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Transcriptomic Basis of Metamorphic Competence in the Salt-Marsh-Dwelling Polychaete *Capitella teleta*

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Abstract. Marine invertebrate larvae typically take hours to weeks after being released into the plankton before becoming "competent" to metamorphose. The mechanisms that govern this transition between the precompetent and metamorphically competent states are unknown. We studied gene expression patterns in precompetent and competent larvae of the salt-marsh-dwelling polychaete worm *Capitella teleta* (Blake, Grassle & Eckelbarger, 2009)—a species in which precompetent larvae are unusually easy to distinguish from competent larvae—to determine differences in gene expression associated with the onset of metamorphic competence. More than 1530 genes were more highly expressed in precompetent larvae, while more than 1060 genes were more highly expressed in competent larvae. Competent larvae downregulated the expression of genes belonging to gene ontologies relating to growth and development and upregulated those associated with ligand-binding transmembrane channels with possible chemo- and mechanosensory functions. Most of these channels were annotated as being from the degenerin/epithelial sodium channel family or the G-protein-coupled receptor family; proteins from these families can have chemosensory functions. Serotonin and GABA (γ-aminobutyric acid) receptors are among the genes that were upregulated in competent larvae; both have been shown to induce larvae of *C. teleta* and other marine invertebrates to metamorphose and are thought to be components of the signal transduction pathway that leads to metamorphosis. Overall, it appears that once larvae of *C. teleta* have completed development of the internal structures and physiology required for juvenile life during the precompetent period, they then upregulate the expression of chemosensory proteins and neurotransmitter receptors that will enable them to detect and transduce a settlement cue signal.

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Abbreviations: 5-HT_6, 5-hydroxytryptamine 6; ASW, artificial seawater; DEG/ENaC, degenerin/epithelial sodium channel; FDR, false discovery rate; GABA, γ-aminobutyric acid; GPCR, G-protein-coupled receptor; HSP, heat-shock protein; NCBI, National Center for Biotechnology Information; NOS, nitric oxide synthase; PKD, polycystic kidney disease; UTR, untranslated region.

Introduction

Many marine invertebrate species from a wide variety of animal phyla depend on their planktonic larvae for dispersal. These microscopic larvae often spend hours to weeks dispersing in the water column before finally settling to the benthos and initiating metamorphosis (Thorson, 1950; Pechenik, 1990). These larvae finally end their planktonic journey once they perceive a settlement cue—a stimulus from the environment that triggers larvae to settle to the benthos and initiate metamorphosis (Stanley *et al.*, 2010; Hadfield, 2011; Lillis *et al.*, 2013). However, the larvae of many species must develop for hours, days, or weeks before becoming responsive to such environmental triggers (Pechenik, 1990; Hadfield *et al.*, 2001).

Time to metamorphic competence thus determines the minimum amount of time that larvae will spend dispersing in the water column—and the distance they will be dispersed—before they are able to perceive a settlement cue, settle, and initiate metamorphosis. While larvae are progressing toward competence in the water column, they are also developing juvenile structures and organs that are essential for life after metamorphosis; by the time larvae reach competence, they are essentially fully formed juveniles waiting to shed larval structures (Hadfield *et al.*, 2001). While some natural settlement cues have been characterized (Pawlik and Faulkner, 1986; Tebben *et al.*, 2011; Swanson *et al.*, 2012; Burns *et al.*, 2014; Shikuma *et al.*, 2014) and various aspects of the physiological control of metamorphosis have been determined (Bishop and Brandhorst, 2001; Leise *et al.*, 2001; Pechenik *et al.*, 2002, 2007; Bishop *et al.*, 2008; Biggers *et al.*, 2012), almost noth-

ing is known about the molecular or physiological mechanisms controlling the onset of metamorphic competence—one of the key processes that determine potential distributions of marine invertebrate species.

To begin understanding the shifts in gene expression that lead to metamorphic competence in marine invertebrates, we have studied the deposit-feeding salt-marsh polychaete *Capitella teleta* (Blake, Grassle & Eckelbarger, 2009). *Capitella teleta* is a model organism with a sequenced and annotated genome that has been used for studying developmental biology, larval settlement, and aspects of the control of metamorphosis (Blake *et al.*, 2009). Larvae of *C. teleta* will rapidly settle and metamorphose once they contact salt-marsh sediment, often in less than 30 min (Dubilier, 1988; Cohen and Pechenik, 1999). While the larvae of many different species become competent after days to weeks of development in the plankton and at widely different times and sizes within each brood (*e.g.*, Pechenik and Heyman, 1987), the larvae of *C. teleta* are competent to metamorphose within minutes of release from their brooding structure, a brood tube (Dubilier, 1988; Cohen and Pechenik, 1999).

Mated females of *C. teleta* soon produce a brood tube out of mucus and sand grains and deposit their embryos on the inner walls of the tube (Blake *et al.*, 2009). Embryos develop about 8 d before they escape and swim as larvae into the water column (Seaver *et al.*, 2005). While most larvae that are released from brood tubes are immediately competent to metamorphose, a brood is occasionally released with larvae that are still precompetent (W. J. Biggers, R. Burns, and J. Pechenik, pers. obs.). These advanced "stage 8" precompetent larvae of *C. teleta* have a conspicuously straight gut morphology, while the "stage 9" competent larvae have a distinctive spiral gut morphology (Seaver *et al.*, 2005). No more than 24 h of development separate these 2 stages (Seaver *et al.*, 2005). Thus, *C. teleta* is especially well suited to explore the shifts in gene expression that cause larvae to become competent: larvae become competent within 30 min of their release into the plankton, and the onset of competence is essentially synchronous within a brood. Once released from the brood tube, the planktonic larvae locomote within the water column using their ciliary trochal bands. On reception of a settlement cue, the larvae then settle to the substrate and initiate metamorphosis. During metamorphosis, the once-planktonic larva's body elongates, trochal cilia are shed, the prostomium elongates, and capillary setae protrude (Biggers *et al.*, 2012). Newly metamorphosed juveniles then begin burrowing and eating sediment.

Here, we took advantage of the already assembled and annotated *C. teleta* genome and easily identifiable precompetent and competent larvae to conduct Illumina mRNA sequencing of these two larval stages, enabling us to identify patterns of differential gene expression at the onset of competence. While we expected some shifts in gene expression to be associated with the onset of metamorphic competence, other shifts may anticipate the individual's needs during or immediately after metamorphosis. By understanding how larvae become competent to metamorphose, we will piece more of the signal transduction cascade that regulates settlement and metamorphosis together. Which genes must be expressed at higher levels in competent larvae to allow signal detection and metamorphosis to occur? These gene products could be present in higher concentrations in competent larvae but be missing or in lower concentrations in precompetent individuals. Precompetent larvae could also be producing inhibitory substances in higher concentrations than larvae that have attained competence (Bishop and Brandhorst, 2001; Pechenik *et al.*, 2007; Biggers *et al.*, 2012). Understanding these transcriptomic changes between two stages that are so very close to each other in developmental time yet functionally very different will aid our understanding of what enables metamorphosis to take place.

Materials and Methods

Animal culture and collection of larvae

Starter cultures of adult *Capitella teleta* were reared in 12.7-cm-diameter glass dishes containing 30 psu of Instant Ocean artificial seawater (ASW) and maintained at 16 °C. The adults were fed sediment collected from the Little Sippewissett Salt Marsh (Falmouth, MA). Salt-marsh sediment was first passed through a 1-mm sieve and frozen at −20 °C for at least 24 h before being used as food or in settlement experiments. Culture dishes were searched regularly for brood tubes, which were then transferred individually to 5-cm-diameter glass dishes containing only ASW. Sand grains were carefully cleared from the side of the brood tubes to allow us to observe the developing embryos. This did not disturb the developing embryos or the brooding mother, as the mucous tube below the sand grains remained intact. Once embryos developed to stage 8 (straight gut) within the brood tube, the tube was carefully cut open; the stage 8 larvae were allowed to swim out and were collected for tissue and experiments. Stage 9 larvae (spiral gut) were collected for tissue preservation and experiments when they naturally escaped from the brood tube. All larval observations were made at 12–50× magnification using a dissecting microscope.

Testing larvae for metamorphic competence

To determine whether stage 8 larvae were indeed precompetent and stage 9 larvae were competent to metamorphose, we placed the larvae in ASW containing salt-marsh sediment and monitored them for metamorphosis. Three replicates of 10 larvae per stage were added to 4 ml of ASW in plastic 16-well plates with each well containing 1.72 cm^3 of salt-marsh sediment. Wells were observed for metamorphosed juveniles every 30 min for 5 h at 50× using a dissecting microscope, with a final observation at 24 h.

