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Developmental priorities shift with ontogeny during the early life stages of the American lobster *Homarus americanus* H. Milne Edwards, 1837 (Decapoda: Astacidea: Nephropidae)

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ABSTRACT

The American lobster (*Homarus americanus* Milne Edwards, 1837) is an ecologically and economically valuable invertebrate in the Northwest Atlantic. Its geographic range is shifting northward due to ocean warming. While extensive research on the thermal tolerance of this species has been performed on adults and postlarvae, there have been few studies focused on its multiple early developmental stages. We applied transcriptomics to investigate transcriptional changes in laboratory-reared American lobster developmental stages I through V. Changes in gene expression were contextualized in the ontogenetic shifts in distribution that these different life history stages experience, with highly active stage IV exhibiting increased cellular metabolism and shell-building processes. We identified differential expression of transcripts related to thermal and UV stress in planktonic stages I-IV compared to benthic stage V, which suggests innate molecular defenses against these stressors. Together, these findings further our understanding of crustacean development in the context of climate change and can be used to inform population distribution modeling efforts. They also provide evidence for the need to investigate the potential trade-offs associated with responding to a changing environment on a stage-by-stage basis.

KEY WORDS: climate change, Crustacea, gene expression, larval development, RNAseq, thermal stress

INTRODUCTION

The American lobster (*Homarus americanus*) is an ecologically and economically important species in the Northwest Atlantic, with the fishery being valued at more than $464 million in Maine alone in 2023 (Waller *et al.*, 2023; Department of Marine Resources Landings Data, 2023). Climate change is reshaping its biogeography, with estimates based on fisheries data placing the rate of the range shift of the species at around 40 miles north per decade (Pinsky *et al.*, 2013; Pershing *et al.*, 2015). As such, the thermal tolerance of adult lobsters in the context of warming waters has been explored extensively. The upper limit for cardiac function in adults is between 25 and 29 °C, depending on thermal acclimation (Camacho *et al.*, 2006). Additionally, adult lobsters acclimated to higher temperatures have a lower scope for cardiac function to its extreme limits, thus offering one explanation for decreasing adult lobster stocks in southern New England, where water temperatures can reach 25 °C and continue to climb as the climate warms (Jury & Watson, 2000; Spees *et al.*, 2002; Camacho *et al.*, 2006). Other drivers of range contraction include increasing rates of epizootic shell disease in the south (Groner *et al.*, 2018) and recruitment failure (Wahle *et al.*, 2009).

Early planktonic life stages are liable to experience more immediate impacts from climate change compared to benthic juveniles and adults, as sea surface temperatures are expected to increase at a faster rate than bottom temperatures (Long *et al.*, 2016; Brickman *et al.*, 2021). Their proximity to the surface also makes them more susceptible to other abiotic stressors, such as exposure to ultraviolet (UV) radiation. There is a documented disconnect between the abundance of adult spawning stock biomass and the abundance of juveniles and young of year in certain areas of the range of this species (Oppenheim, 2016; ALSI, 2022). Such a disconnect illustrates the importance of vulnerability in stages I-V and the direct impact that their success has on adult biomass and landings of this species (Carloni *et al.*, 2018). Settlement is positively correlated with sea surface temperature

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anomalies in southern New England and the Gulf of Maine, but not in the Bay of Fundy (Jaini *et al.,* 2018). Other research has shown several factors, including thermal tolerance, altered molting times, and food availability, influence the population dynamics of marine species, especially at the extreme thermal range limits of a species distribution (Mills *et al.,* 2013). One prey species of particular interest is *Calanus finmarchicus* (Gunnerus, 1770), a copepod that is thought to be an important food source as its abundance is linked to the abundance of postlarval lobsters (Carloni *et al.,* 2018). As such, several climate-driven factors are likely impacting the survival and recruitment of early stages of the American lobster, and understanding the nuanced physiological processes prioritized in each of these vulnerable early stages may allow for a more rigorous assessment of climate-driven range shifts in this and other similar species.

