Expected ocean warming conditions significantly alter the transcriptome of developing postlarval American lobsters (*Homarus americanus*): implications for energetic trade-offs

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1. Abstract

The American lobster (*Homarus americanus*) is one of the most iconic and economically valuable fishery species in the Northwestern Atlantic. Surface ocean temperatures are rapidly increasing across much of the species' range, raising concern about resiliency in the face of environmental change. Warmer temperatures accelerate rates of larval development and enhance survival to the postlarval stage, but the potential costs at the molecular level have rarely been addressed. We explored how exposure to current summer temperatures (16 °C) or temperature regimes mimicking projected moderate or extreme warming scenarios (18 °C and 22 °C, respectively) for the Gulf of Maine during development influences the postlarval lobster transcriptome. After *de novo* assembling the transcriptome, we identified 2,542 differentially expressed (DE; adjusted $p < 0.05$) transcripts in postlarvae exposed to 16 °C vs. 22 °C, and 422 DE transcripts in postlarvae reared at 16 °C vs. 18 °C. Lobsters reared at 16 °C significantly over-expressed transcripts related to cuticle formation and the immune response up to 14.4- and 8.5-fold respectively, relative to those reared at both 18 °C and 22 °C. In contrast, the expression of transcripts affiliated with metabolism increased up to 7.1-fold as treatment temperature increased. These results suggest that lobsters exposed to projected warming scenarios during development experience a shift in the transcriptome that reflects a potential trade-off between maintaining immune defenses and sustaining increased physiological rates under a warming environment. This could have major implications for post-settlement survival through increased risk of mortality due to disease and/or starvation if energetic demands cannot be met.

Keywords: ocean warming, *Homarus americanus*, immunity, transcriptomics, climate change

2. Introduction

Increased anthropogenic carbon dioxide (CO_2) emissions into the atmosphere has caused widespread changes in climatic conditions, including increased rates of ocean warming and acidification. Global ocean surface temperatures have increased by 0.11 °C per decade from 1971 – 2010 (IPCC, 2014), and recent modeling suggest that the oceans are likely warming at a rate faster than previously projected (Alexander et al., 2018; IPCC, 2018; Cheng et al., 2019). The Northwestern Atlantic Ocean is experiencing some of the most rapid rates of warming (Pershing et al., 2015). Specifically, the Gulf of Maine is warming four times the average global rate (i.e., 0.4 °C per decade – Thomas et al., 2017), which has had considerable impacts on the region's commercial finfish (Pershing et al., 2015) and shellfish (Richards et al., 2012; Arnberg et al., 2013) fisheries. Warming ocean temperatures have also been linked to mass mortality events and disease outbreaks in the southern portion of the American lobster's (*Homarus americanus*) geographic range (Pearce and Balcolm, 2005), raising concerns of increased disease susceptibility as the Gulf of Maine continues to warm.

Underlying the impacts of warming on Gulf of Maine species is the effect of temperature on physiological processes (i.e., Q_{10} effects) and the increase in oxygen demand associated with these effects on metabolism (Somero et al., 2015, 2017). Previous experiments have determined general patterns in organismal responses to various environmental stressors, but often did not identify the molecular mechanisms driving observations. As bioinformatics tools continue to advance, next-generation sequencing efforts and wholistic gene expression studies have provided a much greater understanding of how organisms respond to environmental stress (Conesa et al., 2016). Transcriptomics, the study of transcriptomes (RNA-seq; Lesk, 2013; Costa-Silva et al.,

2017), has been implemented in a variety of life-science research studies in the aquatic environment; particularly in the context of understanding cellular responses to climate change (e.g., blue mussels *Mytilus* spp. – Lockwood et al., 2010; corals *Acropora millepora* – Moya et al., 2012; Sydney rock oysters *Saccostrea glomerata* – Goncalves et al., 2017; purple urchins *Strongylocentrotus purpuratus*– Evans et al., 2017; Wong et al., 2018). Transcriptomics is a particularly useful tool to explore gene expression patterns in crustaceans as it does not require that the genome or the transcriptome of a species of interest be fully known (Clark and Greenwood, 2016). Sophisticated software packages allow a user to *de novo* assemble a transcriptome (e.g., Trinity – Grabherr et al., 2011; Haas et al., 2013), which can then serve as a reference for subsequent analyses.

We explored how exposure to projected warming trends for the Gulf of Maine influences the transcriptome of postlarval American lobster. *Homarus americanus* inhabits waters off the Atlantic Coast of North America from North Carolina, USA, to Newfoundland, Canada (Herrick, 1911; Holthuis, 1991). During the summer months, newly hatched lobsters proceed through three pelagic larval stages (I-III) prior to metamorphosing into the final, postlarval stage (IV) that resembles the adult form. The postlarval stage marks the point of transition (settlement) from the pelagic to the benthic stage in the life history of *H. americanus*; how the environment impacts this life history stage may have huge implications for post-settlement survival and recruitment. Although development time varies with larval origin (Quinn et al., 2013) and environmental conditions (Quinn and Rochette, 2015), previous research suggests that exposure to warmer temperatures significantly accelerates larval development time (Hadley, 1906; Templeman, 1936; MacKenzie, 1988; Waller et al., 2017; Barret et al., 2017; Harrington et al., 2019), and

increases survival to stage IV (MacKenzie. 1988; Barret et al., 2017; Harrington et al., 2019). These potential benefits of ocean warming may be outweighed by increased oxygen demand due to elevated metabolic rates (Waller et al., 2017) and elevated levels of stress (Harrington et al., 2019), which could lead to reduced post-settlement survival and subsequent recruitment to the population. Previous genetic analyses of developing *H. americanus* indicate that the constitutive expression of immune-related genes increases and is maximized upon reaching the postlarval stage (Hines et al., 2014), and transcriptomic efforts have identified numerous putative immune-related genes in larval *H. americanus* that remain to be validated (Clark and Greenwood, 2016). Transcriptomic analyses have primarily focused on adult lobsters, particularly in the context of pathogen- and tissue-specific immune responses (Clark et al., 2013a, 2013b, 2013c; Clark et al., 2015) or tissue-specific expression patterns of genes related to biological neural circuits (McGrath et al., 2016), with few exploring the impacts of environmental change on early life history stages.

To our knowledge, this is the first study to address how acute exposure to warming conditions during larval development influences the postlarval *H. americanus* transcriptome. As *H. americanus* lacks a fully sequenced reference genome, our first goal was to *de novo* assemble a postlarval lobster transcriptome. We then characterized the transcriptional profiles across postlarvae exposed to three temperature treatments during larval development. Finally, we compared transcriptome-wide differences across these temperature groups, identifying a large number of differentially expressed transcripts across treatments. We focus our discussion here on transcripts annotated to proteins associated with innate immunity and metabolic turnover as these two broad categories are known to be impacted by a warming environment in adult American

89 lobster. This work aims to provide a better understanding of the molecular mechanisms involved
90 in potential trade-offs between accelerated growth and overall fitness in a warming ocean of a
91 critical life history stage in this important fishery species.
92
93 **3. Materials and methods**
94 *3.1. Larval rearing*
95 This research was part of a larger effort to understand the potential impacts of a warming
96 environment on larval development and survival (Harrington et al., 2019). Egg-bearing female
97 lobsters with at least stage 3 clutches (i.e., mature clutches close to hatching) were collected by
98 the Maine Department of Marine Resources (ME DMR) Ventless Trap Survey in summer 2017
99 from the waters off the midcoast Maine region. Females were transported to the University of
100 Maine's Aquaculture Research Center (ARC) in Orono, ME, and housed individually in
101 recirculating seawater systems (N = 18 females). Systems were filled with artificial seawater that
102 was pre-mixed to a salinity of 34 ppt and maintained at a temperature of 16 °C and a pH of 8.1
103 (via the addition of Kent Marine Superbuffer-dKH^{TM}) as previously described (Harrington et al.,
104 2019). Females were observed at least every 10 h for newly hatched larvae. Upon hatch, larvae
105 were gently netted for removal and counted before being evenly distributed among three
106 temperature treatments: 16, 18, and 22 °C. The 16 °C treatment was selected to represent the
107 current average summer temperatures experienced by larval *H. americanus* in the collection area
108 (The Northeastern Regional Association of Coastal Ocean Observing Systems, NERACOOS,
109 Past X-Band MODIS Satellite Sea Surface Temperature Data; neracoos.org). The 18 °C and 22
110 °C temperature treatments were selected to reflect projected moderate and extreme warming

scenarios, respectively (IPCC, 2019). We used three recirculating seawater systems (one for each temperature treatment) that consisted of four replicate 75 L tanks each. Systems were filled with artificial seawater pre-mixed to conditions similar to the female holding tanks (salinity of 34 ppt and a pH of 8.1) and temperatures were maintained using Delta Star® in-line chillers (Aqua Logic, San Diego, CA, USA). Experimental tanks were stocked evenly across temperatures with larvae from at least three different females over no longer than 48 h to limit size variation among larvae, resulting in stocking densities that ranged from 3.5 – 14 larvae per L depending on the number of individuals available during stocking events. Importantly, there was no significant effect of stocking density on either rate of development or cumulative survival to stage IV across temperature treatments (Harrington et al., 2019). Tanks were heavily aerated, and live *Artemia* spp. (Grade A Brine Shrimp Eggs, Brine Shrimp Direct, Inc., Ogden, UT, USA) were added at a density of 12 mL^{-1} to reduce cannibalism. We conducted daily assessments of water quality (i.e., temperature, salinity, pH, and dissolved oxygen content) and animal husbandry as described in Harrington et al. (2019).