Illumina mRNA sequencing library preparation

Stage 8 and 9 larvae were preserved separately after being released from their brood tubes in RNAlater solution (Thermo Fisher Scientific, Waltham, MA) and stored at −80 °C. Larval mRNA was later extracted by grinding the larvae in 1.5-ml microfuge tubes with a plastic pestle and then purified using Qiagen RNeasy Mini Kits (Qiagen, Hilden, Germany) with a final elution volume of 20 μl. We used a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) to determine that 50 larvae needed to be pooled in one extraction to yield the 1 μg of mRNA required by the Illumina TruSeq mRNA Library Preparation Kit (ver. 2.0) (Illumina, San Diego, CA). Four replicate larval mRNA extractions were made for each larval stage, and each replicate was made into a uniquely barcoded individual mRNA sequencing library using the Illumina TruSeq mRNA Library Preparation Kit (ver. 2.0). The eight individual libraries were then sent to the Tufts University Core Facility, where they were pooled at equal concentrations and sequenced in one Illumina HiSeq 2500 lane to yield 50-bp-long single-ended reads. All libraries were uploaded to the National Center for Biotechnology Information (NCBI) Short Read Archive and are associated with NCBI BioProject PRJNA379706.

Sequence quality control, alignment, and differential expression analysis

Each library was assessed for sequence quality and potential adapter or primer contamination using FastQC software (Babraham Bioinformatics, 2017). All reads were processed with Trimmomatic software (ver. 0.35) in single-end mode to remove contaminating Illumina sequencing adapters and low-quality sequences (Bolger *et al.*, 2014). Here, starting and ending bases of a read with a Phred score less than 3 were trimmed away. After this, a sliding window was applied to cut low-quality sequences away from a read if the average base quality within a 4-bp window fell below a Phred score of 15. Only reads that were at least 36 bp long were retained for alignment. Each trimmed library was then aligned to the *C. teleta* genome obtained from the Ensembl Metazoa database (ver. 82.1) (International Nucleotide Sequence Database Collaboration assembly GCA_000328365.1) (Simakov *et al.*, 2014; Kersey *et al.*, 2016), using the default settings of Bowtie 2 (ver. 2.2.5) (Langmead and Salzberg, 2012) and TopHat 2 (ver. 2.0.14) (Trapnell *et al.*, 2009) software. The percentage of bases aligning to different genomic features, such as coding regions, untranslated regions (UTRs), intronic regions, intergenic regions, and rRNA genes, was determined using the Picard tool CollectRnaSeqMetrics (ver. 2.9.0) (Broad Institute, 2017). HTSEqcount software was used to determine the number of reads that aligned to each gene within each tissue library replicate (Anders *et al.*, 2015). The R package EdgeR was then used to normalize libraries by scaling for library size, estimate dispersions by the quantile-adjusted conditional maximum likelihood method, and calculate differential expression using quasi-likelihood *F*-tests, all of which are recommended for RNA sequencing experiments with a single factor (Robinson and Smyth, 2007a, b; Robinson *et al.*, 2009; Robinson and Oshlack, 2010; Lund *et al.*, 2012; McCarthy *et al.*, 2012; Chen *et al.*, 2014).

Gene ontology analysis

Genes were considered differentially expressed if they had at least a twofold increase or decrease in expression level between developmental stages, a *P*-value of <0.05, and a false discovery rate (FDR) of <0.05. Gene ontology classifications for annotated genes were obtained from the Ensembl Metazoa BioMart *C. teleta* database (ver. 1.0) (Kinsella *et al.*, 2011). A Fisher's exact test was conducted in Blast2GO software to identify significantly (FDR < 0.05) enriched and depleted gene ontology categories between precompetent and competent larvae (Conesa *et al.*, 2005).

Results

Tests of metamorphic competence

All stage 9 larvae metamorphosed and burrowed within 5 h of contact with salt-marsh sediment, while no stage 8 larvae metamorphosed within this time frame (Fig. 1). However, all larvae from both developmental stages had metamorphosed within 24 h after the start of the treatment.

Sequencing and alignment results

A total of 200,064,476 50-bp reads were successfully demultiplexed into 8 separate libraries from the single Illumina HiSeq 2500 sequencing lane. The mean base-call quality of all bases in the demultiplexed reads surpassed a Phred quality of 30 (less than 1/1000 chance a base was called incorrectly). After quality and adapter trimming, the mean library size was 24,677,106 reads; the smallest library contained 19,434,838 reads, while the largest library contained 28,965,035 reads (Table 1). At least 94.9% of reads per trimmed library were successfully aligned to the *Capitella teleta* genome. The majority of bases that aligned for each library had aligned to genomic regions contributing to mRNA (coding regions and UTRs); an average of 74.0% of bases had aligned to these regions. Ribosomal RNA contamination does not appear to have severely affected library construction; a maximum of 3.9% of bases had aligned to rRNA genes (Table 1).

A total of 2605 genes were significantly differentially expressed between the stage 8 precompetent larvae and the stage 9 competent larvae (Fig. A1). Of these, 1536 genes were expressed at higher levels in the precompetent larvae, while 1069 genes were expressed at higher levels in the competent larvae (Table S1, available online). Qualitative multidimensional scaling analysis of all samples revealed that replicates of each larval stage mainly cluster together and that one dimension separates the replicates of each larval stage (Fig. 2).

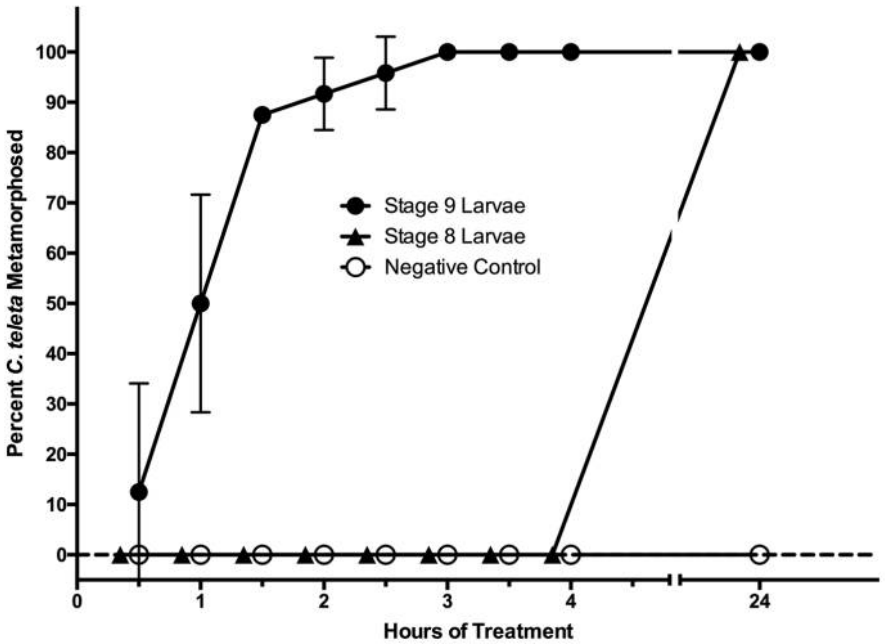

Figure 1. Testing stage 8 and 9 larvae of *Capitella teleta* for metamorphic competence. Each treatment consisted of three replicates of 10 larvae per replicate. Larvae were placed in 4 ml of 30-psu Instant Ocean artificial seawater containing 1.7 cm^3 of salt-marsh sediment. The negative control contained only Instant Ocean seawater and stage 9 larvae. Error bars represent ± 1 SEM.

This indicates that gene expression patterns are more similar for replicates within each larval stage and are more different between larval stages. Of the 2605 differentially expressed genes, 796 were not annotated with a gene ontology in the Ensembl Metazoa BioMart *C. teleta* database (ver. 1.0).

Enriched gene ontologies for each larval stage

Overall, it appears that precompetent larvae are continuing larval morphological and physiological development while also increasing the expression of genes coding for proteins that may inhibit metamorphosis (Table 2). Here, genes involved in processes like protein folding, protein transport, translation initiation, and DNA replication are expressed more highly in precompetent larvae. In contrast, competent larvae are mainly increasing the expression of genes with potential chemosensory functions as well as neurotransmitter receptors that could be involved in the signal transduction cascade leading to metamorphosis (Table 3).

Discussion

Downregulated genes in competent larvae

A fair number of genes were more highly expressed in precompetent larvae than in metamorphically competent larvae.