Vertical distribution of *H. americanus* varies ontogenetically, with each stage experiencing different thermal regimes throughout the water column. Stage I larvae, after hatching at the bottom, are distributed at deeper depths than any of the subsequent stages and are found as deep as 40 meters (Harding *et al.,* 1987), but approximately 15% of the population can be found at the surface during the day (Annis *et al.,* 2007). Stage II and III larvae have a shallower distribution limited to the upper 20 m of the water column (Harding *et al.,* 1987), but they are also in the upper half meter of the water column very infrequently (e.g., Annis *et al.,* 2007). The first postlarval stage (stage IV) is concentrated in the upper 0.5 m of the water column, spending on average 65% of its time there before settling to molt to the first fully benthic stage V (Annis, 2005). As such, the degree of environmental stress (such as temperature and UV extremes) that each stage experiences is likely varied, with important implications for understanding the disconnect between hatch and recruitment numbers.

Higher seawater temperatures during these early life stages are linked to increased mortality and altered development time in crustaceans (Ford *et al.,* 1979; MacKenzie, 1988; Storch *et al.,* 2009; Waller *et al.,* 2017), thereby directly impacting settlement, recruitment, and ultimately population distribution (Palma *et al.,* 1999: Wahle *et al.,* 2013; Annis *et al.,* 2024; Frederich & Lancaster, 2024). Early studies on these stages in lobster established that the first signs of sub-lethal stress in laboratory-reared developmental stages I, III, and IV occur from acute exposures to 26–33.8 °C seawater (Huntsman, 1924), and chronic exposure to temperatures of 23 °C and above resulted in reduced larval performance and growth (Ford *et al.,* 1979). More contemporary studies have corroborated these findings (Quinn *et al.,* 2013) and furthered our understanding of the joint effects of elevated temperature and CO_2 on larvae in the context of climate change, finding complex interactions between both variables with elevated temperature reducing larval viability (Waller *et al.,* 2017). Ontogenetic shifts in thermal tolerance of lab-reared lobsters based on chronic exposures to 8, 18, and 26 °C, demonstrate that stage I lobsters experience reduced survivorship in response to chronic heat stress, whereas stages II-IV experience reduced survivorship in response to cold stress (Annis *et al.,* 2022). Furthermore, shifts in upper and lower critical temperatures at stage II, IV, and V have been identified in acute respirometry trials (Annis *et al.,* 2022).

The effects of UV exposure on crustaceans include changes in color and induction of DNA damage (Gouvela *et al.,* 2005). UV-a indirectly damages DNA by facilitating the production of hydroxyl radicals which lead to strand breakage and the formation of DNA cross-links, while UV-b directly damages DNA by interfering with nucleotide bonding. The impact of UV radiation on planktonic larvae has been investigated in many non-crustacean species as well. For example, the northern anchovy *Engraulis mordax* Girard, 1854 exhibits diel cycles of UV damage and repair that prevent accumulation of derivatives of UV-b damage, with photoenzymes responsible for reparation processes being innately expressed in individuals reared in the dark (Vetter *et al.,* 1999). The effects of UV radiation in *H. americanus* specifically have been investigated to a lesser extent. Tlusty *et al.* (2009) found that stage V laboratory-reared lobsters developed darker shells, or exoskeletons, in the presence of UV light. Another study found that stage I lobsters did not experience significantly higher mortality in a laboratory setting as a result of short-term exposures to UV radiation (Rodriguez *et al.,* 2000). Irradiated larvae were immediately exposed to photo-reactivating light which could serve as a counter to UV damage, and if necessitated in the wild could influence larval behavior and thus exposure to higher temperatures (Rodriguez *et al.,* 2000).