3.2. Sample preservation and RNA extraction

Larval development was assessed daily using a Unitron® Z850 Zoom Stereo Microscope (Unitron, Commack, NY, USA) to examine morphological characteristics (see Harrington et al., 2019). Development was significantly accelerated under warming conditions, with larvae reaching stage IV an average (±SE) of 22.3 ± 1.03, 14.5 ± 0.5, and 11.0 ± 0.4 d post hatch when reared under 16, 18, and 22 °C, respectively (Harrington et al., 2019). Upon reaching stage IV, postlarvae were removed from the experimental tanks. A subset of individuals was immediately

placed individually in DNA/RNA-free microcentrifuge tubes containing 1 mL of RNA*later*™ for preservation, were stored at 4 °C for 24 h after which they were stored at -20 °C until homogenization. Whole animals were homogenized in 1 mL of TRIzol® reagent using a Tissue Tearor (Biospec Products, Inc, Bartlesville, OK, USA) following manufacturer's instructions (see Clark et al., 2013a). To minimize transference of genetic material across samples, the Tissue Tearor was cleaned with three 30 s rinses of reverse osmosis (RO) water followed by a 1 min 30 s rinse with 70% Ethanol. RNA was then isolated using the Qiagen RNeasy® Mini Kit following the manufacturer's protocols, including the optional on-column DNase I treatment. RNA quality and quantity were quantified spectrophotometrically using a Thermo Scientific NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were stored at -80 °C, and material from five replicate animals reared at each temperature were sent to the Genomic Services Lab (GSL) at the HudsonAlpha Institute for Biotechnology (Huntsville, AL, USA) for library preparation and sequencing. Samples were selected based on standard RNA quality and quantity analyses (i.e., A260/A280 values of ~2.0 and A260/A240 values of 1.8-2.2; Table S1) and to ensure that replicate stocking events (and thus larvae from replicate females) were evenly represented across temperature treatments.

3.3. Library preparation and de novo *transcriptome assembly*

Libraries were prepared for all samples by the GSL following internal RNA quality control assays. RNA-seq libraries were prepared using poly(A) enrichment and sequenced on an Illumina HiSeq 2500 sequencer (PE50, 50M reads per sample). Raw sequence reads were uploaded to the Galaxy Server (usegalaxy.org; Afgan et al., 2018; Galaxy Version 18.09). Read

quality was assessed using FastQC (Andrews, 2010; Galaxy Version 0.72), and reads were trimmed using Trim Galore! (Babraham Bioinformatics; Galaxy Version 0.4.3.1). A *de novo* transcriptome was assembled using the Trinity bioinformatics suite (Grabherr et al., 2011; Haas et al., 2013; Galaxy Version 0.0.1). Following the data analysis pipeline of Pertea et al. (2016), reads were aligned to the transcriptome using HiSAT2 (Kim et al., 2015; Galaxy Version 2.1.0+galaxy3) and assembled into full and partial transcripts using StringTie (Pertea et al., 2015; Galaxy Version 1.3.4). StringTie Merge was used to create a set of consistent transcripts across samples (Pertea et al., 2015; Galaxy Version 1.3.4), and featureCounts was used to aggregate the raw counts of mapped reads (Liao et al., 2014; Galaxy Version 1.6.2).

Both DESeq2 (Love et al., 2014; Galaxy Version 2.11.40.2) and edgeR (Robinson et al., 2010; Liu et al., 2015; Galaxy Version 3.20.7.2) were used to assess differential transcript expression (Costa-Silva et al., 2017). We chose these two methods as they are considered robust to the presence of different library sizes and compositions across samples (Dillies et al., 2013) and are recommended for experiments with ≤ 12 replicate samples per group (Nguyen et al., 2018). Both methods use the negative binomial as a reference distribution, but DESeq2 employs a geometric normalization approach and edgeR calculates a weighted mean of log ratios in its normalization step (Dillies et al., 2013). By implementing both analysis tools, we hoped to provide a more robust analysis of differential expression (Nguyen et al., 2018). With both statistical approaches, *p*-values were adjusted for multiple testing with the Benjamini-Hochberg procedure to control for false discovery rate (FDR). For the DESeq2 analyses, factor levels 1, 2, and 3 corresponded to postlarvae reared at 16 °C (current conditions), 18 °C (moderate warming), and 22 °C (extreme warming), respectively; resulting in three pairwise comparisons.

177 Fold change (FC) was calculated for each transcript as a ratio of expression in postlarvae reared
178 at 16 °C relative to 22 °C (current conditions relative to extreme warming), 16 °C relative to 18
179 °C (current conditions relative to moderate warming), and 18 °C relative to 22 °C (moderate
180 relative to extreme warming) in treatment comparisons. Similar pairwise comparisons were set
181 up for the edgeR analyses. All data were graphically represented as $\log_2$ FC for ease of
182 visualization, where positive ($\log_2$ FC > 0) and negative ($\log_2$ FC < 0) values correspond to
183 transcripts over- and under-expressed, respectively, in postlarvae reared at 16 °C relative to 22
184 °C, 16 °C relative to 18 °C, and 18 °C relative to 22 °C in each comparison. We used Venn
185 diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/) to explore similarities in the
186 differentially expressed transcripts identified in each treatment comparison by the different
187 statistical methods.
188
189 *3.4. Annotation and pathway analysis*
190 We used the NCBI BLAST+ blastx algorithm (Galaxy Version 0.3.0) to annotate our
191 transcriptome using the NCBI non-redundant (nr) protein databases (E-value ≤ $1e^{-10}$;
192 downloaded in July 2018). We assigned protein domain information using InterProScan (IPS)
193 and Gene Ontology (GO) functional terms to these annotations using Blast2GO PRO (Götz et al.
194 2008; Version 5.2.5). We used the KEGG Automatic Annotation Server (KAAS; Moriya et al.,
195 2007) for ortholog assignment and pathway analysis of the top 100 significantly (i.e., adjusted *p*-
196 value ≤ 0.05) differentially expressed (DE) and annotated transcripts that were both over- and
197 under-expressed in all treatment comparisons from both the edgeR and DESeq2 analyses. We
198 chose to explore both the top 100 over- and the top 100 under-expressed transcripts in each

199 treatment comparison due to the large number of DE transcripts identified in analyses, and as a
200 means to better capture potential broad scale trade-offs associated with warming. Finally, in a
201 manual synthesis of these data, we identified transcripts annotated to proteins of interest related
202 to immunity, cuticle formation, and metabolism for further discussion. Particular attention was
203 paid to annotations to ensure results reported do not include potentially contaminating eukaryotes
204 (i.e., the majority of top hits were to other crustacean species). We chose these broad categories
205 as they represent processes known to be influenced by temperature in adult *H. americanus*, but
206 have yet to be explored in the context of ocean warming in postlarvae, particularly as they relate
207 to the balance between accelerated growth and overall fitness.
208
209 **4. Results**
210 *4.1. Transcriptome assembly and annotation*
211 Read quality and depth were generally consistent across samples (Table S1). The *de novo*
212 assembly of the postlarval lobster transcriptome via Trinity produced 138,833 transcripts, with
213 maximum and minimum transcript lengths of 26,771 and 201 bp, respectively, and an N50 of
214 1,942, values that are in line with previous transcriptomic analyses of *H. americanus* (Clark and
215 Greenwood, 2016; Northcutt et al., 2016; McGrath et al., 2016). Following the StringTie Merge
216 analysis, a total of 66,962 transcripts were found across all samples. Of these, 16,170 (24.2% of
217 assembled transcripts) were successfully annotated against the NCBI nr protein database, which
218 is comparable to previous research (Northcutt et al., 2016; McGrath et al., 2016). Mapping these
219 gene IDs to GO annotations identified 1,711 unique GO categories represented in our
220 transcriptome: 335 attributed to cellular components, 458 attributed to molecular function, and

221 918 attributed to biological processes. Of these, the top ten GO terms attributed to the greatest
222 number of transcripts (in descending order) were integral component of membrane
223 (GO:0016021), oxidation-reduction process (GO:0055114), membrane (GO:0016020),
224 transmembrane transport (GO:0055085), nucleus (GO:0005634), proteolysis (GO:0006508), zinc
225 ion binding (GO:0008270), regulation of transcription (DNA-templated; GO:0006355), protein
226 phosphorylation (GO:0006468), and transmembrane transporter activity (GO:0022857).
227
228 *4.2. Differential expression*
229 We observed a distinct clustering of samples by temperature whereby postlarvae reared
230 under 16 °C were most dissimilar from those reared under 22 °C, with those exposed to 18 °C
231 overlapping in the middle of these two groups (Fig. S1). Using DESeq2 analysis, we detected a
232 total of 2,542 differentially expressed (DE; adjusted $p < 0.05$) transcripts in the 16 °C vs. 22 °C
233 treatment comparison, 422 DE transcripts in the 16 °C vs. 18 °C treatment comparison, and 33
234 DE transcripts in the 18 °C vs. 22 °C treatment comparison (Table 1; Fig. 1). Of these DE
235 transcripts, none were shared across all three treatment comparisons, but 375 were shared
236 between the 16 °C vs. 22 °C and 16 °C vs. 18 °C treatment comparisons (Fig. 2). Results of the
237 edgeR analysis identified 805, three, and zero DE transcripts in the 16 °C vs. 22 °C, 16 °C vs. 18
238 °C, and 18 °C vs. 22 °C treatment comparisons, respectively (Table 1). All three of the DE
239 transcripts identified in the 16 °C vs. 18 °C treatment using edgeR were also identified by
240 DESeq2, and we omit the edgeR results from subsequent analyses. However, edgeR identified 14
241 DE transcripts in the 16 °C vs. 22 °C that were not identified by DESeq2 (Fig. 3), and as such we

include both analyses for this treatment comparison in the following sections to provide a more robust analysis of these data.

4.3. Top 100 DE transcripts

4.3.1. Comparison of current conditions and extreme warming. In both the DESeq2 and edgeR analyses, slightly more than half of the identified DE transcripts were over-expressed in postlarvae reared at 16 °C relative to 22 °C (Table 1), and the top 10 GO terms affiliated with the greatest number of DE transcripts were consistent (Table S2). The KAAS pathway analysis indicated that over-expressed transcripts in the 16 °C relative to the 22 °C treatment included proteins broadly involved in signaling and cellular processes, transcription, and the coagulation cascade (Table S3). Moreover, common IPS protein domains associated with these transcripts included those involved in transcription, chitin-binding, immunity, and cellular signaling (Fig. 4). In contrast, KAAS pathway analysis indicated that the top 100 under-expressed transcripts in the 16 °C treatment relative to postlarvae reared at 22 °C included proteins involved in a variety of metabolic processes, DNA repair and replication processes, calcium ion signaling, the cell cycle, ribosome biogenesis, and tRNA biogenesis (Table S3). Similarly, these transcripts included IPS domains related to metabolism, as well as DNA replication and repair (Fig. 4).

