

Transcriptome dynamics of an oyster larval response to a conspecific cue-mediated settlement induction in Pacific oyster *Crassostrea gigas*

Mary Grace Sedanza^{1,2,*}, Jalal Alshaweesh^{3,4}, Yi-Li Gao⁵, Asami Yoshida¹, Hee-Jin Kim¹, Kenichi Yamaguchi¹, Cyril Glenn Satuito^{1,6}

¹ Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki 852-8521, Japan; y-asami@nagasaki-u.ac.jp (A.Y.); heejin@nagasaki-u.ac.jp (H.-J.K.); kenichi@nagasaki-u.ac.jp (K.Y.); satuito@nagasaki-u.ac.jp (C.G.S.)

² Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao Iloilo 5023, Philippines

³ Department of Parasitology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki 852-8523, Japan; bb55418852@ms.nagasaki-u.ac.jp

⁴ Graduate School of Biomedical Science, Doctoral Leadership Program, Nagasaki University, Nagasaki 852-8523, Japan

⁵ College of Science and Technology, Ningbo University, Ningbo 315300, China; loootutugaoyili@163.com (Y.-L.G.)

⁶ Organization for Marine Science and Technology, Nagasaki University, Nagasaki 852-8521, Japan

* Correspondence: bb53419802@ms.nagasaki-u.ac.jp or mcsedanza@up.edu.ph; Tel.: +81-95-819-2853