Table 1

Sequencing library quality control statistics after quality trimming and alignment

Sample	Sequenced reads	Reads remaining after cleaning	% cleaned reads aligned	% coding bases	% UTR bases	% mRNA bases	% intronic bases	% intergenic bases	% rRNA bases
C1	28,767,983	28,458,569	95.1	57.94	14.83	72.77	5.58	20.78	0.86
C2	19,655,572	19,434,838	95.3	60.31	14.04	74.35	5.23	19.88	0.55
C3	21,763,337	21,464,080	95.2	52.64	15.40	68.04	7.49	22.57	1.90
C4	23,376,691	23,056,643	94.9	57.06	14.97	72.03	5.50	21.81	0.66
P1	25,878,108	25,278,205	95.5	59.04	13.82	72.86	4.59	18.62	3.93
P2	22,300,488	22,016,305	95.4	60.11	14.76	74.87	5.00	19.28	0.85
P3	29,055,316	28,743,172	95.8	66.58	11.98	78.56	4.49	16.29	0.66
P4	29,266,981	28,965,035	95.9	67.10	11.83	78.92	4.46	15.74	0.87

The "% bases" columns represent the percentage of aligned bases that aligned to the given genomic region for that sample. Samples C1–C4 are replicate libraries of competent larvae, while samples P1–P4 are replicate libraries of precompetent larvae. UTR, untranslated region.

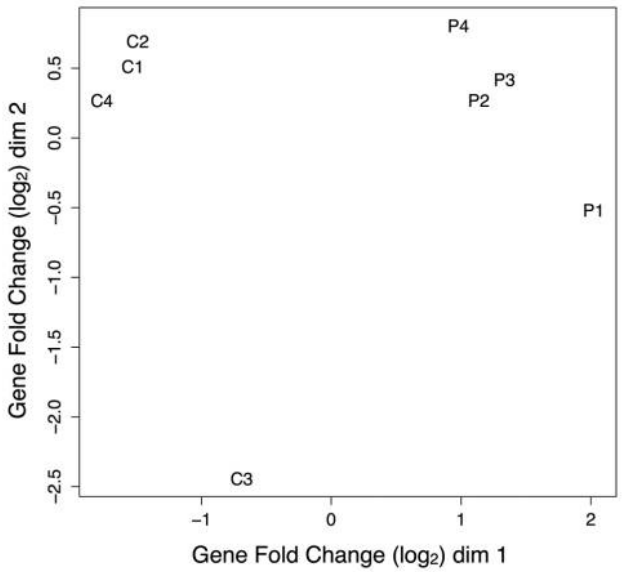

Figure 2. Multidimensional scaling plot of all samples based on gene expression data. Distances between each pair of samples are computed using $\log_2$ fold change expression values. Replicate samples within each larval stage mainly cluster together. Replicates of each larval stage are separated by dimension 1.

Most of the gene ontologies that were more highly expressed by precompetent larvae are associated with growth and development. These gene ontologies include categories involved with DNA replication, cell division, transcription, translation, protein folding, and other protein modifications (Table 2). These results agree with Hadfield *et al.*'s (2001) suggestion that all juvenile structures required for postmetamorphosis juvenile life must be developed before a larva attains metamorphic competence. Through metamorphosis larval-specific structures are shed, and then, because juvenile features required for benthic life are already developed, individuals can immediately proceed further with growth and development into adults. Indeed, gut development is not completed in precompetent stage 8 larvae of *Capitella teleta*, and circular, longitudinal, and oblique muscles are not well developed prior to the stage 9 competent larvae of *C. teleta* (Seaver *et al.*, 2005); these features are required for successful burrowing and deposit feeding in *C. teleta* juveniles. In addition, embryos and larvae of *C. teleta* continue producing new segments until they reach metamorphic competence at stage 9, with released larvae halting further segmentation until metamorphosing with a total of 12–14 segments (Seaver *et al.*, 2005). Even development of the animal's nervous system halts once the larva reaches competent stage 9. Surprisingly, the development of *C. teleta*'s nervous system could be considered complete once larvae reach stage 9, as the nervous system changes little between stage 9 larvae and the newly metamorphosed juveniles (Meyer *et al.*, 2015).

Some genes responsible for the synthesis of nitric oxide and other processes that could inhibit metamorphosis were also more highly expressed in the precompetent larvae of *C. teleta*. The competent larvae of *C. teleta*, along with those of some molluscs, ascidians, and echinoderms, are inhibited from metamorphosing by endogenously produced nitric oxide (Bishop and Brandhorst, 2001; Pechenik *et al.*, 2007; Biggers *et al.*, 2012; for examples of nitric oxide stimulation of metamorphosis in an ascidian and an abalone, see Ueda and Degnan, 2013, 2014). Not surprisingly, in many animals nitric oxide synthase (NOS) requires an interaction with heat-shock protein 90 (HSP90) to keep the essential heme cofactor bound (Bishop and Brandhorst, 2003). However, some competent hydroid and gastropod larvae metamorphosed after being stressed with increased temperatures (Kroiher *et al.*, 1992; Gaudette *et al.*, 2001; Bishop and Brandhorst, 2003), probably because HSP90 proteins were diverted from NOS to renature proteins that had denatured from thermal stress (Bishop and Brandhorst, 2003). Both HSP70 and HSP90 were more highly expressed in precompetent larvae of *C. teleta* and were downregulated in competent larvae (Table A1). While these proteins also play roles in developmental processes, it is possible that increased expression of HSP90 could ensure sufficiently high endogenous concentrations of nitric oxide to prevent metamorphosis from occurring.

Four heme peroxidases were also more highly expressed in precompetent larvae and downregulated in competent larvae (Table A2). Heme peroxidases are known to play roles in innate immunity, hormone synthesis, and prevention of oxidative stress in the cell. Heme peroxidases work by using hydrogen peroxide to oxidize various substrates to produce an oxidized product and water. Hydrogen peroxide has stimulated at least partial metamorphosis in larvae from a number of marine invertebrates (Pires and Hadfield, 1991; Boettcher and Targett, 1998). Two superoxide dismutases, which produce either oxygen or hydrogen peroxide from the reactive oxygen species superoxide, were also more highly expressed in precompetent larvae of *C. teleta* (Table A2). Precompetent larvae of *C. teleta* may be inactivating the reactive oxygen species hydrogen peroxide by catalyzing its breakdown into water. Reactive oxygen species such as hydrogen peroxide can inhibit endogenous nitric oxide production (Murad, 2006), and such inhibition of the inhibitory endogenous nitric oxide in marine invertebrates could lead to metamorphosis.

Upregulated genes in competent larvae

The most significantly enriched gene ontology categories within the competent larvae of *C. teleta* have to do with the gene ontology processes and functions of sodium ion channels and sodium transport. Within these two ontologies, 20 genes have been annotated as being amiloride-sensitive sodium channels (Table A3). In addition, five other genes that were upregulated in competent larvae of *C. teleta* have been annotated as having similarities to polycystic kidney disease (PKD) proteins (Table A4). Both amiloride-sensitive sodium channels and PKD channels belong to a large family of channels called the degenerin/epithelial sodium channel family (DEG/ENaC