We identified a total of 38 proteins of interest in this treatment comparison, 26 of which were uniquely identified in this treatment comparison (Table 2; Fig. 5). Twenty-six transcripts were over-expressed in postlarvae reared at 16 °C relative to 22 °C, with 16 annotated to proteins involved in cuticle formation or chitin degradation, and 10 annotated to proteins involved in the innate immune response (Table 2; Fig. 5). In contrast, 12 proteins of interest were affiliated with

264 transcripts significantly under-expressed in postlarvae reared at 16 °C relative to 22 °C,
265 including eight annotated to proteins involved in cell division and DNA replication and five
266 annotated to proteins affiliated with metabolism or energy demanding processes (Table 2; Fig.
267 5).
268
269 4.3.2. Comparison of current conditions and moderate warming. Nearly 60% of the DE
270 transcripts in this comparison were over-expressed in postlarvae reared at 16 °C relative to 18 °C
271 (Table 1). The top 10 GO terms in all categories were similar to those observed in the 16 °C vs.
272 22 °C comparison (Table S2), and proteins annotated to DE transcripts in this treatment
273 comparison were generally involved in similar pathways identified via KAAS for the 16 °C vs.
274 22 °C comparison (Table S3). The IPS domains associated with the top 100 DE transcripts in the
275 16 °C relative to the 18 °C treatment were similar to those identified in 16 °C vs. 22 °C
276 comparison, but included some additional domains associated with cellular signaling and
277 transcription (Fig. S2).
278 We identified 25 transcripts annotated to proteins of interest in postlarvae exposed to 16
279 °C vs. 18 °C, 13 of which were uniquely detected in this treatment (Table 2; Fig. 5). Nineteen
280 were over-expressed in postlarvae reared at 16 °C relative to 18 °C, including eight annotated to
281 proteins involved in cuticle formation, six annotated to proteins involved in innate immunity, and
282 three annotated to proteins affiliated with developmental processes (Table 2; Fig. 5). In contrast,
283 proteins of interest were affiliated with eight transcripts significantly under-expressed in
284 postlarvae reared at 16 °C relative to 18 °C, including four annotated to proteins related to, or
285 dependent upon, calcium ion binding (Table 2; Fig. 5).

4.3.3. Comparison of moderate and extreme warming scenarios. Due to the lack of DE transcripts in the 18 °C vs. 22 °C comparison, most of the GO terms affiliated with either significantly over- or under-expressed transcripts occurred only once (Table S2). All DE transcripts in this treatment comparison were broadly affiliated with proteins involved in metabolism and the ubiquitin system according to KAAS pathway analysis (Table S3). However, IPS protein domains associated with or regulated by calcium ions and protein domains associated with oxygen transport were more prominent in transcripts that were under-expressed in postlarvae reared at 18 °C compared to 22 °C (Fig. S3). We also identified a total of four proteins of interest associated with the DE transcripts that were unique to this treatment comparison (Table 2; Fig. 5). Transcripts over-expressed in postlarvae reared at 18 °C relative to 22 °C were annotated to proteins involved in chitin binding, whereas transcripts under-expressed in postlarvae reared at 18 °C relative to 22 °C were annotated to proteins affiliated with lipid transport and calcium-dependent activity (Table 2; Fig. 5).

5. Discussion

5.1. Compromised innate immunity

Although invertebrates lack adaptive immunity, they possess a complex innate immune system that is capable of distinguishing self from non-self, eliminating pathogens, and healing wounds while repairing cellular damage via humoral and cellular mechanisms (Hoffmann, 2003; Royet, 2004; Cerenius and Söderhäll, 2011). In *H. americanus*, this system consists of a variety of pattern recognition receptors (PRRs) and effector cells, antimicrobial peptides (AMPs),

coagulation and melanization pathways, and lectins that induce agglutination and phagocytosis (Zasloff et al., 2002; Ghosh et al., 2011; Clark and Greenwood, 2016; Bowden, 2017). Postlarvae reared under current conditions (16 °C) significantly over-expressed transcripts associated with the innate immune response 2.2- to 8.3-fold (log_2 FC values of 1.1 – 3.0) higher when compared to postlarvae reared under predicted warming regimes. These included transcripts annotated to proteins that exhibit peptidase inhibitor activity (AMP type 2 precursor, crustin, crustin 2 – Rosa et al., 2007; Pisuttharachai et al., 2009; Kim et al., 2013); essential components of the TLR-mediated Dorsal/Relish pathways that induces an immune response via immune gene expression regulation (Spätzle 1, 3, and 4 – Wang et al., 2012; Clark and Greenwood, 2016); non-specific protease inhibitors that bind to, and neutralize, pathogenic proteases (A2M and A2M isoform 2 – Lin et al, 2008; Ma et al., 2010); and an anti-apoptosis and anti-inflammatory factor (hormone receptor – Wang et al., 2018; Tables 2, S3; Fig. 2). This indicates that ocean warming reduces the expression of components involved in a variety of immune pathways, potentially increasing disease susceptibility under warming, a trend also suggested in kuruma shrimp (*Marsupenaeus japonicus*) exposed to heat stress (Zheng et al., 2019). Research suggests postlarvae are at risk for a variety of microbial diseases (Fisher et al., 1978), the spread of which could be impacted by increasing temperature (Hernroth and Baden, 2018; Shields, 2019). In adult *H. americanus*, warming events have been linked to mass die offs and an increased incidence of epizootic shell disease (ESD) across the southern extent of the species range (Pearce and Balcolm, 2005; Wahle et al., 2009). While ESD prevalence has remained < 2% in lobsters sampled along the Maine coast (relative to 20 – 30 % in Southern New England), the highest levels of disease prevalence occurred in 2013 and 2017, which followed the two warmest years in the region since 2005 (ME

330 DMR, 2017; Reardon et al., 2018). Progression of ESD is accelerated under warmer
331 temperatures (Barris et al., 2018), and estimates predict that population-level impacts of ESD
332 may increase as ocean temperatures continue to rise (Groner et al., 2018). Although much of the
333 research on ESD in *H. americanus* has focused on adults, postlarvae may be vulnerable to this
334 disease due to the relatively thin exoskeleton at this stage (Fisher et al., 1976, 1978). This may be
335 particularly important in the context of ocean warming as we found that postlarvae reared under
336 current conditions also expressed transcripts involved in cuticle formation and chitin metabolism
337 at levels 4.5 – 14.4 (log_2 FC values of 2.2 – 3.9) times greater than those reared at under both
338 moderate and extreme warming regimes (Tables 2, S3; Fig. 5), a pattern also demonstrated in
339 northern krill (*Meganyctiphaes norvegica*) exposed to a short-term temperature stress (i.e., 9 °C
340 control vs. 12 °C warming scenario; Blanco-Bercial and Maas, 2018). These transcripts were
341 annotated to proteins involved in the mineralization of the pre-exuvial cuticle (early cuticle
342 proteins 2, 5, and 6 – Shafer et al., 2009), calcification of the exoskeleton (calcification-
343 associated soluble matrix protein 2 – Inoue et al., 2008), ecdysis (chitinase and chitinase 2 –
344 Fujitani et al., 2014), and chitin-binding processes (cuticle protein and cuticle-like protein –
345 Andersen, 1999; Inoue et al., 2003), suggesting that multiple aspects of proper exoskeletal
346 formation may be compromised under warming conditions. Although postlarvae have a much
347 higher molt frequency, and presumably greater chance of removing ESD than adults, they may
348 encounter difficulties during molting due to adhesion of tissues to the cuticle (Fisher et al.,
349 1978), which could be compounded by improper cuticle development and potentially lead to
350 molt death syndrome.

Together, these data indicate that postlarval *H. americanus* exposed to both moderate (18 °C) and extreme (22 °C) warming scenarios during development may be at a greater risk to pathogens due to suppression of both the primary defense against pathogens (the exoskeleton) and multiple components of the innate immune system. Previous molecular studies of adult *H. americanus* have demonstrated the ability of lobsters to mount both pathogen- and tissue-specific immune responses (Clark et al., 2013a, 2013b, 2013c; Clark et al., 2015). To our knowledge, similar studies have not been conducted on the postlarval stage, especially in the context of environmental change. Future research would therefore benefit from assessing changes to the larval transcriptome following an immune challenge to more fully understand how a changing climate might impact disease susceptibility.

5.2. Elevated energetic demands

Physiological processes are highly dependent on temperature, and an increase in temperature by just 1 °C may increase metabolic rates by 5 – 10%, greatly increasing oxygen and energy demands (Somero et al., 2015, 2017). In order to meet these energetic demands, organisms must generate ATP by either substrate-level phosphorylation (via glycolysis and Krebs cycle) or oxidative phosphorylation (via the ATP synthase complex; Sokolova et al, 2012; Somero et al., 2017). When compared to postlarvae reared under the extreme warming scenario (22 °C), postlarvae exposed to 16 °C significantly under-expressed transcripts related to amino acid metabolism, carbohydrate metabolism, the Krebs cycle, glycolysis/gluconeogenesis, pyruvate metabolism, and lipid metabolism (as indicated via KAAS pathway analysis; Table S3). Similarly, northern krill (Blanco-Bercial and Maas, 2018) and a calanoid copepod (*Temora*