Supplementary Materials

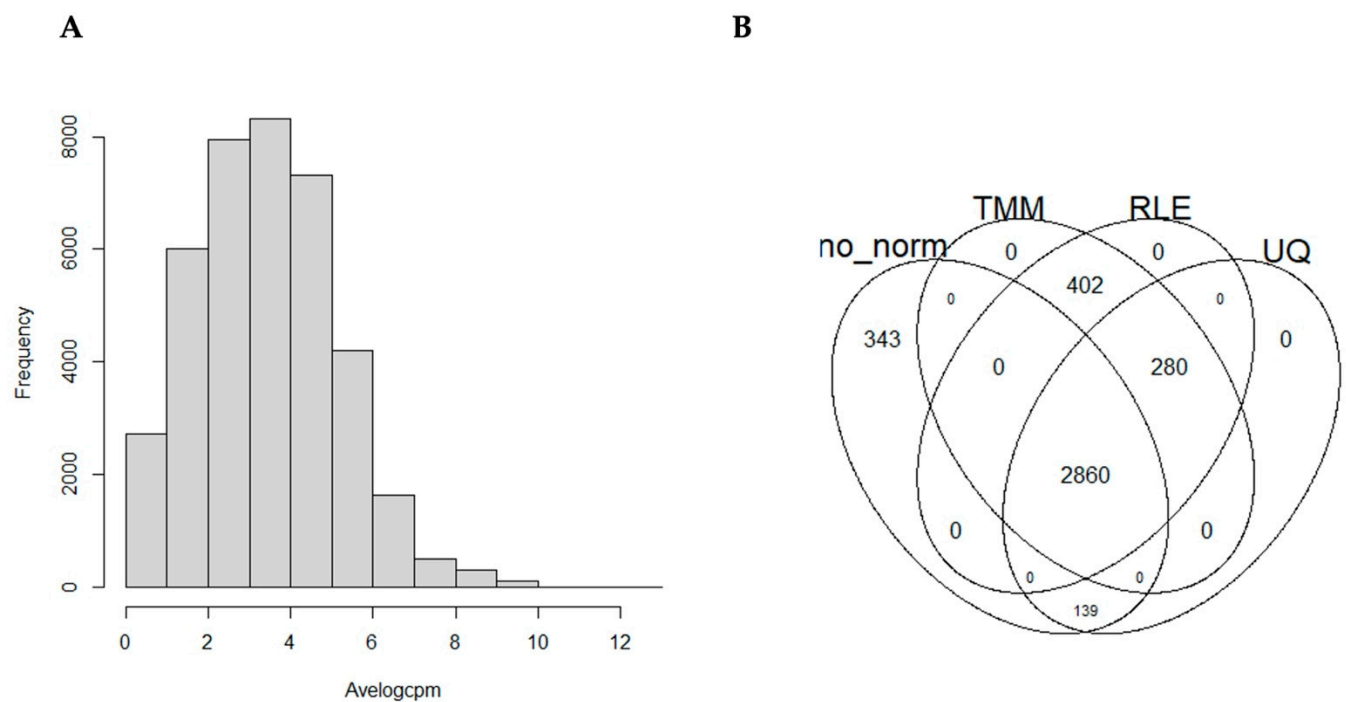


Figure S1. Histogram profile of the average log count-per-million (CPM) of the mapped transcript sequences **(A)** and the Venn diagram of transcripts with differential expression using various normalization models **(B)**. Abbreviations: no_norm = no normalization; TMM = Trimmed Mean of M-values; RLE = Relative Log Expression; UQ = Upper Quartile.

Table S1. Full list of transcripts used for differential expression analysis in the Pacific oyster *Crassostrea gigas* transcriptome.

The table is provided as a separate **Table S1.xls** document

Table S2. Results of the Blastx homology search for the thirty differential transcript expression in the *C. gigas* transcriptome.

| <i>C. gigas</i> Accession No. (Subject) | <i>C. gigas</i> Annotation | Homolog Protein ID (Query) | Species | Homolog Accession No. | Identities (Query/ Subject) | Homology (%) | BlastX E-value | Protein Length (Query) |
|---|--|---|--------------------------------|--------------------------|-----------------------------------|-----------------|------------------------|------------------------------|
| LOC105335414 | Abscisic acid and environmental stress-inducible protein | Abscisic acid and environmental stress-inducible protein (AESIP) | <i>Crassostrea gigas</i> | XP_011437564.2 | 84/84 | 100 | 0 | 126 |
| LOC105341000 | Coadhesin | Coadhesin | <i>Crassostrea gigas</i> | XP_034329440.1 | 831/831 | 100 | 0 | 831 |
| LOC105324992 | SCO-spondin | SCO-spondin | <i>Crassostrea gigas</i> | XP_034330544.1 | 3201/3201 | 100 | 0 | 3201 |
| LOC105338148 | Uncharacterized LOC105338148, Isoform X1 | General transcriptional corepressor trfA-like,1 (trfA-like,1) | <i>Mercenaria mercenaria</i> | XP_045201952.1 | 96/290 | 33 | 3 x 10 ⁻²⁹ | 796 |
| LOC105347520 | A disintegrin and metalloproteinase with thrombospondin motifs adt-1 | A disintegrin and metalloproteinase with thrombospondin motifs adt-1 (ADAM-1) | <i>Crassostrea gigas</i> | XP_034329445.1 | 595/595 | 100 | 0 | 595 |
| LOC105324594 | Uncharacterized LOC105324594 | Calcium-dependent protein kinase 7 (CPK7) | <i>Crassostrea gigas</i> | XP_011427257.2 | 70/136 | 51 | 2 x 10 ⁻³⁷ | 133 |
| LOC117688777 | Spidroin-1-like | Spidroin-1-like,1 | <i>Crassostrea gigas</i> | XP_034323067.1 | 46/46 | 100 | 2 x 10 ⁻⁰⁸ | 308 |
| LOC105348066 | Uncharacterized LOC105348066 | Stereocilin | <i>Mizuhopecten yessoensis</i> | OWF40357.1 | 483/1126 | 43 | 0 | 1412 |
| LOC105328424 | Uncharacterized LOC105328424 | IgGFC-binding protein | <i>Crassostrea gigas</i> | XP_011422608.2 | 267/678 | 39 | 6 x 10 ⁻¹³⁵ | 708 |
| LOC105340987 | Uncharacterized LOC105340987, isoform X1 | SCO-spondin-like,1 | <i>Crassostrea virginica</i> | XP_022339848.1 | 808/866 | 93 | 0 | 1957 |
| LOC105340987 | Uncharacterized LOC105340987, isoform X2 | SCO-spondin-like,2 | <i>Crassostrea virginica</i> | XP_022339848.1 | 808/866 | 93 | 0 | 1957 |
| LOC109618369 | Uncharacterized LOC109618369 | General transcriptional corepressor trfA-like,2 (trfA-like,1) | <i>Mercenaria mercenaria</i> | XP_045201952.1 | 97/290 | 33 | 9 x 10 ⁻³¹ | 796 |