The vulnerability to each of these stressors varies with ontogeny and subsequent distribution as do the primary processes that could be impacted and thus the energetic trade-offs associated with each life stage. Expression of immune-related genes increases through development from stage I to stage IV based on an oligonucleotide microarray study of *H. americanus* (Hines *et al.,* 2014). It was found that genes related to cuticle formation and muscle development were differentially expressed from stage III to stage IV (Hines *et al.,* 2014). More recent approaches to transcriptome profiling, namely RNA-seq, further our ability to probe nuances in the molecular biology of larval crustaceans (Wang *et al.,* 2009). Through an RNA-seq approach, stage-specific changes in transcription have been documented in a related decapod crustacean, Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931). Wei *et al.* (2014) outlined key points in cuticle and muscle development related to spatial distribution for each life stage. The transition from nauplius to zoea stage in *L. vannamei* is marked by an upregulation of digestive enzymes and metabolism, whereas the transition into the mysis stage is marked by an upregulation of genes related to muscle development. Metamorphosis into the postlarval stage of *L. vannamei* is marked by an upregulation of chitin protein, potentially preparing the animal for life at deeper depths (Wei *et al.,* 2014).

The RNA-seq approach has been used in several gene expression studies of *H. americanus* framed in the context of climate change, but they only focus on stage IV postlarvae (Harrington *et al.,* 2020; Lopez-Anido *et al.,* 2021; Niemisto *et al.,* 2021). For example, exposure of stage IV to projected future warming scenarios may compromise their ability to reallocate energy to important physiological functions like preventing and repairing cellular damage and cuticle formation (Harrington *et al.,* 2020; Lopez-Anido *et al.,* 2021). Regulation of genes related to shell-building processes and immune function has also been found to be altered under scenarios of warming and elevated CO_2 in lobster postlarvae (Niemisto *et al.,* 2021). Characterization of transcriptional changes on a stage-by-stage basis using an RNA-seq approach, especially in the context of resilience to environmental stressors, is useful for informing the disconnect

between spawning stock biomass and recruitment numbers. It has also been recommended to make climate-driven fisheries models more robust (Mills *et al.,* 2013).

To identify the most significant innate molecular processes that could be subject to energetic trade-offs throughout development, we utilized laboratory-reared, unstressed larval stages I-III, post-larval stage IV, and early benthic phase stage V lobsters and applied transcriptomics to identify cellular pathways and their changes throughout the larval development. We also surveyed our data for genes related to thermal and UV stress to provide DNA sequences of the molecular markers for these stressors with the intent to facilitate further research into the early stages of *H. americanus* in the context of climate change. To the best of our knowledge, our study is the first to use RNA-seq to investigate changes in gene transcription in *H. americanus* on a stage-by-stage basis as they relate to climate-driven environmental stressors.

METHODS

Animal husbandry

Eight ovigerous female lobsters were caught near Boothbay Harbor in the Gulf of Maine and were held under the State of Maine Department of Marine Resources Special License #2022-19-04 at the Bigelow Laboratory for Ocean Sciences, East Boothbay, ME. These adults were held individually in a flow-through tank fed by water from the Damariscotta River estuary (mean temperature 14.4 °C; salinity 30–32 ppt). Hatchlings were isolated within 24 h in individual 400 ml glass jars filled with 0.45 μm filtered seawater. Jarred individuals were acclimated to the documented optimal rearing temperature of 18 ^{0}C (Quinn, 2017). Approximately 90% of the water in each jar was changed three times per week. Water was not aerated, but regular water changes yielded mean dissolved oxygen levels of 7.2 mg l^{-1}. Larvae were exposed to light approximately 3 h per day during molt checks and water changes. Larvae were fed freshly hatched *Artemia* ad libitum.

Sample preservation

Lobsters across stages I to V (N = 5 per stage) were sampled 48–60 h after each molt, with time to molt from hatch averaging 6 ± 2, 11 ± 6, 20 ± 4, and 47 ± 8 d for stages II, III, IV, and V, respectively. Sampled lobsters were rinsed with milli-Q water before preservation in DNA-RNA-free microcentrifuge tubes containing 600 μl of RNAlater solution (Ambion, Austin, TX, USA). We chose to investigate whole-body gene expression rather than isolating individual organs. While this introduced certain limitations, i.e., different ratios of organs from stage to stage, it also reduced the number of samples to be analyzed, and organ-specific analyses would have been challenging, especially in the very early and smaller life stages. Samples were held at 8 °C for 12 h to allow the preservative to permeate the tissue before being transferred to −80 °C for storage until analysis. Samples were shipped on dry ice to Novogene (Sacramento, CA, USA) for extraction, sequencing, and analysis.