373 *longicornis* – Semmouri et al., 2019) demonstrated an increase in expression of proteins related
374 to metabolic processes in response to warmer temperatures. We also found that lobsters reared
375 under 22 °C expressed transcripts annotated to acyl-CoA Δ-9 desaturase and Δ-9 desaturase,
376 proteins involved in fatty-acid metabolism (Guo et al., 2013), at levels that were 2.9 and 3.1
377 times greater, respectively (calculated from the inverse of FC values), than postlarvae reared
378 under current conditions (Fig. 5). These proteins play an essential role in maintaining membrane
379 fluidity in response to temperature stress (Zheng et al., 2019), but their over-expression in
380 response to warming may also suggest a potential shift in metabolism to mobilize energy
381 reserves that merits attention in future research (Semmouri et al., 2019; Zheng et al., 2019).
382 Furthermore, expression of transcripts annotated to NADH-dehydrogenase subunit 2 (also
383 termed NADH-ubiquinone oxidoreductase chain 2, ND2) was 7.1- and 4.9-fold higher
384 (calculated from the inverse of FC values) in postlarvae reared under extreme and moderate
385 warming scenarios, respectively, relative to those reared under current conditions. The protein
386 ND2 functions as the core subunit of Complex I in the electron transport chain and is responsible
387 for the initial transfer of electrons from NADH to the immediate receptor, ubiquinone (Kim et
388 al., 2011; Somero et al., 2017), which suggests an increase in electron transport under warming,
389 a trend also exhibited by *T. longicornis* under heat stress (Semmouri et al., 2019). Similarly,
390 purple urchins (*S. purpuratus*) collected from southern portions of the species' distribution along
391 the West Coast of the USA exhibited higher expression levels of genes related to metabolism,
392 electron transport, and protein translation termination relative to urchins collected from northern
393 sites that were 5 – 8 °C cooler in temperature when reared under common laboratory conditions
394 (Pespeni et al., 2013). Moreover, Pespeni et al. (2013) demonstrated that southern *S. purpuratus*

likely possess a greater scope for growth (the difference between energy input as food and output as respiratory metabolism) based on these genetic differences, which was corroborated by a 10% increase in the rate of re-growth of urchin spines relative to northern urchins. Larval development time in *H. americanus* is significantly reduced in lobsters reared under 22 °C relative to 16 °C (Harrington et al., 2019), and postlarvae reared at 16 °C significantly under-expressed transcripts affiliated with DNA repair and replication processes, cell cycle (cell growth and death), and tRNA biogenesis (Table S3) relative to those reared at 22 °C (as seen in *T. longicornis* – Semmouri et al., 2019). Additionally, postlarvae reared at 22 °C expressed transcripts annotated to proteins involved in DNA replication initiation and elongation (DNA primase-like protein and DNA replication licensing factors MCM2, MCM3, MCM5, and MCM7) and transcription elongation (FACT complex subunit SPT16) at levels that were 2.2 – 3.4 times greater than those reared at 16 °C (calculated from inverse FC values; Fig. 5). These data suggest that postlarvae were likely able to meet the ATP demands associated with development and growth under warming conditions in a laboratory setting. However, elevated aerobic metabolism cannot be maintained if ATP supply (i.e., food availability) does not match ATP demand (Sokolova et al., 2012). Here, developing lobsters were fed live *Artemia* spp. to satiation and were thus not food limited up to stage IV; however, research suggests a potential mismatch between the natural timing of *H. americanus* settlement and the peak abundance of a major food source, *Calanus finmarchicus*, under warming conditions (Carloni et al., 2018). It is therefore possible that postlarval *H. americanus* will be unable to meet the increasing energy demands associated with ocean warming due to declines in prey availability, which could result in reduced post-settlement survival in the face of future change.

417

418 *5.3. Caveats*

419 One major caveat associated with transcriptomics is that expression of mRNAs and
420 corresponding protein expression cannot be considered proportional without validation due to the
421 differential lifetimes and translation rates of mRNAs (Lesk, 2013; Evans, 2015). Additional
422 challenges arise for non-model organisms that lack a completely sequenced genome, as the
423 amount of functionally annotated genetic information available on searchable databases is
424 generally lacking and restricted to highly conserved pathways (Conesa et al., 2016). This
425 presents challenges in discovering novel genetic adaptations that are unique to groups found in
426 challenging environments (Clark and Greenwood, 2016). It has also become difficult to
427 guarantee or determine which tool for differential expression analysis is the most accurate or best
428 option for a given project as the number of developed programs continues to increase (Dillies et
429 al., 2013; Nguyen et al., 2018). One potential strategy is to employ multiple tools to provide a
430 more robust analysis, which is the approach we followed. The quantitative and qualitative
431 differences of these two methods demonstrate the benefit of using multiple tools in analyses, but
432 also suggest that this variation should be further explored in subsequent and future analyses.
433 Transcriptomics alone may not always indicate physiological changes in response to a changing
434 environment as it focuses primarily on differentially expressed genes with large fold changes.
435 These genes are often considered dispensable and redundant in function and may contribute only
436 marginally to overall fitness levels relative to constitutively expressed “hub” genes that exhibit
437 stable expression levels but have huge impacts on the expression or post-translational
438 modifications of downstream genes in response to environmental stressors (Evans, 2015).

439 Understanding protein activity and changes in energy allocation also provides a more robust
440 assessment of fitness during environmental stress (Evans, 2015; Pan et al., 2015), demonstrating
441 the importance of supplementing transcriptomic data with other metrics of physiology in order to
442 understand the full organismal response to a changing environment. Finally, it is important to
443 acknowledge the potential for contaminating eukaryotes to influence the results of transcriptomic
444 analyses. Exercising caution, as was done in this project, while reviewing blastx hits during the
445 annotation process can help eliminate suspect annotations prior to analyses, particularly in non-
446 model species.
447
448 **6. Conclusions**
449 We observed a shift in the postlarval lobster transcriptome as a result of exposure to
450 predicted warming scenarios during development. Postlarvae reared under current average
451 summer temperatures (16 °C) for midcoast Maine exhibited significantly higher expression of
452 transcripts associated with the immune response and cuticle formation compared to lobsters
453 exposed to temperatures that were 2 °C and 6 °C warmer. As rearing temperature increased, the
454 abundance of transcripts affiliated with metabolic turnover increased, suggesting a cellular focus
455 on meeting the demands of increased metabolic rates under warming at the potential expense of
456 the immune response (Zheng et al.. 2019). Postlarvae may be particularly vulnerable to disease
457 when experiencing dietary deficiencies (Fisher et al., 1976), which may already be contributing
458 to post-settlement mortality in *H. americanus* as the zooplankton assemblage shifts as a
459 consequence of warming (Carloni et al., 2018). Insufficient resources may also increase post-
460 settlement mortality if lobsters are unable to meet the energetic and oxygen demands of a

461 warming environment due to Q_{10} effects. Postlarvae will not face ocean warming in isolation and
462 we suggest that future research should also explore changes in the transcriptome in the context of
463 multiple environmental factors.
464 Supplementary data to this article can be found online.
465
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467 The authors declare no conflict of interest.
468
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481
482 **Availability of data and materials**

RNA sequencing data reported will be deposited in the GenBank SRA and the University of Maine's Dataverse and made available upon acceptance of the manuscript.

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Table 1. Number of differentially expressed (DE; adjusted p-value ≤ 0.05) transcripts over- and under-expressed in each temperature comparison using both DESeq2 (**A**) and edgeR (**B**) analyses. For each comparison, the number of over- and under-expressed transcripts are expressed as the first temperature treatment relative to the second temperature treatment (e.g., in the 16 °C vs. 22 °C comparison, transcripts are over- or under-expressed in postlarvae reared at 16 °C relative to those reared at 22 °C).

Comparison	Total DE (#)	Over-expressed (#)	Under-expressed (#)
(A)			
16 °C vs. 22 °C	2,542	1,354	1,188
16 °C vs. 18 °C	422	326	96
18 °C vs. 22 °C	33	16	17
(B)			
16 °C vs. 22 °C	805	468	337
16 °C vs. 18 °C	3	1	2
18 °C vs. 22 °C	0	-	-

Table 2. List of proteins to which differentially expressed transcripts were annotated whose expression was higher (**A**) or lower (**B**) in all treatment comparisons. Proteins with an asterisk (*) were common across both the 16 °C vs. 22 °C and the 16 °C vs. 18 °C treatment comparisons. For the 16 °C vs. 22 °C comparison, additional transcripts identified by edgeR are indicated by an obelisk (†).

	Comparison	Transcript Annotated Protein Description	Function of Protein
(A)	16 °C vs. 22 °C	Strongly chitin-binding protein*	Structural constituent of cuticle
		Chitin-binding protein*	Structural constituent of cuticle
		Cuticle protein 19.8*	Structural constituent of cuticle
		Gastrolith protein 18.2	Formation of chitin-based gastrolith matrix
		Chitinase	Breaks down glycosidic bonds in chitin
		Chitinase 2, partial	Chitin binding; Hydrolase activity; Carbohydrate metabolic process
		Calcification-associated soluble matrix protein 2 (Casp-2)	Chitin binding domain; Calcification of the cuticle
		Peritrophin*	Chitin binding; Chitin metabolic processes
		Peritrophin 44-like protein	Chitin binding; Involved in antibacterial innate immunity via peritrophic membrane formation
		rly cuticle protein 2	Structural constituent of cuticle
		Early cuticle protein 5	Structural constituent of cuticle

Table 2 Continued

	Early cuticle protein 6	Structural constituent of cuticle
	Cuticle protein*	Structural constituent of cuticle
	Cuticle-like protein*	Structural constituent of cuticle
	Cuticle 7-like protein	Structural constituent of cuticle
	Cuticle protein 7†	Structural constituent of cuticle
	Antimicrobial peptide type 2 precursor	Peptidase inhibitor activity; Involved in innate immunity
	Crustin	Antimicrobial peptide; Involved in innate immunity
	Spätzle 1 (Spz1)*	Spätzle-like protein 1; Involved in innate immunity
	Spätzle 3 (Spz3)*	Spätzle-like protein 3; Involved in innate immunity
	Spätzle 4†	Spätzle-like protein 4; Involved in innate immunity
	Proclotting enzyme-like*	Serine-type endopeptidase; Proteolysis; Involved in innate immunity
	Alpha 2-macroglobulin (A2M)*	Non-specific protease inhibitor; Involved in innate immunity
	Octopamine receptor β-2R†	Adrenergic receptor activity; Neuromodulator and neurotransmitter
	Alpha 2-macroglobulin isoform 2†	Non-specific protease inhibitor; Involved in innate immunity
	Hormone receptor, partial†	Anti-apoptosis and anti-inflammatory roles in innate immunity
16 °C vs. 18 °C	Chitin-binding protein, partial	Structural constituent of cuticle
	Cuticle protein AMP13.4	Structural constituent of cuticle; Antimicrobial peptide; Involved in