| | | | | | | | | |
|--------------|---|---|------------------------------|----------------|---------|-----|----------------------|------|
| LOC117688778 | Spidroin-1-like | Spidroin-1-like,2 | <i>Crassostrea gigas</i> | XP_034323070.1 | 63/63 | 100 | 7×10^{-13} | 238 |
| LOC117688748 | Uncharacterized LOC117688748 | Uncharacterized LOC117688748 | <i>Crassostrea gigas</i> | XP_034322956.1 | 292/292 | 100 | 0 | 292 |
| LOC117688749 | Uncharacterized LOC117688749 | Uncharacterized LOC117688749 | <i>Crassostrea gigas</i> | XP_034322955.1 | 292/292 | 100 | 0 | 292 |
| LOC105327237 | Uncharacterized LOC105327237 | SCO-spondin-like,3 | <i>Crassostrea virginica</i> | XP_022339848.1 | 552/806 | 68 | 0 | 1957 |
| LOC117684686 | ADP-ribosylation factor 1-like | ADP-ribosylation factor 1- like | <i>Crassostrea gigas</i> | XP_034313686.1 | 189/189 | 100 | 3×10^{-136} | 189 |
| LOC105327534 | Mediator of DNA damage checkpoint protein 1 | Mediator of DNA damage checkpoint protein 1 (MDC1) | <i>Crassostrea gigas</i> | XP_011426386.2 | 590/590 | 100 | 0 | 590 |
| LOC105344419 | Glycine-rich cell wall structural protein 1.8 transcript var X2 | Glycine-rich cell wall structural protein 1.8 transcript isoform X2 (GRP 1.8-X2) | <i>Crassostrea gigas</i> | XP_034323479.1 | 141/141 | 100 | 0 | 141 |
| LOC105344419 | Glycine-rich cell wall structural protein 1.8 transcript var X1 | Glycine-rich cell wall structural protein 1.8 transcript isoform X1 (GRP 1.8-X1) | <i>Crassostrea gigas</i> | XP_011450501.3 | 155/155 | 100 | 0 | 155 |
| LOC105329902 | Uncharacterized LOC105329902 | Tyrp-1 | <i>Hyriopsis cumingii</i> | APC92582.1 | 282/731 | 39 | 2×10^{-157} | 778 |
| LOC105334609 | Collagen alpha-2(I) chain | Collagen alpha-2(I) chain | <i>Crassostrea gigas</i> | XP_011436430.2 | 173/173 | 100 | 6×10^{-37} | 173 |
| LOC105324991 | IgGFC-binding protein | IgGFC-binding protein | <i>Crassostrea gigas</i> | XP_011422608.2 | 687/687 | 100 | 0 | 708 |
| LOC105345892 | Leucine-rich repeat- containing protein 4C | Leucine-rich repeat- containing protein 4C (LRRC4C) | <i>Crassostrea gigas</i> | XP_011452538.2 | 391/391 | 100 | 0 | 391 |
| LOC105322623 | Uncharacterized LOC105322623 | Uncharacterized LOC105322623 | <i>Crassostrea gigas</i> | XP_011419747.2 | 400/400 | 100 | 0 | 400 |
| LOC105330463 | Spidroin-1-like | Spidroin-1-like,3 | <i>Crassostrea gigas</i> | XP_034323071.1 | 30/30 | 100 | 3×10^{-08} | 236 |
| LOC105345888 | Uncharacterized LOC105345888 | CD283 | <i>Mytilus edulis</i> | CAG2230452.1 | 131/372 | 35 | 3×10^{-59} | 392 |
| LOC105319426 | Acanthoscurrin-2 | Acanthoscurrin-2 | <i>Crassostrea gigas</i> | XP_011415271.2 | 161/161 | 100 | 0 | 161 |

| | | | | | | | | |
|--------------|--|---|--------------------------------|----------------|---------|-----|---------------------|-----|
| LOC105345889 | Leucine-rich repeat LGI family member 2 | Leucine-rich repeat LGI family member 2 (LGI2) | <i>Crassostrea gigas</i> | XP_011452536.2 | 195/195 | 100 | 0 | 195 |
| LOC117688175 | Uncharacterized LOC117688175 | Serine/arginine repetitive matrix protein 1-like (SRRM1-like) | <i>Mizuhopecten yessoensis</i> | XP_021357253.1 | 108/218 | 50 | 9×10^{-62} | 760 |