Table 2

Gene ontologies (GO) enriched in precompetent larvae of Capitella teleta

GO ID	GO term	Type[a]	No. of genes[b]	FDR[c]
GO:0006457	Protein folding	P	33	3.00E−19
GO:0004298	Threonine-type endopeptidase activity	F	17	1.50E−16
GO:0051082	Unfolded protein binding	F	20	1.70E−12
GO:0005524	ATP binding	F	120	8.50E−10
GO:0005852	Eukaryotic translation initiation factor 3 complex	C	10	1.60E−08
GO:0003743	Translation initiation factor activity	F	13	1.30E−07
GO:0019773	Proteasome core complex, α-subunit complex	C	7	2.40E−07
GO:0006270	DNA replication initiation	P	7	1.90E−05
GO:0000398	mRNA splicing, *via* spliceosome	P	11	2.10E−05
GO:0003755	Peptidyl-prolyl cis-trans isomerase activity	F	7	3.40E−04
GO:0000413	Protein peptidyl-prolyl isomerization	P	7	3.40E−04
GO:0006511	Ubiquitin-dependent protein catabolic process	P	15	4.70E−04
GO:0004579	Dolichyl-diphosphooligosaccharide-protein glycotransferase activity	F	4	5.90E−04
GO:0042555	MCM complex	C	5	6.80E−04
GO:0006779	Porphyrin-containing compound biosynthetic process	P	6	1.40E−03
GO:0045454	Cell redox homeostasis	P	12	1.50E−03
GO:0003678	DNA helicase activity	F	10	1.70E−03
GO:0015671	Oxygen transport	P	11	5.40E−03
GO:0005732	Small nucleolar ribonucleoprotein complex	C	5	5.80E−03
GO:0042176	Regulation of protein catabolic process	P	3	7.50E−03
GO:0022624	Proteasome accessory complex	C	3	7.50E−03
GO:0008250	Oligosaccharyltransferase complex	C	3	7.50E−03
GO:0005885	Arp2/3 protein complex	C	4	1.30E−02
GO:0016272	Prefoldin complex	C	4	1.30E−02
GO:0008641	Small-protein-activating enzyme activity	F	5	1.40E−02
GO:0006886	Intracellular protein transport	P	24	1.50E−02
GO:0016051	Carbohydrate biosynthetic process	P	14	1.90E−02
GO:0008017	Microtubule binding	F	12	2.30E−02
GO:0004814	Arginine-tRNA ligase activity	F	3	2.30E−02
GO:0005092	GDP-dissociation inhibitor activity	F	3	2.30E−02
GO:0006465	Signal peptide processing	P	3	2.30E−02
GO:0006420	Arginyl-tRNA aminoacylation	P	3	2.30E−02
GO:0006281	DNA repair	P	22	2.40E−02
GO:0008173	RNA methyltransferase activity	F	6	2.60E−02
GO:0020037	Heme binding	F	26	2.70E−02
GO:0006221	Pyrimidine nucleotide biosynthetic process	P	6	3.30E−02
GO:0000070	Mitotic sister chromatid segregation	P	6	3.30E−02
GO:0006739	NADP metabolic process	P	4	3.50E−02
GO:0031981	Nuclear lumen	C	15	4.80E−02
GO:0005871	Kinesin complex	C	10	4.90E−02
GO:0051920	Peroxiredoxin activity	F	3	4.90E−02
GO:0006890	Retrograde vesicle-mediated transport, Golgi to ER	P	3	4.90E−02
GO:0051087	Chaperone binding	F	3	4.90E−02
GO:0003918	DNA topoisomerase type II (ATP-hydrolyzing) activity	F	3	4.90E−02

[a] GO category that each GO term is derived from (C, cellular component; F, biological function; P, biological process).
[b] Number of annotated, differentially expressed genes in the given GO.
[c] Benjamini-Hochberg-corrected *P*-value as calculated by a Fisher's exact test. FDR, false discovery rate.

family) (Ben-Shahar, 2011). These DEG/ENaC channels perform a variety of sensory functions in vertebrates and invertebrates, including chemo- and mechanosensation. These channels are involved in the reception of salt, sour, and "water" tastes in a variety of different organisms, from humans to the fruit fly *Drosophila melanogaster*. Mechanosensory functions of these proteins range from skin pressure reception to pain response from applied acids (Ben-Shahar, 2011). Mammalian genomes encode for only eight or nine of these proteins, while the genomes of such invertebrates as *D. melanogaster* and the nematode *Caenorhabditis elegans* encode for 30 or 31, making them one of the largest protein families represented among invertebrate genomes; these proteins may have evolved to perform many specialized sensory functions in invertebrates (Ben-Shahar, 2011). Amiloride appeared to be weakly inductive to larvae of the hydroid *Hydractinia echinata*: a 600 μmol l^{-1} solution of amiloride in seawater induced ~30% of the tested larvae to metamorphose (Berking, 1988). The role that DEG/

Table 3

Gene ontologies (GO) enriched in competent larvae of Capitella teleta

GO ID	GO term	Type[a]	No. of genes[b]	FDR[c]
GO:0005272	Sodium channel activity	F	24	3.20E−12
GO:0006814	Sodium ion transport	P	25	1.30E−10
GO:0005509	Calcium ion binding	F	45	1.30E−04
GO:0016020	Membrane	C	174	1.40E−04
GO:0003840	γ-Glutamyltransferase activity	F	5	2.40E−03
GO:0006749	Glutathione metabolic process	P	6	1.40E−02
GO:0004129	Cytochrome *c* oxidase activity	F	5	1.60E−02
GO:0080019	Fatty-acyl-CoA reductase (alcohol-forming) activity	F	4	1.60E−02

[a] GO category that each GO term is derived from (C, cellular component; F, biological function; P, biological process).
[b] Number of annotated, differentially expressed genes in the given GO.
[c] Benjamini-Hochberg-corrected *P*-value as calculated by a Fisher's exact test. FDR, false discovery rate.

ENaC channels play in settlement and metamorphosis has not been explored in any other marine invertebrate species. By expressing these channels at higher levels at competence, larvae of *C. teleta* could be preparing to navigate and assess complex pelagic and benthic landscapes as they prepare for metamorphosis.

The greatest number of genes that were upregulated in competent larvae are found within the membrane cellular component ontology (Table 3). This category contains the sodium channels described above as well as other relevant receptors. The most abundant of these receptors were the 44 G-protein-coupled receptors (GPCRs) (Table A5). GPCRs are a large family of transmembrane chemosensory receptors that are commonly involved in chemical sensation, including taste and smell. This result is similar to GPCR transcription patterns that have been reported for the demosponge *Amphimedon queenslandica*, in which many GPCRs were well expressed in pelagic larvae and then downregulated in benthic juveniles (Conaco *et al.*, 2012). Here, Conaco *et al.* (2012) argue that GPCRs may be used by larvae to sense appropriate settlement cues and environments for settlement and metamorphosis (Conaco *et al.*, 2012). GPCRs have often been studied as putative receptors for settlement cues in marine invertebrate larvae (Hadfield, 2011). While GPCRs do not mediate settlement and metamorphosis in larvae of the polychaete *Hydriodes elegans* or the coral *Montipora capitate* (Holm *et al.*, 1998; Tran and Hadfield, 2012), metamorphosis of the larvae of the marine mollusc *Haliotis rufescens* (abalone) did appear to be modulated by a GPCR (Baxter and Morse, 1987). Among the GPCRs expressed in *C. teleta*, 39 were annotated as rhodopsin-like GPCRs, including a 5-hydroxytryptamine 6 (5-HT_6) receptor, a glycoprotein hormone receptor, a thyrotropin-releasing hormone receptor, and a galanin receptor. The upregulation of a galanin receptor in competent larvae of *C. teleta* is intriguing, as the function of the short neuropeptide galanin in invertebrates is not well understood (Liu *et al.*, 2010). In mammalian species, galanin is involved in regulating food intake, pain reception, and nerve regeneration (Branchek *et al.*, 2000). Interestingly, mammalian genomes (humans, mice, and rats) also encode three nicotinic acid receptors in the A11 subfamily of rhodopsin-like GPCRs (genes *HCAR1*, *HCAR2*, and *HCAR3*). Nicotinic acid and the related B vitamin nicotinamide rapidly stimulated larvae of *C. teleta* to metamorphose (Burns *et al.*, 2014). However, when BLASTP searches using these human protein sequences were conducted within the *C. teleta* proteome, no high-quality matches were found (lowest *E*-value = 3.5E−14; highest percent identity = 32.6%). Perhaps the ligand-binding channel that larvae of *C. teleta* use to sense nicotinamide and nicotinic acid is not a GPCR (Burns *et al.*, 2014).

The upregulation of the 5-HT_6 serotonin receptor in competent larvae of *C. teleta* is exciting because treating these larvae with serotonin or the selective serotonin reuptake inhibitor fluoxetine stimulates them to metamorphose (Biggers *et al.*, 2012). In addition, the serotonin receptor antagonist ketanserin is known to inhibit larvae from metamorphosing in response to NOS inhibitors and nicotinamide (Biggers *et al.*, 2012; Burns *et al.*, 2014). Larvae of *C. teleta* may require serotonin to transduce signals stimulated by the reception of a settlement cue leading to the initiation of metamorphosis, something that could be explored in future studies.

Other GPCRs included five γ-aminobutyric acid (GABA) B receptors. While not a GPCR, a GABA A receptor was also upregulated (Table A6). GABA is an inhibitory amino acid neurotransmitter that has stimulated some species of marine invertebrate larvae to metamorphose (Morse *et al.*, 1979; Pearce and Scheibling, 1990; García-Lavandeira *et al.*, 2005; Yang *et al.*, 2015). Like serotonin, perhaps a component of the signal transduction cascade leading to metamorphosis in *C. teleta* is mediated by GABA. Other upregulated receptors in competent larvae included seven ionotropic glutamate receptors, two nicotinic acetylcholine–gated receptors, two neurotransmitter-gated ion channels, and a gustatory receptor (Table A6). Although it is not clear what substance this gus-

tatory receptor is responsible for sensing, this is more evidence that competent larvae of *C. teleta* have upregulated another gene responsible for environmental chemosensation.