Table 2 Continued

		innate immunity
	Spätzle	Spätzle-like protein; Involved in innate immunity
	Crustin 2	Antimicrobial peptide; Involved in innate immunity
	Arrestin	Regulates signal transduction at G-protein coupled receptors
	Glutamate-gated chloride channel, partial	Extracellular ligand-gated ion channel activity; Transmembrane signaling receptor activity
	Juvenile hormone esterase-like carboxylesterase 1	Hydrolase activity
	DNA/RNA non-specific endonuclease	Nucleic acid and metal ion binding; Cleaves phosphodiester bonds within polynucleotide chains
	Hsc70-interacting protein, isoform X2	Chaperone binding; May facilitate interaction of HSC70 with target proteins
	Vrille	DNA-binding transcription factor activity
	Cytochrome P450	Heme and iron binding; Monooxygenase activity; Oxidoreductase activity
18 °C vs. 22 °C	Ubiquitin family protein, putative	Ubiquitination of proteins
	Obstructor F	Chitin binding; Chitin metabolic processes
	Spondin 2-like	May bind to bacteria and act as an opsonin

Table 2 Continued

(B)	16 °C vs. 22 °C	Acyl-CoA Δ-9 desaturase	Fatty acid metabolism; Cell membrane fluidity regulation; Oxidoreductase activity
		Δ-9 desaturase†	Lipid metabolism; Fatty acid biosynthetic process
		L-fucose kinase	ATP binding, kinase activity
		NADH dehydrogenase subunit 2*	Core subunit of mitochondrial membrane respiratory chain NADH dehydrogenase; Electron transport chain
		Sarco-endoplasmic reticulum Ca^{2+}-ATPase pump (SERCA)*	Calcium-transporting ATPase activity; ATP binding; Calcium ion transmembrane transport
		Mitotic checkpoint protein*	Cell cycle arrest protein
		BRCA1-associated RING domain protein 1-like	Protein ubiquitination; Positive regulation of apoptotic process
		DNA primase-like protein	Involved in DNA replication; Synthesizes small RNA primers for Okazaki fragments
		p53 protein	DNA-binding transcription factor activity; Apoptotic processes
		DNA replication licensing factor MCM2†	DNA replication initiation; Negative regulation of DNA helicase activity
		DNA replication licensing factor MCM3†	DNA binding; DNA replication initiation
		NA replication licensing factor MCM5†	DNA replication initiation
		DNA replication licensing factor MCM7†	DNA replication initiation
		FACT complex subunit SPT16†	Heterodimeric protein complex that impacts RNA polymerase II

Table 2 Continued

		transcription elongation
	Selenium independent glutathione peroxidase	Involved in oxidative stress response
	Hemocyanin subunit 2	Metal ion binding; Oxygen carrier activity
	Heat shock protein (Hsp20)	Small heat shock protein (HSP20) family
16 °C vs. 18 °C	Calmodulin	Binds to Ca^{2+}; Regulates enzymes, ion channels, and aquaporins
	Calcium-activated chloride channel regulator 2, partial	Modulates chloride current across plasma membrane in Ca^{2+}-dependent manner
	Calcium/calmodulin-dependent protein kinase type 1 isoform X2	Cell cycle regulation; Calmodulin binding
	Apoptosis-linked gene 2 (ALG-2) interacting protein x	Interacts with ALG-2 to regulate apoptosis
	Ubiquitin family protein, putative	Ubiquitination of proteins
	Ubiquitin carboxyl-terminal hydrolase 14	Proteasome-associated deubiquitinase; Regulation of ubiquitin regeneration
	HSP90A, predicted	Unfolded protein binding; Molecular chaperone
	Heat shock 70 kDa protein	ATP binding; Molecular chaperone - folding and transport of newly synthesized polypeptides, activation of proteolysis
	Alpha 2-macroglobulin (A2M)	Non-specific protease inhibitor; Involved in innate immunity
18 °C vs. 22 °C	Hemocyanin subunit 2	Metal ion binding; Oxygen carrier activity

Table 2 Continued

	Pseudohemocyanin, partial	Oxidoreductase activity; Related to molt cycle
	Ubiquitin-conjugating enzyme E2Q-like	Ubiquitination of proteins; Proteasome
	Clottable protein 2	Lipid transporter activity; Lipid transport
	Calpain-B-like protein, partial	Calcium-dependent cysteine-type endopeptidase activity; Ca^{2+} binding; Proteolysis

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°C as identified by DESeq2 (**C**) and edgeR (**D**). Asterisks (*) indicate protein domains uniquely identified via edgeR analysis.

Fig. 5. Expression levels (log_2 Fold Change; mean + SE) of proteins of interest unique to treatment comparisons: current conditions (16 °C) vs. extreme warming (22 °C) scenario (**A**); current conditions vs. moderate warming (18 °C) scenario (**B**); and moderate vs. extreme warming scenarios (**C**). Positive and negative log_2 FC values indicate over- and under-expressed transcripts, respectively, in postlarvae reared at 16 °C relative to those reared at 22 °C; postlarvae reared at 16 °C relative to those reared at 18 °C; and postlarvae reared at 18 °C relative to those reared at 22 °C. Labels refer to Blast2GO PRO descriptions.

Fig. S1. Similarities of the replicate samples within each treatment temperature, and across temperature treatments. Results of a Principal Component Analysis (PCA) in panel (A): red, green, and blue circles represent postlarvae reared at 22, 18, and 16 °C, respectively. Panel (B) shows a heatmap representation of the Euclidian distance matrix of these samples (B), whereby samples that are most similar are in darker shades of blue and those that are least similar are indicated by lighter shades of blue. In both panels, each of the five replicates is labeled by both temperature and replicate number. For example, "16_Rep1" indicates biological replicate 1 from the 16 °C temperature treatment group.

Fig. S2. Distribution of InterProScan protein domains associated with differentially expressed transcripts of the current conditions (16 °C) vs. moderate (18 °C) warming scenario comparison. Panels depict domains associated with transcripts that were over-expressed (A) and under-expressed (B) in postlarvae reared at 16°C relative to 18°C as identified by DESeq2.

Fig. S3. Distribution of InterProScan protein domains associated with differentially expressed transcripts of the moderate (18 °C) vs. extreme (22 °C) warming scenario comparison. Panels depict domains associated with transcript that were over-expressed (A) and under-expressed (B) in postlarvae reared at 18°C relative to 22°C as identified by DESeq2.

Fig. 1.

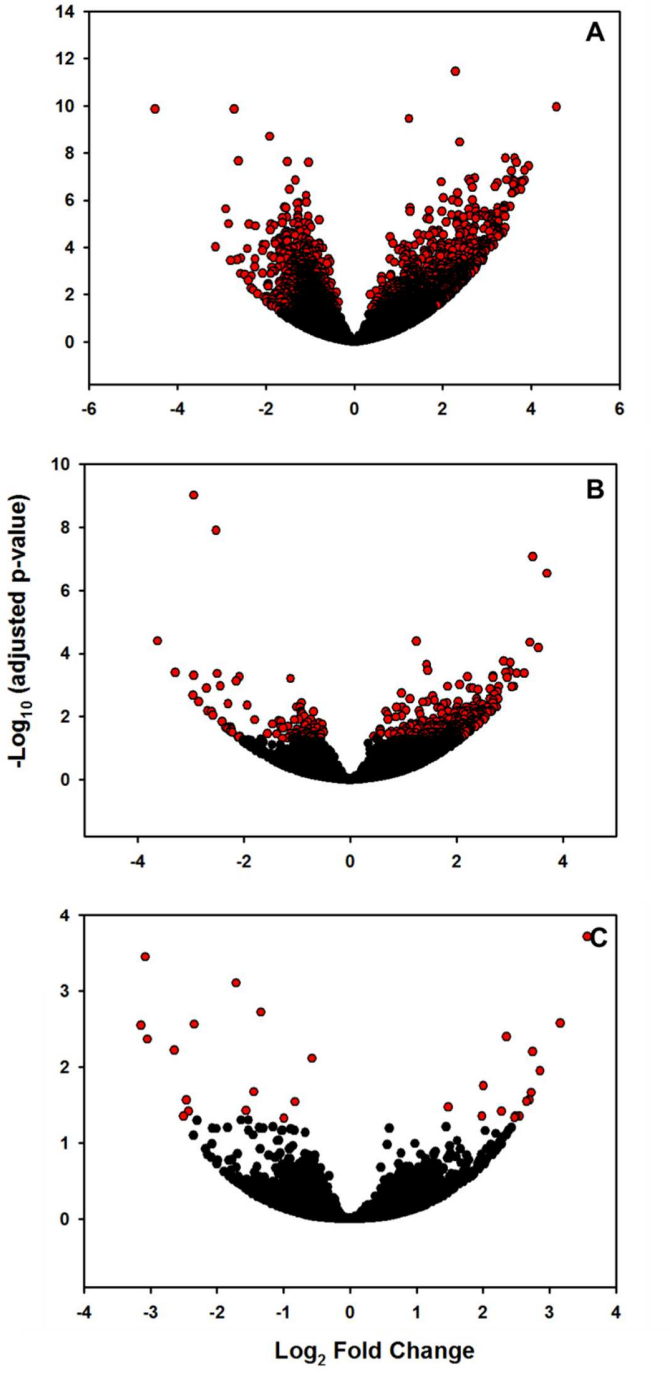
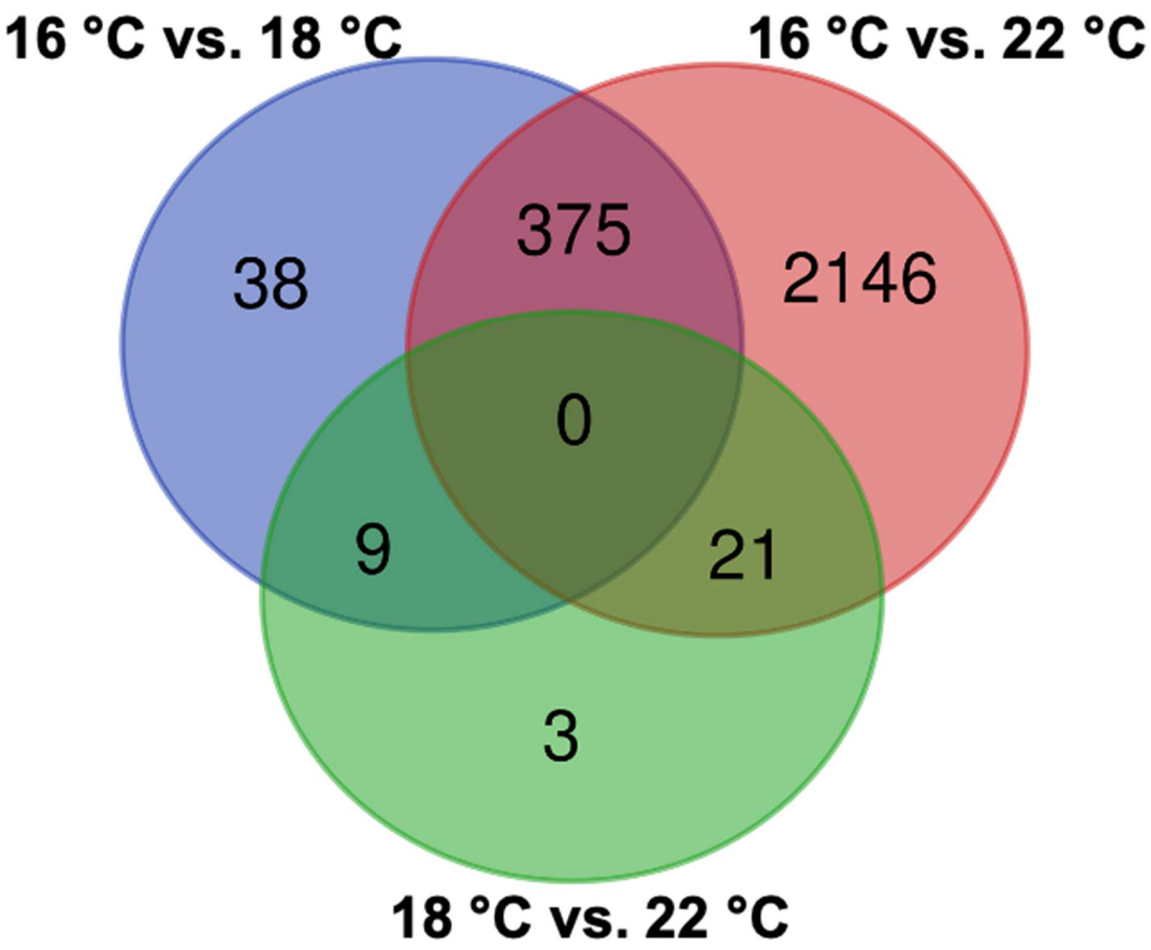