RNA extraction and library preparation

RNA extraction was performed according to Novogene's internal protocols, which include an automated homogenization method followed by a filtration column. Sample integrity and quantity were determined using an Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) and sample purity was checked using a Nanodrop spectrophotometer (ThermoFisher, Wilmington, DE, USA). Libraries were prepared using poly-A enrichment to select for mRNA following Novogene's internal quality control assays. Sequencing was done using the Illumina NovaSeq platform (Parkhomchuk *et al.,* 2009). Samples were sequenced as paired-end reads of 150 base pairs, and those reads in the fastq format were processed for downstream analyses or "cleaned" by removing reads containing adaptors, reads with > 10% uncertain nucleotides, and reads with >50% low quality nucleotides, defined as base quality < 5. Reads were aligned to a reference sequence for American lobster (Polinski *et al.,* 2021) using HISAT2 (version 2.0.5; Mortazavi *et al.,* 2008) and assembled into transcripts using StringTie (version 1.3.3b; Pertea *et al.,* 2015). Raw counts of map reads were aggregated using featureCounts (version 1.5.0-p3; Liao *et al.,* 2014). Raw data have been deposited in GenBank Sequence Read Archive, SRA, accession number PRJNA1087720.

Differential expression analysis

Raw read counts were normalized to correct for sequencing depth before differential transcript expression was analyzed using DESeq2 software (Anders & Huber, 2010). DESeq2 uses the negative binomial as the reference distribution and takes a geometric normalization approach. False discovery rate (FDR) was controlled for by adjusting p-values for multiple testing with the Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995), and the differential transcript screening threshold was $|\log2(\text{FoldChange})| \geq 1$ and padj ≤ 0.05. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using clusterProfiler (version 3.8.1) (Ashburner *et al.,* 2000). The KEGG pathway enrichment analysis was restricted solely to *H. americanus* (hame).

RESULTS

Transcriptome quality and mapping

Samples showed consistent read quality and depth. Across stages, 299,072,812 raw reads were generated, of which, an average of 97.5% were clean reads and 92.1% were able to be aligned with the reference genome (Table 1). Mapping gene IDs to the GO database identified 2,921 unique GO terms represented in our transcriptome; mapping gene IDs to the KEGG database identified 133 unique KEGG pathways represented in our transcriptome.

Differential transcript expression across stages

There were 11,653 transcripts commonly expressed among all the first five developmental stages. Stage I exhibited 899 uniquely expressed transcripts, stage II 311, stage III 59, and stages IV and V 157 and 901, respectively (Fig.1). Molt to stage II was marked by the upregulation of 384 transcripts and the highest degree of downregulation at 1,347 transcripts. Molt to stage III was marked by the upregulation of 95 transcripts and the downregulation of 190 transcripts. Stages II and III showed the most similar patterns of expression. Molt to stage IV was characterized by the upregulation of 1,443 transcripts and the downregulation

Table 1. Read quality parameters for each developmental stage in the American lobster, *Homarus americanus* ($N = 5$ per stage).

Stage	Mean Raw Reads	Mean percentage of clean reads	Mean percentage of reads aligned to genome
I	66,489,408	97.5	92.4
II	69,914,350	98.1	92.8
III	61,520,640	97.7	92.2
IV	56,246,590	97.0	92.6
V	44,901,824	97.0	90.3
Overall	299,072,812	97.5	92.1