Overall, it appears that precompetent larvae of *C. teleta* are completing the development of structures required for survival as deposit-feeding juveniles and exhibiting increased expression of genes belonging to gene ontologies that reflect this status of continued growth and development (Table 2). However, once this development is completed, competent larvae of this species appear to then downregulate genes related to overall development and growth and subsequently increase the expression of ligand-binding transmembrane channels and other potential chemosensory proteins. Although we do not know to which ligands these proteins bind, the potential for chemosensation is greater in competent larvae because of the increased expression of these genes.

In combination with a potential for greater chemosensation, competent larvae also showed increased expression of many neurotransmitter receptors. Here, serotonin, GABA, glutamate, acetylcholine, and other neurotransmitters may play a role in transducing the signal perceived from a settlement cue to initiate settlement and metamorphosis. Perhaps chemosensory receptors and other components of the signal transduction cascade leading to metamorphosis are highly expressed at competence as a safety mechanism: if individuals of *C. teleta* were to metamorphose before their muscles, gut, or nervous system were fully functional, the resulting juvenile would not be likely to survive in the benthos. Future studies should examine the role that GPCRs play in modulating settlement and metamorphosis in *C. teleta*, as so many of these receptors were expressed at higher levels in competent larvae. In addition, the potential for amiloride-sensitive sodium channels to act as chemosensory channels should be explored in the larvae of *C. teleta* and other marine invertebrates because of their substantially higher expression level in competent larvae of *C. teleta*. To our knowledge, the possibility that these channels play roles in the control of metamorphosis in the larvae of marine invertebrates has not been thoroughly explored.

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Literature Cited

Anders, S., P. T. Pyl, and W. Huber. 2015. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31:** 166–169.

Babraham Bioinformatics. 2017. FastQC: a quality control tool for high throughput sequence data. [Online]. Available: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ [2017, June 20].

Baxter, G., and D. E. Morse. 1987. G protein and diacylglycerol regulate metamorphosis of planktonic molluscan larvae. *Proc. Natl. Acad. Sci. U.S.A.* **84:** 1867–1870.

Ben-Shahar, Y. 2011. Sensory functions for degenerin/epithelial sodium channels (DEG/ENaC). *Adv. Genet.* **76:** 1–26.

Berking, S. 1988. Ammonia, tetraethylammonium, barium and amiloride induce metamorphosis in the marine hydroid *Hydractinia*. *Roux. Arch. Dev. Biol.* **197:** 1–9.

Biggers, W. J., A. Pires, J. A. Pechenik, E. Johns, P. Patel, T. Polson, and J. Polson. 2012. Inhibitors of nitric oxide synthase induce larval settlement and metamorphosis of the polychaete annelid *Capitella teleta*. *Invertebr. Reprod. Dev.* **56:** 1–13.

Bishop, C. D., and B. P. Brandhorst. 2001. NO/cGMP signaling and HSP90 activity represses metamorphosis in the sea urchin *Lytechinus pictus*. *Biol. Bull.* **201:** 394–404.

Bishop, C. D., and B. P. Brandhorst. 2003. On nitric oxide signaling, metamorphosis, and the evolution of biphasic life cycles. *Evol. Dev.* **5:** 542–550.

Bishop, C. D., A. Pires, S.-W. Norby, D. Boudko, L. L. Moroz, and M. G. Hadfield. 2008. Analysis of nitric oxide–cyclic guanosine monophosphate signaling during metamorphosis of the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opisthobranchia). *Evol. Dev.* **10:** 288–299.

Blake, J. A., J. P. Grassle, and K. J. Eckelbarger. 2009. *Capitella teleta*, a new species designation for the opportunistic and experimental *Capitella* sp. I, with a review of the literature for confirmed records. *Zoosymposia* **2:** 25–53.

Boettcher, A. A., and N. M. Targett. 1998. Role of chemical inducers in larval metamorphosis of queen conch, *Strombus gigas* Linnaeus: relationship to other marine invertebrate systems. *Biol. Bull.* **194:** 132–142.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30:** 2114–2120.

Branchek, T. A., K. E. Smith, C. Gerald, and M. W. Walker. 2000. Galanin receptor subtypes. *Trends Pharmacol. Sci.* **21:** 109–117.

Broad Institute. 2017. Picard: a set of command line tools (in Java) for manipulating high-throughput sequencing (HTS) data and formats such as SAM/BAM/CRAM/and VCF. [Online]. Available: http://broadinstitute.github.io/picard [2017, June 20].

Burns, R. T., J. A. Pechenik, W. J. Biggers, G. Scavo, and C. Lehman. 2014. The B vitamins nicotinamide (B3) and riboflavin (B2) stimulate metamorphosis in larvae of the deposit-feeding polychaete *Capitella teleta*: implications for a sensory ligand-gated ion channel. *PLoS One* **9:** e109535.

Chen, Y., A. Lun, and G. K. Smyth. 2014. Differential expression analysis of complex RNA-seq experiments using edgeR. Pp. 51–74 in *Statistical Analysis of Next Generation Sequencing Data*, S. Datta and D. Nettleton, eds. Springer, Basel.

Cohen, R. A., and J. A. Pechenik. 1999. Relationship between sediment organic content, metamorphosis, and postlarval performance in the deposit-feeding polychaete *Capitella* sp. I. *J. Exp. Mar. Biol. Ecol.* **240:** 1–18.

Conaco, C., P. Neveu, H. Zhou, M. L. Arcila, S. M. Degnan, B. M. Degnan, and K. S. Kosik. 2012. Transcriptome profiling of the demosponge *Amphimedon queenslandica* reveals genome-wide events that accompany major life cycle transitions. *BMC Genomics* **13:** 209.

Conesa, A., S. Gotz, J. M. Garcia-Gomez, J. Terol, M. Talon, and M. Robles. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21:** 3674–3676.

Dubilier, N. 1988. H_2S—a settlement cue or a toxic substance for *Capitella* sp. I larvae? *Biol. Bull.* **174:** 30–38.

García-Lavandeira, M., A. Silva, M. Abad, A. J. Pazos, J. L. Sánchez, and M. Luz Pérez-Parallé. 2005. Effects of GABA and epinephrine on the settlement and metamorphosis of the larvae of four species of bivalve molluscs. *J. Exp. Mar. Biol. Ecol.* **316:** 149–156.

Gaudette, M. F., J. L. Lowther, and J. A. Pechenik. 2001. Heat shock induces metamorphosis in the larvae of the prosobranch gastropod *Crepidula fornicata*. *J. Exp. Mar. Biol. Ecol.* **266:** 151–164.

Hadfield, M. G. 2011. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annu. Rev. Mar. Sci.* **3:** 453–470.

Hadfield, M. G., E. J. Carpizo-Ituarte, K. del Carmen, and B. T. Nedved. 2001. Metamorphic competence, a major adaptive convergence in marine invertebrate larvae. *Am. Zool.* **41:** 1123–1131.

Holm, E. R., B. T. Nedved, E. Carpizo-Ituarte, and M. G. Hadfield. 1998. Metamorphic-signal transduction in *Hydroides elegans* (Polychaeta: Serpulidae) is not mediated by a G protein. *Biol. Bull.* **195:** 21–29.

Kersey, P. J., J. E. Allen, I. Armean, S. Boddu, B. J. Bolt, D. Carvalho-Silva, M. Christensen, P. Davis, L. J. Falin, C. Grabmueller, *et al.* 2016. Ensembl Genomes 2016: more genomes, more complexity. *Nucleic Acids Res.* **44:** 574–580.

Kinsella, R. J., A. Kähäri, S. Haider, J. Zamora, G. Proctor, G. Spudich, J. Almeida-King, D Staines, P Derwent, A Kerhornou, *et al.* 2011. Ensembl BioMarts: a hub for data retrieval across taxonomic space. *Database* **2011:** bar030.

Kroiher, M., M. Walther, and S. Berking. 1992. Heat shock as inducer of metamorphosis in marine invertebrates. *Roux. Arch. Dev. Biol.* **201:** 169–172.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Meth.* **9:** 357–359.

Leise, E. M., K. Thavaradhara, N. R. Durham, and B. E. Turner. 2001. Serotonin and nitric oxide regulate metamorphosis in the marine snail *Ilyanassa obsoleta*. *Am. Zool.* **41:** 258–267.