Fig. 2.



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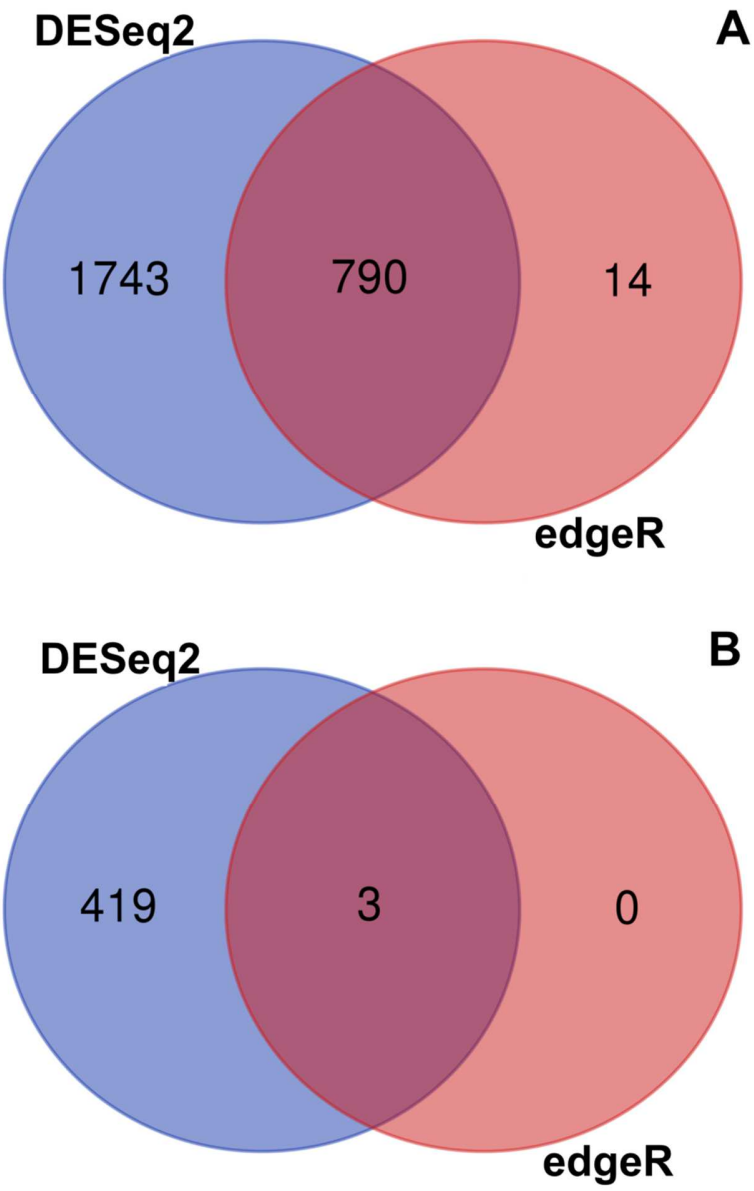

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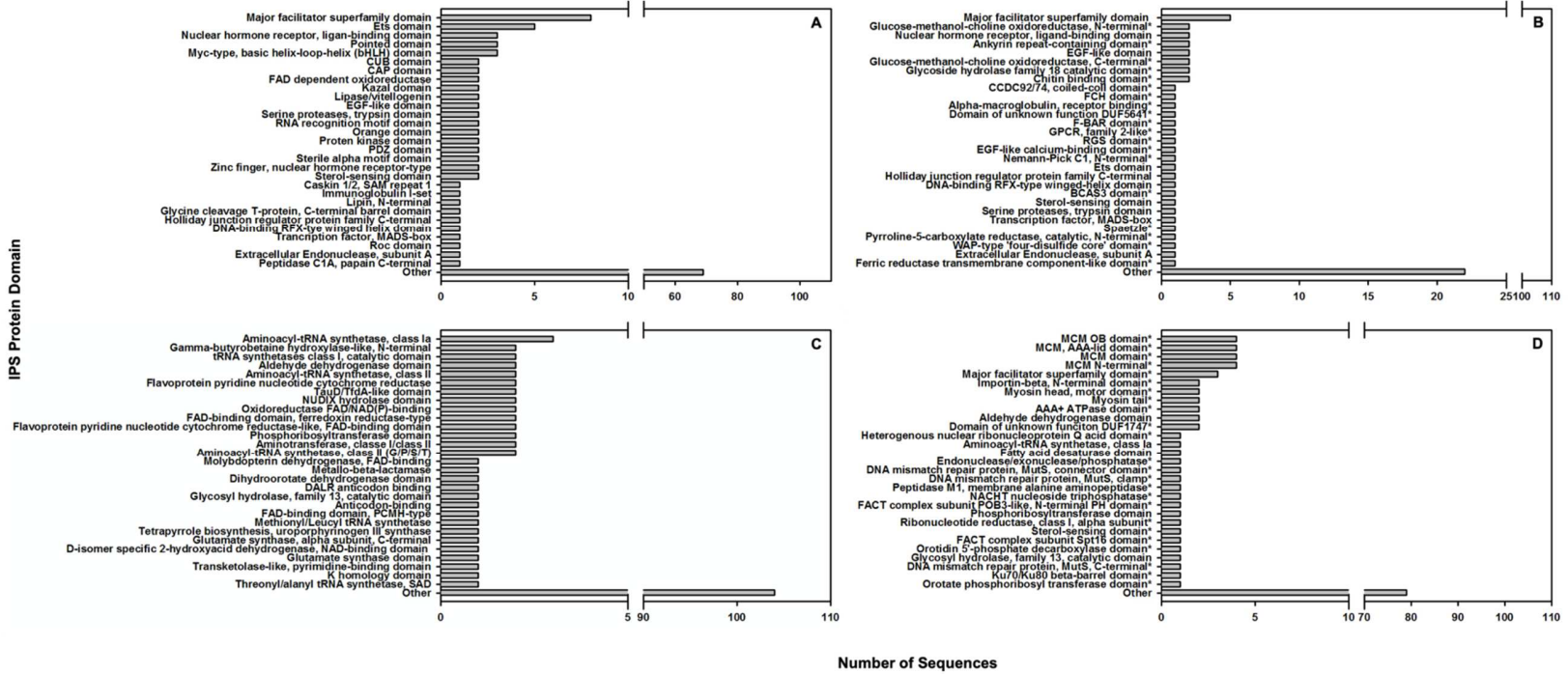
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Ets domain
Nuclear hormone receptor, ligan-binding domain
Pointed domain
Myc-type, basic helix-loop-helix (bHLH) domain
CUB domain
CAP domain
FAD dependent oxidoreductase
Kazal domain
Lipase/vitellogenin
EGF-like domain
Serine proteases, trypsin domain
RNA recognition motif domain
Orange domain
Proten kinase domain
PDZ domain
Sterile alpha motif domain
Zinc finger, nuclear hormone receptor-type
Sterol-sensing domain
Caskin 1/2, SAM repeat 1
Immunoglobulin I-set
Lipin, N-terminal
Glycine cleavage T-protein, C-terminal barrel domain
Holliday junction regulator protein family C-terminal
DNA-binding RFX-tye winged helix domain
Trancription factor, MADS-box
Roc domain
Extracellular Endonuclease, subunit A
Peptidase C1A, papain C-terminal
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100
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Major facilitator superfamily domain
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Nuclear hormone receptor, ligand-binding domain
Ankyrin repeat-containing domain*
EGF-like domain
Glucose-methanol-choline oxidoreductase, C-terminal*
Glycoside hydrolase family 18 catalytic domain*
Chitin binding domain*
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FCH domain*
Alpha-macroglobulin, receptor binding*
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F-BAR domain*
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Aldehyde dehydrogenase domain
Aminoacyl-tRNA synthetase, class II
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TauD/TfdA-like domain
NUDIX hydrolase domain
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FAD-binding domain, ferredoxin reductase-type
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Phosphoribosyltransferase domain
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Aminoacyl-tRNA synthetase, class II (G/P/S/T)
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Metallo-beta-lactamase
Dihydroorotate dehydrogenase domain
DALR anticodon binding
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Anticodon-binding
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MCM N-terminal*
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Myosin tail*
AAA+ ATPase domain*
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DNA mismatch repair protein, MutS, clamp*
Peptidase M1, membrane alanine aminopeptidase*
NACHT nucleoside triphosphatase*
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Phosphoribosyltransferase domain
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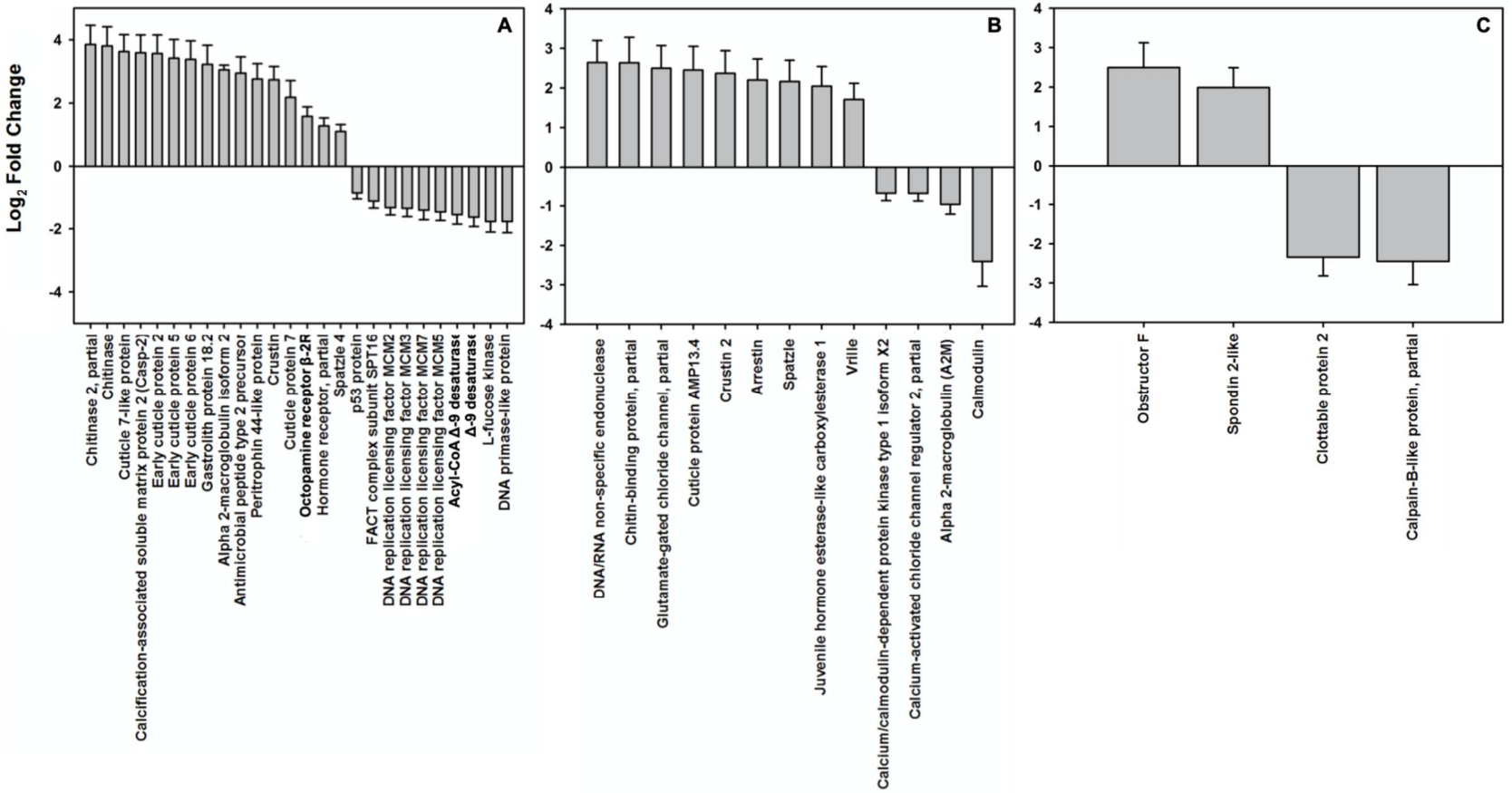
Number of Sequences