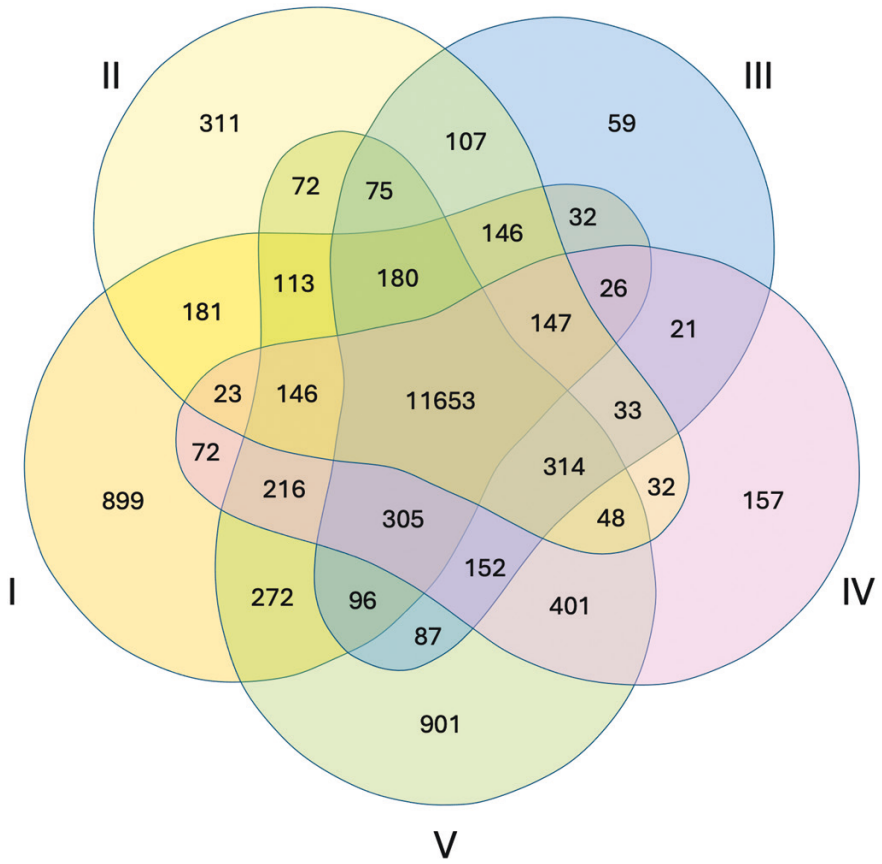

Figure 1. Venn diagram showing the number of uniquely expressed genes in each developmental stage of *Homarus americanus*. Stage I is represented in orange at left, with representation of subsequent stages (stage II in yellow, III in blue, IV in pink, and V in green) proceeding clockwise. Commonly expressed transcripts among stages are represented in overlapping sections.

of 590 transcripts. Molt to stage V was marked by the highest degree of upregulation, at 2,352 transcripts, and the downregulation of 841 transcripts. Stages IV and V had the least similar patterns of expression (Fig. 2).

GO term and KEGG pathway enrichment analysis

Stage I vs. II. After molt to stage II, 3 GO terms and 2 KEGG pathways were significantly enriched. Within these, transcripts related to 1) extracellular processes, 2) structural constituents of the cuticle, and 3) chitin binding, were largely upregulated in stage II larvae. The pathway for non-homologous end-joining was also upregulated. Transcripts related to amino and nucleotide sugar metabolism were largely downregulated in stage II.

Stage II vs. III. After molt to stage III, 11 GO terms were significantly enriched. The top five most significant, in order, included (1) proteolysis, (2) sulfotransferase activity, and (3) transferase activity transferring sulfur-containing groups, in which the majority of transcripts were downregulated, and (4) structural constituent of cuticle, in which differentially expressed transcripts were largely upregulated. Five (5) serine-type peptidase activity was also significantly enriched, and there was an equal level of up- and downregulation in the differentially expressed transcripts annotated to this term. No KEGG pathways were significantly enriched in this comparison.

Stage III vs. IV. After molt to stage IV, 23 GO terms and 9 KEGG pathways were significantly enriched. The top five GO terms were (1) carbohydrate metabolic process, (2) proteolysis, (3) extracellular region, and (4) structural constituent of cuticle, in which the majority of differentially expressed transcripts were upregulated. Additionally, transcripts annotated to the term (5)