Lillis, A., D. B. Eggleston, and D. R. Bohnenstiehl. 2013. Oyster larvae settle in response to habitat-associated underwater sounds. *PLoS One* **8:** e79337.

Liu, Z., Y. Xu, L. Wu, and S. Zhang. 2010. Evolution of galanin receptor genes: insights from the deuterostome genomes. *J. Biomol. Struct. Dyn.* **28:** 97–106.

Lund, S. P., D. Nettleton, D. J. McCarthy, and G. K. Smyth. 2012. Detecting differential expression in RNA-sequence data using quasi-likelihood with shrunken dispersion estimates. *Stat. Appl. Genet. Mol. Biol.* **11:** 8.

McCarthy, D. J., Y. Chen, and G. K. Smyth. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res.* **40:** 4288–4297.

Meyer, N. P., A. Carrillo-Baltodano, R. E. Moore, and E. C. Seaver. 2015. Nervous system development in lecithotrophic larval and juvenile stages of the annelid *Capitella teleta*. *Front. Zool.* **12:** 13.

Morse, D. E., N. Hooker, H. Duncan, and L. Jensen. 1979. γ-Aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. *Science* **204:** 407–410.

Murad, F. 2006. Nitric oxide and cyclic GMP in cell signaling and drug development. *N. Engl. J. Med.* **355:** 2003–2011.

Pawlik, J. R., and D. J. Faulkner. 1986. Specific free fatty acids induce larval settlement and metamorphosis of the reef-building tube worm *Phragmatopoma califomica* (Fewkes). *J. Exp. Mar. Biol. Ecol.* **102:** 301–310.

Pearce, C. M., and R. E. Scheibling. 1990. Induction of metamorphosis of larvae of the green sea-urchin, *Strongylocentrotus droebachiensis*, by coralline red algae. *Biol. Bull.* **179:** 304–311.

Pechenik, J. A. 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: Does it occur? Is there a price to pay? *Ophelia* **32:** 63–94.

Pechenik, J. A., and W. D. Heyman. 1987. Using KCl to determine size at competence for larvae of the marine gastropod *Crepidula fornicata* (L.). *J. Exp. Mar. Biol. Ecol.* **112:** 27–38.

Pechenik, J. A., W. Li, and D. E. Cochrane. 2002. Timing is everything: The effects of putative dopamine antagonists on metamorphosis vary with larval age and experimental duration in the prosobranch gastropod *Crepidula fornicata*. *Biol. Bull.* **202:** 137–147.

Pechenik, J. A., D. E. Cochrane, W. Li, E. T. West, A. Pires, and M. Leppo. 2007. Nitric oxide inhibits metamorphosis in larvae of *Crepidula fornicata*, the slippershell snail. *Biol. Bull.* **213:** 160–171.

Pires, A., and M. G. Hadfield. 1991. Oxidative breakdown products of catecholamines and hydrogen peroxide induce partial metamorphosis in the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opisthobranchia). *Biol. Bull.* **180:** 310–317.

Robinson, M. D., and A. Oshlack. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol.* **11:** R25.

Robinson, M. D., and G. K. Smyth. 2007a. Moderated statistical tests for assessing differences in tag abundance. *Bioinformatics* **23:** 2881–2887.

Robinson, M. D., and G. K. Smyth. 2007b. Small-sample estimation of negative binomial dispersion, with applications to SAGE data. *Biostatistics* **9:** 321–332.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2009. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26:** 139–140.

Seaver, E. C., K. Thamm, and S. D. Hill. 2005. Growth patterns during segmentation in the two polychaete annelids, *Capitella* sp. I and *Hydroides elegans*: comparisons at distinct life history stages. *Evol. Dev.* **7:** 312–326.

Shikuma, N. J., M. Pilhofer, G. L. Weiss, M. G. Hadfield, G. J. Jensen, and D. K. Newman. 2014. Marine tubeworm metamorphosis induced by arrays of bacterial phage tail-like structures. *Science* **343:** 529–533.

Simakov, O., F. Marletaz, S.-J. Cho, E. Edsinger-Gonzales, P. Havlak, U. Hellsten, D.-H. Kuo, T. Larsson, J. Lv, D. Arendt, *et al.* 2014. Insights into bilaterian evolution from three spiralian genomes. *Nature* **493:** 526–531.

Stanley, J. A., C. A. Radford, and A. G. Jeffs. 2010. Induction of settlement in crab megalopae by ambient underwater reef sound. *Behav. Ecol.* **21:** 113–120.

Swanson, R. L., M. Byrne, T. A. A. Prowse, B. Mos, S. A. Dworjanyn, and P. D. Steinberg. 2012. Dissolved histamine: a potential habitat marker promoting settlement and metamorphosis in sea urchin larvae. *Mar. Biol.* **159:** 915–925.

Tebben, J., D. M. Tapiolas, C. A. Motti, D. Abrego, A. P. Negri, L. L. Blackall, P. D. Steinberg, and T. Harder. 2011. Induction of larval metamorphosis of the coral *Acropora millepora* by tetrabromopyrrole isolated from a *Pseudoalteromonas* bacterium. *PLoS One* **6:** e19082.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25:** 1–45.

Tran, C., and M. G. Hadfield. 2012. Are G-protein-coupled receptors involved in mediating larval settlement and metamorphosis of coral planulae? *Biol. Bull.* **222:** 128–136.

Trapnell, C., L. Pachter, and S. L. Salzberg. 2009. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* **25:** 1105–1111.

Ueda, N., and S. M. Degnan. 2013. Nitric oxide acts as a positive regulator to induce metamorphosis of the ascidian *Herdmania momus*. *PLoS One* **8:** e72797.

Ueda, N., and S. M. Degnan. 2014. Nitric oxide is not a negative regulator of metamorphic induction in the abalone *Haliotis asinina*. *Front. Mar. Sci.* **1:** 21.

Yang, Z., H. Yu, R. Yu, and Q. Li. 2015. Induced metamorphosis in larvae of the veined rapa whelk *Rapana venosa* using chemical cues. *Mar. Biol. Res.* **11:** 1085–1092.

Appendix

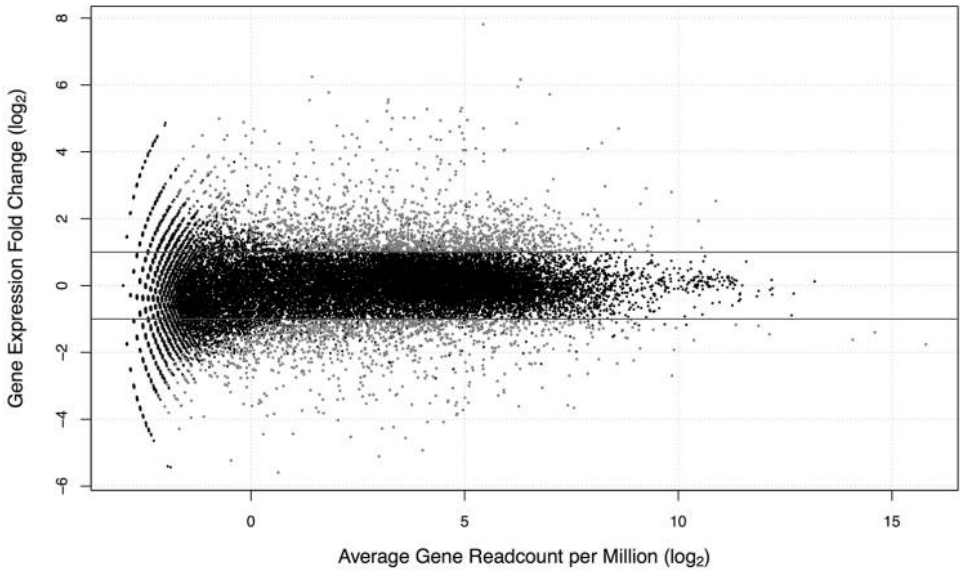

Figure A1. Fold change in gene expression *versus* average expression level per gene (mean normalized read count). Each circle represents one gene. Gray circles indicate that a gene is significantly differentially expressed (Benjamini-Hochberg false discovery rate of <0.05, $\log_2$ fold change of >1). Black circles are genes that were not significantly differentially expressed. The horizontal lines indicate a twofold change in gene expression.