Fig. 5.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Early cuticle protein 5
Early cuticle protein 6
Gastrolith protein 18.2
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Hormone receptor, partial
Spatzle 4
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DNA replication licensing factor MCM5
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Δ-9 desaturase
L-fucose kinase
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DNA/RNA non-specific endonuclease
Chitin-binding protein, partial
Glutamate-gated chloride channel, partial
Cuticle protein AMP13.4
Crustin 2
Arrestin
Spatzle
Juvenile hormone esterase-like carboxylesterase 1
Vrille
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Calcium-activated chloride channel regulator 2, partial
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Calmodulin
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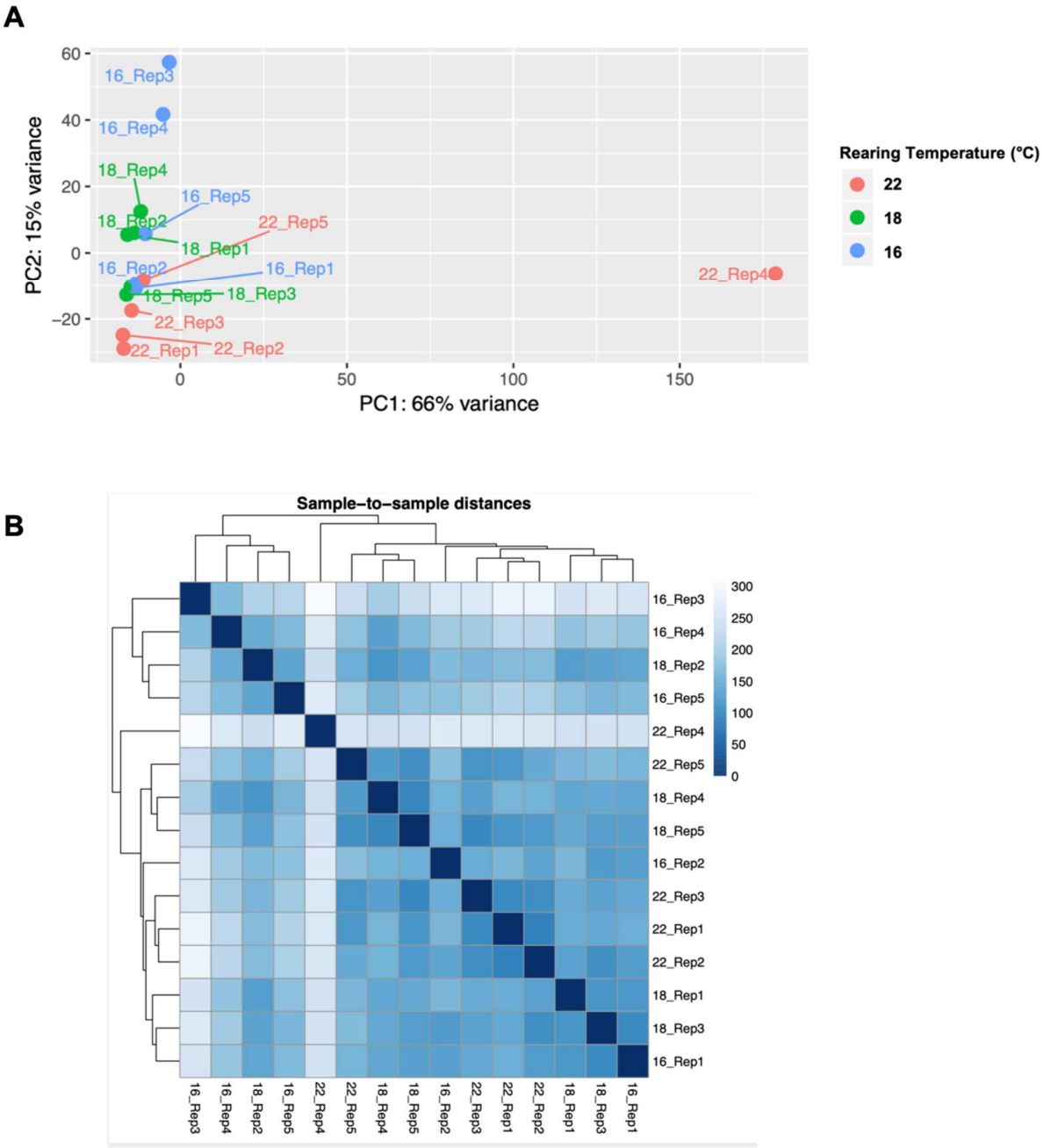
Clottable protein 2
Calpain-B-like protein, partial