Table S3. Results of the Blastx homology search for the differential transcript expression involved in the Gene Ontology enriched processes of the *C. gigas* transcriptome.

| <i>C. gigas</i> Accession No. (Subject) | Protein ID (Query) | Species | Homolog Accession No. | Identities (Query/Subject) | Homology (%) | BlastX E- value | Protein Length (Query) |
|--|---|--------------------------------|-----------------------|-------------------------------|-----------------|----------------------|------------------------------|
| Chitin binding (GO:0008061) | | | | | | | |
| LOC105326593 | cgPif97 | <i>Crassostrea gigas</i> | AFT63504.1 | 881/1024 | 86 | 0 | 438 |
| LOC105317660 | Peritrophin-44 | <i>Crassostrea gigas</i> | XP_011412673.1 | 288/288 | 100 | 3×10^{-98} | 138 |
| LOC105346858 | Lactadherin, 1 | <i>Mizuhopecten yessoensis</i> | OWF43623.1 | 40/141 | 28 | 1×10^{-06} | 565 |
| LOC105346858 | Lactadherin, 2 | <i>Mizuhopecten yessoensis</i> | OWF43623.1 | 40/141 | 28 | 1×10^{-06} | 565 |
| LOC105331104 | Collagen alpha 1(XII) chain | <i>Mytilus coruscus</i> | CAC5362153.1 | 373/693 | 54 | 0 | 797 |
| LOC105336951 | Peritrophin-1-like | <i>Mizuhopecten yessoensis</i> | XP_021368929.1 | 101/233 | 43 | 4×10^{-53} | 239 |
| LOC105329937 | Peritrophin-1-like | <i>Gigantopelta aegis</i> | XP_041349873.1 | 67/144 | 47 | 9×10^{-35} | 229 |
| LOC105332576 | Cleavage and polyadenylation factor I subunit 1 | <i>Crassostrea gigas</i> | NP_001292250.1 | 472/472 | 100 | 0 | 472 |
| LOC105332577 | Putative chitinase 1 precursor | <i>Crassostrea gigas</i> | NP_001292252.1 | 475/475 | 100 | 0 | 475 |
| LOC105332575 | Putative chitinase | <i>Crassostrea gigas</i> | NP_001295799.1 | 471/471 | 100 | 0 | 471 |
| Calcium ion binding (GO:0005509) | | | | | | | |
| LOC105323227 | DNA ligase 1 Isoform X1 | <i>Crassostrea gigas</i> | XP_011420555.2 | 843/843 | 100 | 0 | 843 |
| | DNA ligase 1 Isoform X2 | <i>Crassostrea gigas</i> | XP_011420556.2 | 843/843 | 100 | 0 | 843 |
| | DNA ligase 1 Isoform X3 | <i>Crassostrea gigas</i> | XP_011420558.2 | 811/811 | 100 | 0 | 811 |
| | DNA ligase 1 Isoform X4 | <i>Crassostrea gigas</i> | XP_011420559 | 811/811 | 100 | 0 | 811 |
| | DNA ligase 1 Isoform X5 | <i>Crassostrea gigas</i> | XP_011420561 | 795/795 | 100 | 0 | 795 |
| | DNA ligase 1 Isoform X6 | <i>Crassostrea gigas</i> | XP_034305628 | 766/766 | 100 | 0 | 766 |
| LOC105345764 | EF-hand domain-containing protein 1 | <i>Crassostrea gigas</i> | XP_011452353.1 | 641/641 | 100 | 0 | 641 |
| LOC105331896 | Calmodulin-4 transcript var X1 | <i>Crassostrea gigas</i> | XP_011432565.2 | 178/178 | 100 | 4×10^{-122} | 178 |
| | Calmodulin-4 transcript var X2 | <i>Crassostrea gigas</i> | XP_011432571.2 | 176/176 | 100 | 1×10^{-122} | 176 |
| | Calmodulin-4 transcript var X3 | <i>Crassostrea gigas</i> | XP_011432579.2 | 175/175 | 100 | 8×10^{-122} | 175 |