Table A1

Heat-shock proteins upregulated in precompetent larvae of Capitella teleta

Gene ID[a]	Log_2 FC[b]	Log_2 CPM[c]	F[d]	P-value[e]	FDR[f]	InterPro descriptors[g]
CapteG18512	2.91	9.25	124.33	1.39E−07	3.74E−05	Heat-shock protein 70 family
CapteG179511	2.60	6.43	84.46	1.07E−06	1.20E−04	Heat-shock protein 70 family
CapteG159587	2.45	9.12	176.43	2.06E−08	1.45E−05	Heat-shock protein HSP90 family
CapteG221226	2.33	6.96	134.77	8.98E−08	2.95E−05	Heat-shock protein 70 family
CapteG223688	1.96	2.99	23.11	4.60E−04	8.42E−03	Heat-shock protein 70 family
CapteG223831	1.51	6.74	130.54	1.07E−07	3.20E−05	Heat-shock protein HSP90 family

[a] Ensembl Metazoa gene ID.

[b] Log_2 fold change (FC) in gene expression between precompetent and competent larvae of *C. teleta*. Positive values are expressed more highly in precompetent larvae, while negative values are expressed more highly in competent larvae.

[c] Average $\log_2$ read count per gene per million reads mapped (CPM).

[d] Differential expression test F-statistic.

[e] Differential expression test P-value.

[f] Benjamini-Hochberg-adjusted P-value to control type I error. FDR, false discovery rate.

[g] InterPro annotation descriptions for each gene.

Table A2

Heme peroxidases and superoxide dismutases upregulated in precompetent larvae of Capitella teleta

Gene ID[a]	Log_2 FC[b]	Log_2 CPM[c]	F[d]	P-value[e]	FDR[f]	InterPro descriptors[g]
CapteG134931	4.08	3.93	106.04	3.25E−07	5.60E−05	Heme peroxidase
CapteG72445	2.67	2.57	44.57	2.58E−05	1.04E−03	Heme peroxidase
CapteG146425	2.30	1.88	29.96	1.56E−04	3.83E−03	Heme peroxidase
CapteG191097	1.48	3.54	27.15	2.37E−04	5.20E−03	Heme peroxidase
CapteG229194	1.12	5.32	41.42	3.63E−05	1.35E−03	Manganese/iron superoxide dismutase
CapteG148692	1.12	3.22	23.22	4.51E−04	8.29E−03	Superoxide dismutase (Cu/Zn)/superoxide dismutase copper chaperone

[a] Ensembl Metazoa gene ID.
[b] Log_2 fold change (FC) in gene expression between precompetent and competent larvae of *C. teleta*. Positive values are expressed more highly in precompetent larvae, while negative values are expressed more highly in competent larvae.
[c] Average log_2 read count per gene per million reads mapped (CPM).
[d] Differential expression test *F*-statistic.
[e] Differential expression test *P*-value.
[f] Benjamini-Hochberg-adjusted *P*-value to control type I error. FDR, false discovery rate.
[g] InterPro annotation descriptions for each gene.

Table A3

Amiloride-sensitive ion channels upregulated in competent larvae of Capitella teleta

Gene ID[a]	Log_2 FC[b]	Log_2 CPM[c]	F[d]	P-value[e]	FDR[f]	InterPro descriptors[g]
CapteG204744	−1.09	3.04	14.92	2.36E−03	2.76E−02	Na^+ channel, amiloride sensitive
CapteG200711	−1.10	3.36	16.35	1.71E−03	2.18E−02	Na^+ channel, amiloride sensitive
CapteG228582	−1.11	3.73	15.12	2.26E−03	2.67E−02	Na^+ channel, amiloride sensitive
CapteG146895	−1.21	2.85	12.12	4.70E−03	4.52E−02	Na^+ channel, amiloride sensitive
CapteG187791	−1.25	0.11	14.18	4.35E−03	4.27E−02	Na^+ channel, amiloride sensitive
CapteG135400	−1.26	1.31	18.74	1.04E−03	1.54E−02	Na^+ channel, amiloride sensitive
CapteG191757	−1.28	−0.06	11.58	5.43E−03	4.99E−02	Na^+ channel, amiloride sensitive
CapteG63286	−1.41	0.76	16.02	1.84E−03	2.30E−02	Na^+ channel, amiloride sensitive
CapteG227962	−1.42	0.93	28.81	2.47E−04	5.35E−03	Na^+ channel, amiloride sensitive
CapteG218320	−1.45	1.35	17.83	1.25E−03	1.75E−02	Na^+ channel, amiloride sensitive
CapteG207658	−1.48	−0.33	11.73	5.20E−03	4.85E−02	Na^+ channel, amiloride sensitive
CapteG188964	−1.56	1.62	28.56	1.91E−04	4.44E−03	Na^+ channel, amiloride sensitive
CapteG195930	−1.68	0.82	38.26	5.24E−05	1.73E−03	Na^+ channel, amiloride sensitive
CapteG212550	−1.68	0.66	29.43	1.68E−04	4.03E−03	Na^+ channel, amiloride sensitive
CapteG73713	−1.71	0.69	15.52	2.06E−03	2.50E−02	Na^+ channel, amiloride sensitive
CapteG192207	−1.73	0.44	17.10	1.46E−03	1.95E−02	Na^+ channel, amiloride sensitive
CapteG195931	−1.75	0.85	24.02	3.93E−04	7.50E−03	Na^+ channel, amiloride sensitive
CapteG215630	−1.79	2.26	23.38	4.39E−04	8.13E−03	Na^+ channel, amiloride sensitive
CapteG220280	−1.82	0.93	27.56	2.22E−04	4.98E−03	Na^+ channel, amiloride sensitive
CapteG201141	−2.25	3.79	88.46	8.44E−07	1.04E−04	Na^+ channel, amiloride sensitive

[a] Ensembl Metazoa gene ID.
[b] Log_2 fold change (FC) in gene expression between precompetent and competent larvae of *C. teleta*. Positive values are expressed more highly in precompetent larvae, while negative values are expressed more highly in competent larvae.
[c] Average log_2 read count per gene per million reads mapped (CPM).
[d] Differential expression test *F*-statistic.
[e] Differential expression test *P*-value.
[f] Benjamini-Hochberg-adjusted *P*-value to control type I error. FDR, false discovery rate.
[g] InterPro annotation descriptions for each gene.

Table A4

Polycystic kidney disease-like genes upregulated in competent larvae of Capitella teleta

Gene ID[a]	Log_2 FC[b]	Log_2 CPM[c]	F[d]	P-value[e]	FDR[f]	InterPro descriptors[g]
CapteG169289	−1.41	7.96	72.56	2.34E−06	2.00E−04	Polycystic kidney disease type 2 protein
CapteG119174	−2.06	5.11	111.96	2.43E−07	4.77E−05	Polycystin cation channel, PKD1/PKD2
CapteG89348	−2.17	2.54	58.06	7.18E−06	4.31E−04	Polycystin cation channel, PKD1/PKD2
CapteG185815	−2.39	5.43	108.51	2.88E−07	5.27E−05	Polycystic kidney disease type 2 protein
CapteG89699	−2.90	5.12	53.23	1.10E−05	5.84E−04	Polycystic kidney disease type 2 protein

[a] Ensembl Metazoa gene ID.
[b] Log_2 fold change (FC) in gene expression between precompetent and competent larvae of *C. teleta*. Positive values are expressed more highly in precompetent larvae, while negative values are expressed more highly in competent larvae.
[c] Average log_2 read count per gene per million reads mapped (CPM).
[d] Differential expression test *F*-statistic.
[e] Differential expression test *P*-value.
[f] Benjamini-Hochberg-adjusted *P*-value to control type I error. FDR, false discovery rate.
[g] InterPro annotation descriptions for each gene.