Fig. S1.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PC2: 15% variance
PC1: 66% variance
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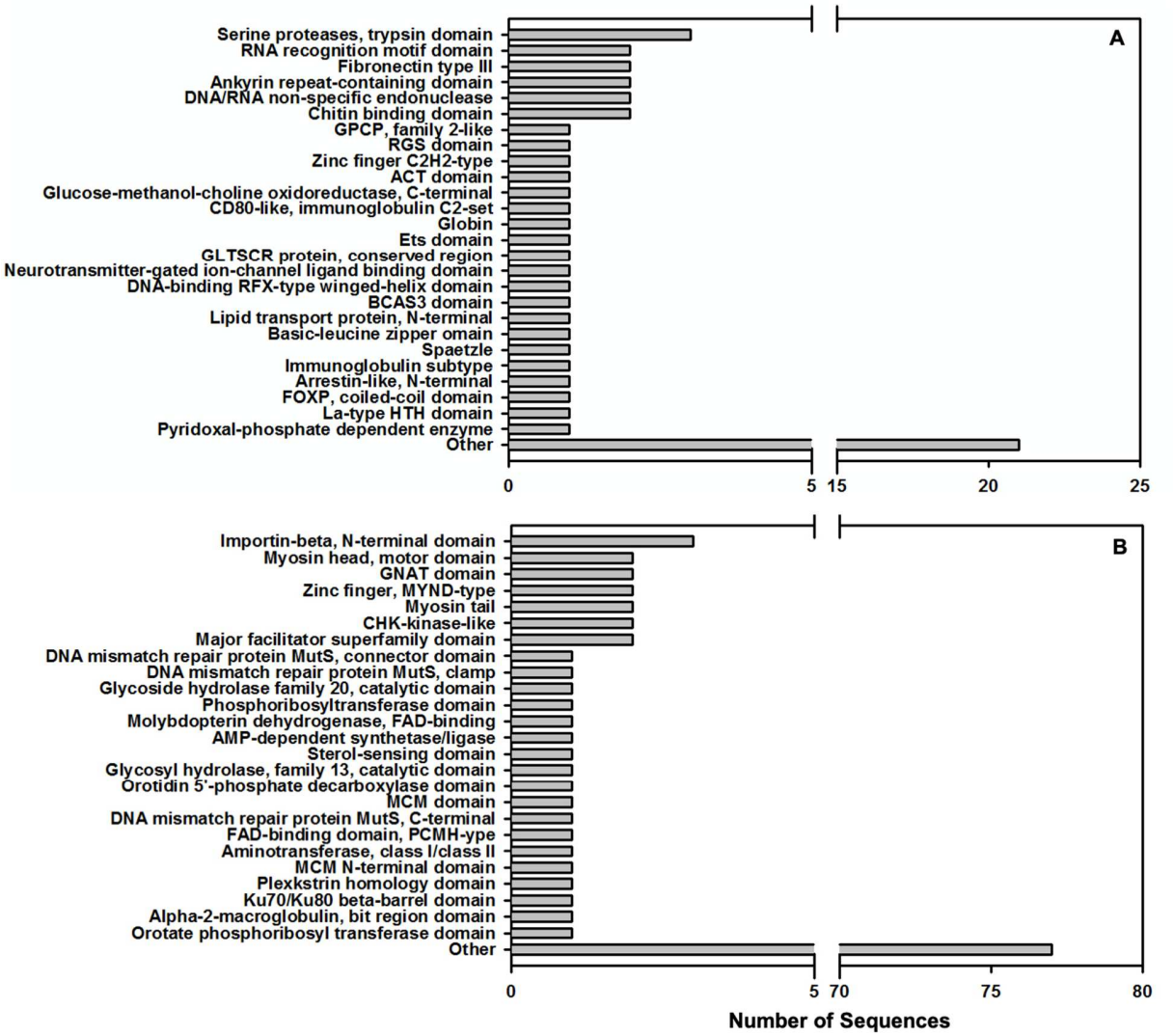
Rearing Temperature (°C)
22
18
16
B
Sample–to–sample distances

Fig. S2.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RGS domain
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ACT domain
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Globin
Ets domain
GLTSCR protein, conserved region
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GNAT domain
Zinc finger, MYND-type
Myosin tail
CHK-kinase-like
Major facilitator superfamily domain
DNA mismatch repair protein MutS, connector domain
DNA mismatch repair protein MutS, clamp
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Orotidin 5'-phosphate decarboxylase domain
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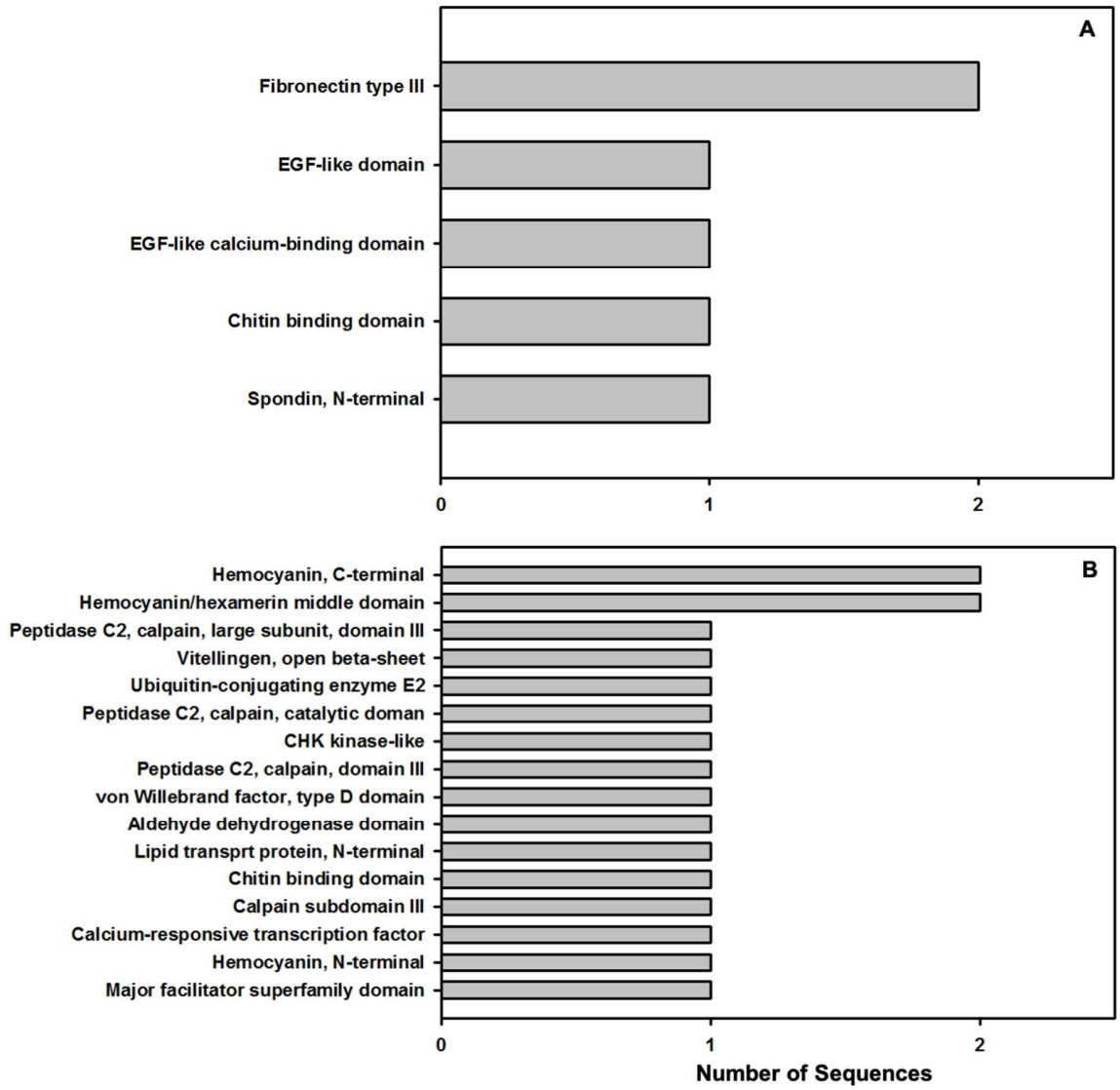
Fig. S3.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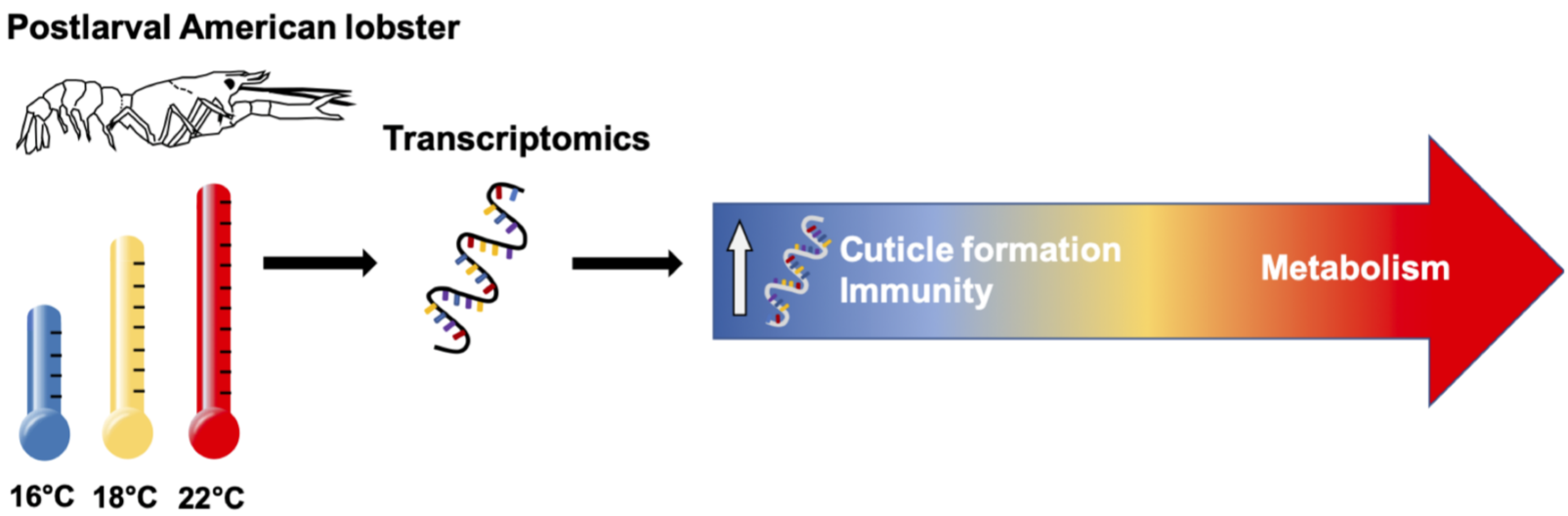
B
Hemocyanin, C-terminal
Hemocyanin/hexamerin middle domain
Peptidase C2, calpain, large subunit, domain III
Vitellingen, open beta-sheet
Ubiquitin-conjugating enzyme E2
Peptidase C2, calpain, catalytic doman
CHK kinase-like
Peptidase C2, calpain, domain III
von Willebrand factor, type D domain
Aldehyde dehydrogenase domain
Lipid transprt protein, N-terminal
Chitin binding domain
Calpain subdomain III
Calcium-responsive transcription factor
Hemocyanin, N-terminal
Major facilitator superfamily domain
0
1
2
Number of Sequences


Postlarval American lobster
Transcriptomics
16°C
18°C
22°C
Cuticle formation
Immunity
Metabolism