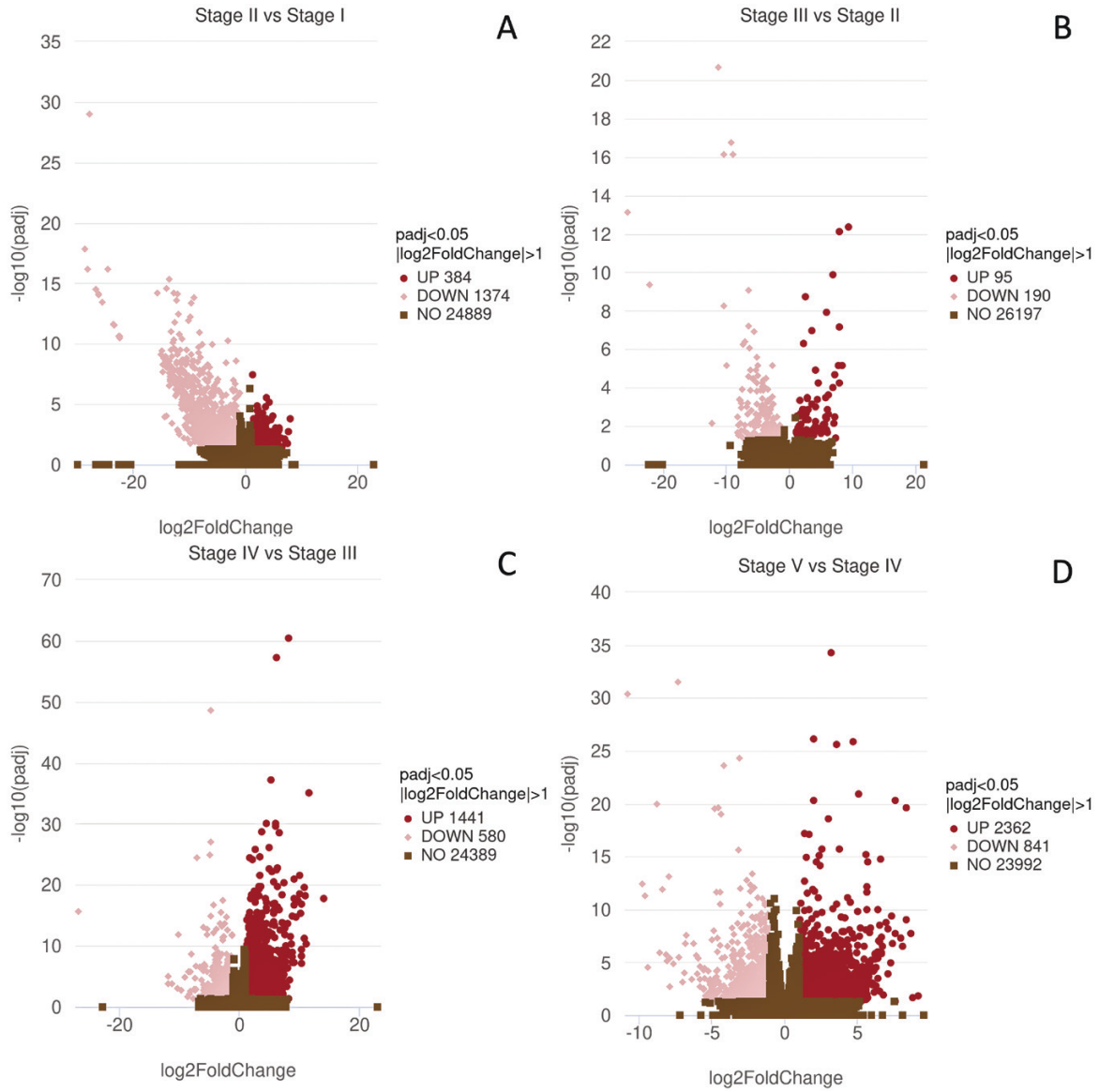

Figure 2. Volcano plots show differential gene expression in *Homarus americanus* between stages I and II (**A**), stages II and III (**B**), stage III and IV (**C**), and stages IV and V (**D**) after each respective molt. The brown squares represent genes that did not meet the threshold for differential gene expression. Red dots represent up-regulated transcripts and light pink diamonds represent down-regulated transcripts. Note that axes are scaled differently from panel to panel.

response to oxidative stress were markedly downregulated. The majority of differentially expressed transcripts within each of the nine significantly enriched KEGG pathways were upregulated; these included amino sugar and nucleotide sugar metabolism, glycosaminoglycan biosynthesis - chondroitin sulfate/dermatan sulfate, and more.