Table A5

G-protein-coupled receptors upregulated in competent larvae of Capitella teleta

Gene ID[a]	Log_2 FC[b]	Log_2 CPM[c]	F[d]	P-value[e]	FDR[f]	InterPro descriptors[g]
CapteG190026	−1.01	2.61	19.94	1.38E−03	1.88E−02	GPCR, rhodopsin-like, 7TM
CapteG147802	−1.01	2.59	24.90	1.36E−03	1.86E−02	G-protein-coupled receptor, rhodopsin-like|galanin receptor family
CapteG34777	−1.01	2.39	28.89	1.64E−03	2.12E−02	G-protein-coupled receptor, rhodopsin-like|neuropeptide Y receptor family
CapteG196014	−1.03	3.69	39.69	2.21E−04	4.96E−03	GPCR, rhodopsin-like, 7TM
CapteG196564	−1.04	2.01	12.37	4.40E−03	4.31E−02	GPCR, rhodopsin-like, 7TM
CapteG213150	−1.06	3.28	36.89	2.54E−04	5.45E−03	GPCR, rhodopsin-like, 7TM
CapteG115578	−1.08	2.66	27.63	5.66E−04	9.77E−03	GPCR, rhodopsin-like, 7TM
CapteG90908	−1.11	2.96	30.33	2.47E−04	5.35E−03	G-protein-coupled receptor, rhodopsin-like|thyrotropin-releasing hormone receptor
CapteG152907	−1.15	3.88	48.11	2.77E−05	1.09E−03	GPCR, rhodopsin-like, 7TM
CapteG224577	−1.15	5.40	53.86	1.04E−05	5.66E−04	GPCR, rhodopsin-like, 7TM
CapteG201805	−1.17	0.51	12.77	3.98E−03	4.00E−02	GPCR, rhodopsin-like, 7TM
CapteG196425	−1.20	1.17	19.53	1.21E−03	1.72E−02	GPCR, family 3, γ-aminobutyric acid receptor, type B
CapteG194428	−1.23	1.46	23.08	5.96E−04	1.02E−02	G-protein-coupled receptor, rhodopsin-like
CapteG227407	−1.24	1.93	27.01	2.96E−04	6.11E−03	GPCR, rhodopsin-like, 7TM
CapteG196555	−1.25	1.13	19.31	9.27E−04	1.41E−02	GPCR, rhodopsin-like, 7TM
CapteG141683	−1.25	2.83	31.67	1.22E−04	3.14E−03	G-protein-coupled receptor, rhodopsin-like|5-hydroxytryptamine 6 receptor
CapteG198894	−1.26	1.13	22.73	7.51E−04	1.21E−02	GPCR, rhodopsin-like, 7TM
CapteG195782	−1.30	1.36	25.28	3.45E−04	6.84E−03	GPCR, rhodopsin-like, 7TM
CapteG209584	−1.32	1.32	28.60	3.12E−04	6.33E−03	GPCR, rhodopsin-like, 7TM
CapteG26928	−1.35	1.40	19.95	8.19E−04	1.29E−02	GPCR, rhodopsin-like, 7TM
CapteG205658	−1.38	1.09	17.22	1.42E−03	1.91E−02	GPCR, family 3, γ-aminobutyric acid receptor, type B
CapteG140881	−1.39	1.15	18.98	9.91E−04	1.48E−02	GPCR, family 3, γ-aminobutyric acid receptor, type B
CapteG194422	−1.40	0.84	18.38	1.12E−03	1.61E−02	GPCR, rhodopsin-like, 7TM
CapteG134768	−1.43	3.88	23.84	4.05E−04	7.65E−03	G-protein-coupled receptor, rhodopsin-like|glycoprotein hormone receptor family
CapteG98913	−1.44	0.56	16.59	1.63E−03	2.10E−02	GPCR, rhodopsin-like, 7TM

Table A5 (*Continued*)

Gene ID[a]	Log_2 FC[b]	Log_2 CPM[c]	F[d]	P-value[e]	FDR[f]	InterPro descriptors[g]
CapteG188220	−1.48	0.16	18.47	1.10E−03	1.59E−02	GPCR, rhodopsin-like, 7TM
CapteG192959	−1.49	2.52	20.79	6.98E−04	1.14E−02	GPCR, family 3, γ-aminobutyric acid receptor, type B
CapteG199664	−1.51	2.18	32.76	1.05E−04	2.82E−03	GPCR, rhodopsin-like, 7TM
CapteG215255	−1.53	2.03	20.90	6.84E−04	1.13E−02	GPCR, rhodopsin-like, 7TM
CapteG110011	−1.60	1.28	46.07	2.20E−05	9.30E−04	GPCR, rhodopsin-like, 7TM
CapteG213630	−1.61	1.81	36.12	6.81E−05	2.11E−03	GPCR, rhodopsin-like, 7TM
CapteG216885	−1.75	3.24	35.03	7.82E−05	2.31E−03	GPCR, rhodopsin-like, 7TM
CapteG145194	−1.76	2.26	64.37	4.30E−06	3.09E−04	GPCR, family 3, γ-aminobutyric acid receptor, type B
CapteG192499	−1.76	1.72	50.85	1.37E−05	6.78E−04	GPCR, rhodopsin-like, 7TM
CapteG131608	−1.97	−0.71	17.02	1.48E−03	1.97E−02	GPCR, rhodopsin-like, 7TM
CapteG108341	−2.18	−1.54	11.92	4.96E−03	4.69E−02	GPCR, rhodopsin-like, 7TM
CapteG199650	−2.40	−0.90	20.28	7.69E−04	1.23E−02	GPCR, rhodopsin-like, 7TM
CapteG201394	−2.41	−1.40	13.26	3.52E−03	3.68E−02	GPCR, rhodopsin-like, 7TM
CapteG195933	−2.45	−0.25	20.98	6.74E−04	1.11E−02	GPCR, rhodopsin-like, 7TM
CapteG199130	−2.52	−1.18	22.55	5.07E−04	9.02E−03	GPCR, rhodopsin-like, 7TM
CapteG193146	−2.90	−1.31	17.04	1.47E−03	1.96E−02	GPCR, rhodopsin-like, 7TM
CapteG37194	−2.91	−1.47	17.86	1.24E−03	1.74E−02	GPCR, rhodopsin-like, 7TM
CapteG201967	−3.20	−1.54	16.79	1.56E−03	2.04E−02	GPCR, rhodopsin-like, 7TM
CapteG205921	−3.35	5.01	28.48	1.94E−04	4.48E−03	GPCR, rhodopsin-like, 7TM

[a] Ensembl Metazoa gene ID.

[b] Log_2 fold change (FC) in gene expression between precompetent and competent larvae of *C. teleta*. Positive values are expressed more highly in precompetent larvae, while negative values are expressed more highly in competent larvae.

[c] Average log_2 read count per gene per million reads mapped (CPM).

[d] Differential expression test *F*-statistic.

[e] Differential expression test *P*-value.

[f] Benjamini-Hochberg-adjusted *P*-value to control type I error. FDR, false discovery rate.

[g] InterPro annotation descriptions for each gene. GPCR, G-protein-coupled receptor; TM, transmembrane (*i.e.*, 7TM indicates that there are 7 transmembrane domains in the protein).

Table A6

Other receptors upregulated in competent larvae of Capitella teleta

Gene ID[a]	Log_2 FC[b]	Log_2 CPM[c]	F[d]	P-value[e]	FDR[f]	InterPro descriptors[g]
CapteG148064	−1.02	2.56	32.92	1.25E−03	1.75E−02	γ-Aminobutyric acid A receptor/glycine receptor α
CapteG192796	−1.06	2.03	22.09	1.69E−03	2.17E−02	Ionotropic glutamate receptor
CapteG219746	−1.12	2.85	34.09	2.64E−04	5.61E−03	Ionotropic glutamate receptor
CapteG152072	−1.15	5.02	48.78	1.68E−05	7.71E−04	Ionotropic glutamate receptor
CapteG188571	−1.17	3.89	41.37	3.65E−05	1.35E−03	Ionotropic glutamate receptor
CapteG204388	−1.66	2.19	44.63	2.56E−05	1.04E−03	Ionotropic glutamate receptor
CapteG98250	−1.85	−0.67	17.42	1.36E−03	1.86E−02	Ionotropic glutamate receptor
CapteG204976	−2.25	−1.65	11.62	5.36E−03	4.95E−02	Ionotropic glutamate receptor
CapteG205451	−1.14	0.76	11.66	5.31E−03	4.92E−02	Nicotinic acetylcholine–gated receptor, transmembrane domain
CapteG123873	−1.62	0.88	23.06	4.64E−04	8.47E−03	Nicotinic acetylcholine–gated receptor, transmembrane domain
CapteG221712	−1.05	4.27	40.09	5.92E−05	1.91E−03	Neurotransmitter-gated ion channel
CapteG217687	−1.82	0.93	38.69	4.97E−05	1.67E−03	Neurotransmitter-gated ion channel
CapteG214390	−1.69	−0.57	12.10	4.73E−03	4.54E−02	Gustatory receptor

[a] Ensembl Metazoa gene ID.

[b] Log_2 fold change (FC) in gene expression between precompetent and competent larvae of *C. teleta*. Positive values are expressed more highly in precompetent larvae, while negative values are expressed more highly in competent larvae.

[c] Average log_2 read count per gene per million reads mapped (CPM).

[d] Differential expression test *F*-statistic.

[e] Differential expression test *P*-value.

[f] Benjamini-Hochberg-adjusted *P*-value to control type I error. FDR, false discovery rate.

[g] InterPro annotation descriptions for each gene.