| | | | | | | | |
|--|--|------------------------------|----------------|---------|-----|----------------------|------|
| LOC105337218 | Calcium-binding protein E63-1-like | <i>Haliotis rubra</i> | XP_046557623.1 | 26/70 | 37 | 0.002 | 144 |
| LOC105328000 | Calmodulin-A isoform X1 | <i>Crassostrea gigas</i> | XP_011427010.1 | 159/159 | 100 | 4×10^{-95} | 210 |
| | Calmodulin-A isoform X2 | <i>Crassostrea gigas</i> | XP_011427011.1 | 153/153 | 100 | 4×10^{-90} | 204 |
| LOC105320395 | Leucine-rich repeat-containing protein 74B isoform X1 | <i>Crassostrea gigas</i> | XP_011416624.1 | 601/601 | 100 | 0 | 601 |
| | Leucine-rich repeat-containing protein 74B isoform X2 | <i>Crassostrea gigas</i> | XP_011416625.1 | 601/601 | 100 | 0 | 601 |
| | Leucine-rich repeat-containing protein 74B isoform X3 | <i>Crassostrea gigas</i> | XP_034323188.1 | 601/601 | 100 | 0 | 601 |
| | Leucine-rich repeat-containing protein 74B isoform X4 | <i>Crassostrea gigas</i> | XP_011416626.1 | 574/574 | 100 | 0 | 574 |
| | Leucine-rich repeat-containing protein 74B isoform X5 | <i>Crassostrea gigas</i> | XP_011416627.1 | 563/563 | 100 | 0 | 563 |
| LOC105332695 | Sarcoplasmic calcium-binding protein | <i>Crassostrea gigas</i> | XP_011433684.1 | 179/179 | 100 | 0 | 179 |
| LOC105338472 | Myosin | <i>Crassostrea gigas</i> | XP_011441922.1 | 162/162 | 100 | 0 | 162 |
| LOC105330202 | EGF-like repeat and discoidin I-like domain-containing protein 3 | <i>Mercenaria mercenaria</i> | XP_045201990.1 | 150/321 | 65 | 9×10^{-89} | 322 |
| LOC105341555 | Mammalian ependymin-related protein 1 | <i>Crassostrea gigas</i> | XP_011446436.2 | 205/205 | 100 | 0 | 205 |
| LOC105327335 | EF-hand domain-containing family member B | <i>Crassostrea gigas</i> | XP_011426045.1 | 602/602 | 100 | 0 | 602 |
| LOC105327434 | Neurocalcin homolog | <i>Crassostrea gigas</i> | XP_011426213.2 | 185/185 | 100 | 0 | 185 |
| LOC105334536 | Sarcoplasmic calcium-binding protein isoform X2 | <i>Crassostrea gigas</i> | XP_011436355.1 | 178/178 | 100 | 0 | 178 |
| LOC105335092 | Neurocalcin homolog | <i>Crassostrea gigas</i> | XP_011437085.1 | 185/185 | 100 | 0 | 185 |
| LOC105336644 | Regucalcin isoform X1 | <i>Crassostrea gigas</i> | XP_011439353.1 | 308/308 | 100 | 0 | 308 |
| | Regucalcin isoform X2 | <i>Crassostrea gigas</i> | XP_019926340.2 | 308/308 | 100 | 0 | 308 |
| LOC105336648 | Regucalcin transcript variant X1 | <i>Crassostrea gigas</i> | XP_011439356.2 | 300/300 | 100 | 0 | 300 |
| | Regucalcin variant X3 | <i>Crassostrea gigas</i> | XP_011439357.2 | 300/300 | 100 | 0 | 300 |
| LOC105335791 | Annexin A4 | <i>Crassostrea gigas</i> | XP_011438182.2 | 311/311 | 100 | 0 | 311 |
| Extracellular region (GO:0005576) | | | | | | | |
| LOC105321645 | Poly(3-hydroxyalkanoate) depolymerase C | <i>Orchesella cincta</i> | ODN05040.1 | 145/328 | 44 | 4×10^{-83} | 350 |
| | Poly(3-hydroxyalkanoate) depolymerase C | <i>Orchesella cincta</i> | ODN05040.1 | 106/233 | 45 | 6×10^{-65} | 350 |
| LOC105326593 | Pif97 | <i>Crassostrea gigas</i> | AFT63504.1 | 438/438 | 100 | 0 | 438 |
| LOC105342849 | Trithorax group protein osa-like (for transcript variant X1) | <i>Crassostrea virginica</i> | XP_022308844.1 | 368/697 | 53 | 4×10^{-158} | 1637 |
| | Trithorax group protein osa-like (for transcript variant X2) | <i>Crassostrea virginica</i> | XP_022308844.1 | 368/697 | 53 | 3×10^{-158} | 1637 |
| LOC105340688 | NPC intracellular cholesterol transporter 2 | <i>Crassostrea gigas</i> | XP_011445166.2 | 144/144 | 100 | 0 | 144 |

