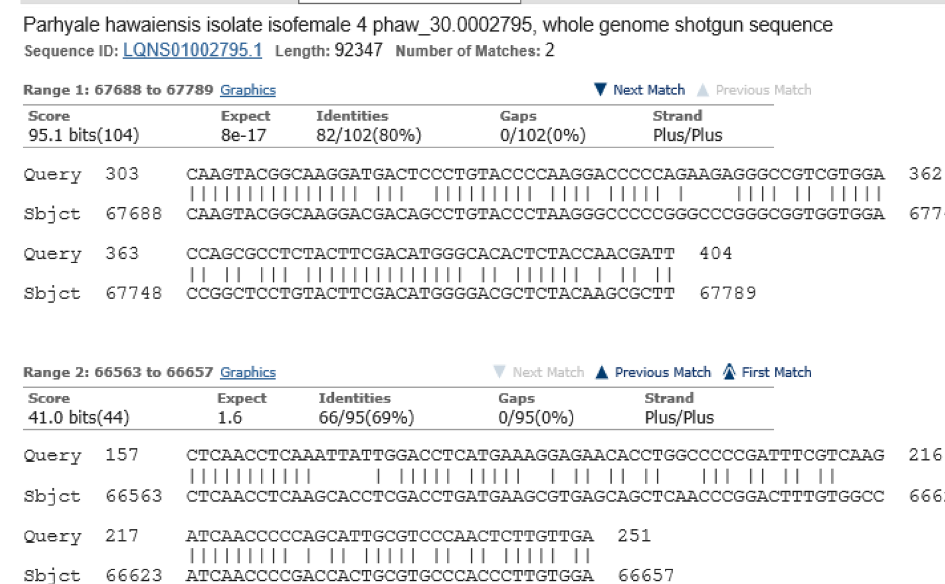

Figure 5: Sample BLAST result > 80% (*L. lineolaris* aligned with *P. hawaiensis*)

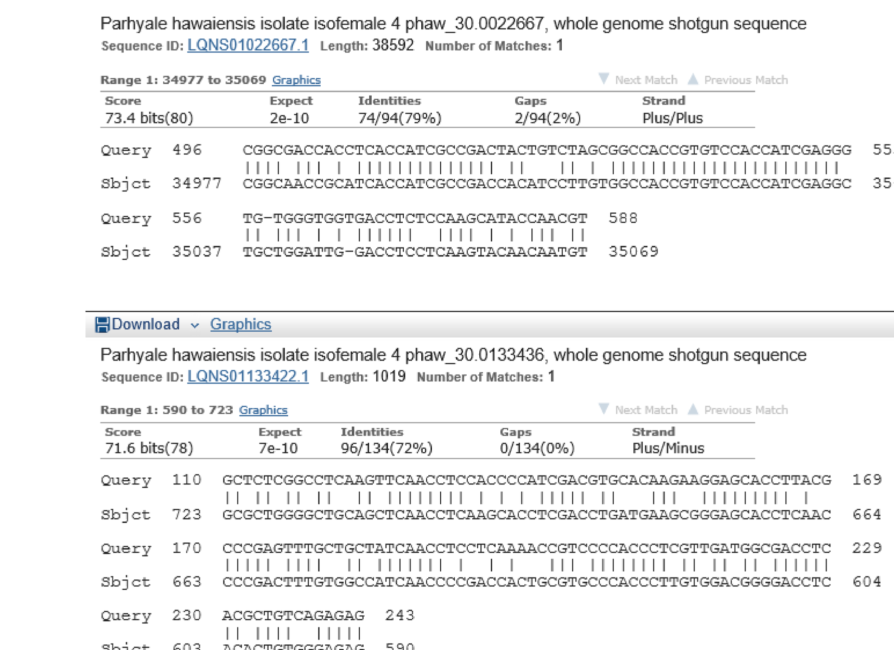

Figure 6: Sample BLAST result: alignment % <80% (*G. pulex* with *P. hdawaiensis*)

Name	Length	Score
KM023725:*Penaeus monodon*	867	68
KF781517.1: *Portunus trituberculatus*	1008	84.2
HQ414581.1: *Procambus clarkii*	812	68
DQ315381.1: *Lygus lineolaris*	889	96.1
EH271669.1: *Gammarus pulex*	630	24.7

Figure 7: Alignment ascension numbers, organisms, and alignment percentages

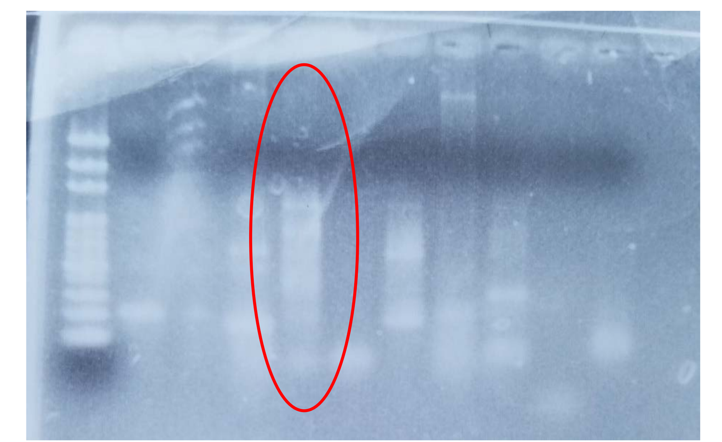

Figure 8: Gel electrophoresis of primers 7/8 with *P. hawaiensis* DNA

Conclusion

When conducting this experiment the goal alignment percentage was 80%, this indicated a highly conserved gene meaning that the gene was more easily recognized in the *P. hawaiensis* genome. Only *L. lineolaris* and *P. trituberculatus* had 84% and 96.1% alignments percentages, respectively. Given the conflicting percentages obtained, the gene was not able to be said to be easily recognized in the *P. hawaiensis* genome.

GST was determined to be present in the *P. hawaiensis* genome when the sequence suspected provided by colleagues was compared to known GST sequences from other organisms. Due to the recent identification of the GST sequence and the confirmation of the sequence in *P. hawaiensis* further work will be completed to ensure that it is the correct gene. Furthermore, the assay will be refined and testing with heavy metals will be performed to ascertain the upregulation of the gene in specific conditions.

References

Hansen, Bjørn Henrik, Dag Altin, Siv-Hege Vang, Trond Nordtug, and Anders J. Olsen. "Effects of Naphthalene on Gene Transcription in Calanus Finmarchicus (Crustacea: Copepoda)." Aquatic Toxicology 86.2 (2008): 157-65. Web. 4 Feb. 2016.

"Potential for Human Exposure". Agency for Toxic Substances and Disease Registry 60.6: 139-180. 2003.

"Toxic Mud from Brazil Mine Spills into Atlantic Ocean." ABC News. N.p., 21 Nov. 2015. Web. 04 Feb. 2016.

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