| | | | | | | | |
|--------------|--|--------------------------------|----------------|---------|-----|---------------------|-----|
| LOC105341555 | Mammalian ependymin-related protein 1 | <i>Crassostrea gigas</i> | XP_011446436.2 | 205/205 | 100 | 0 | 205 |
| LOC105317660 | Peritrophin-44 | <i>Crassostrea gigas</i> | XP_011412673.1 | 288/288 | 100 | 3×10^{-98} | 138 |
| LOC105346858 | Lactadherin, 1 | <i>Mizuhopecten yessoensis</i> | OWF43623.1 | 40/141 | 28 | 1×10^{-06} | 565 |
| | Lactadherin, 2 | <i>Mizuhopecten yessoensis</i> | OWF43623.1 | 40/141 | 28 | 1×10^{-06} | 565 |
| LOC105318009 | Golgi-associated plant pathogenesis-related protein 1 | <i>Crassostrea gigas</i> | XP_011413171.2 | 236/236 | 100 | 0 | 236 |
| LOC105331104 | Collagen alpha 1(XII) chain | <i>Mytilus coruscus</i> | CAC5362153.1 | 373/693 | 54 | 0 | 797 |
| LOC105345005 | Dermatopontin isoform X1 | <i>Crassostrea gigas</i> | XP_011451270.1 | 192/192 | 100 | 0 | 192 |
| | Dermatopontin isoform X2 | <i>Crassostrea gigas</i> | XP_034301840.1 | 182/182 | 100 | 0 | 182 |
| LOC105338558 | Metalloproteinase inhibitor 3 | <i>Crassostrea gigas</i> | XP_011442039.2 | 251/251 | 100 | 0 | 251 |
| LOC105336951 | Peritrophin-1-like | <i>Mizuhopecten yessoensis</i> | XP_021368929.1 | 101/233 | 43 | 4×10^{-53} | 239 |
| LOC105329937 | Peritrophin-1-like | <i>Gigantopelta aegis</i> | XP_041349873.1 | 67/144 | 47 | 9×10^{-35} | 229 |
| LOC105345185 | Inactive pancreatic lipase-related protein 1 | <i>Crassostrea gigas</i> | XP_011451564.2 | 357/357 | 100 | 0 | 357 |
| LOC105331423 | Pancreatic lipase-related protein 2 | <i>Crassostrea gigas</i> | XP_011431904.2 | 518/518 | 100 | 0 | 158 |
| LOC105343616 | Adenosine deaminase AGSA isoform X1, X2 | <i>Crassostrea gigas</i> | XP_011449339.2 | 612/612 | 100 | 0 | 645 |
| LOC105327922 | von Willebrand factor C domain-containing protein 2-like | <i>Crassostrea gigas</i> | XP_011426902.2 | 137/137 | 100 | 0 | 137 |