Stage IV vs. V. The transition to the first benthic stage yielded significant enrichment of 22 GO terms and 15 KEGG pathways. Transcripts were largely upregulated within GO terms for (1) carbohydrate metabolic process, (2) oxidation-reduction process, (3) proteolysis, (4) hydrolase activity, and (5) hydrolyzing O-glycosyl compounds. The majority of transcripts within the significantly enriched pathways were also upregulated, including those related to lysosome, retinol metabolism, and amino sugar and nucleotide sugar metabolism. A detailed list of significantly enriched GO terms and KEGG pathways for all larval stages is available in Supplementary material Tables S1 and S2.

Genes involved in response to environmental stressors

We analyzed GO:0031072, heat shock binding proteins, to investigate respective changes to relevant gene expression in the context of a warming ocean and the different thermal conditions within the water column. Four out of five transcripts annotated to this term were differentially expressed in at least one subsequent stage comparison. None of the transcripts within this GO term showed a significant difference in expression between stage I and II or II and III (see Supplementary material Table S3 for stage-specific FPKM values; Supplementary material Table S4 for sequences). Expression of one DnaJ homolog, member 1-like gene (LOC121867731), was greater in stage IV postlarvae relative to stage III and significantly ($P < 0.05$) lower in stage

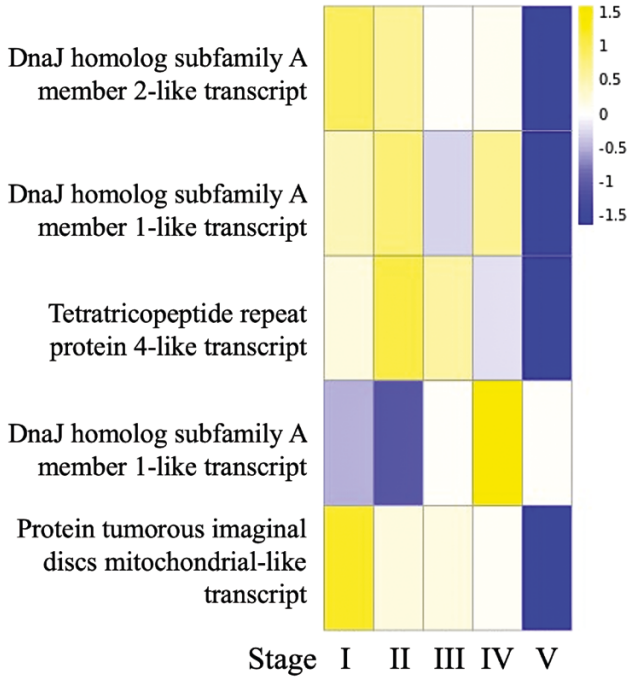

Figure 3. Heatmap showing expression of transcripts in GO:0031072 related to heat stress. Gene descriptions are listed at left with stages I through V represented by the columns left to right. Coloration represents the relative FPKM to the prior stage.

V relative to stage IV. Expression of two other DnaJ homologs (LOC121875628 and LOC121858298) as well as a tetratricopeptide repeat protein 4-like transcript variant was significantly ($P < 0.05$) decreased in stage V relative to stage IV (Fig. 3).

DISCUSSION

We identified stage-specific whole body gene expression patterns in lobster larvae with transcripts related to cuticle formation most upregulated in stages I, IV, and V, and transcripts related to energy metabolism and growth most upregulated in stages IV and V, respectively. These changes in expression are easily contextualized in the known ontogenetic shifts in distribution and ecology these larvae experience. We also identified differential expression of genes related to thermal and UV stress, which, found in laboratory-reared, un-stressed lobsters suggest innate molecular defenses against these stressors. Of particular interest is DnaJ, which has been successfully used as a marker for drought resistance in maize (Dong *et al.*, 2022) and should be investigated further as a marker for thermal stress resistance in crustaceans. While thermal stress is inherently tied to the warming of the world's oceans, reduced cloud cover leading to unusually high UV indices and subsequent increased risk of UV stress for organisms around the world has also been attributed to climate change (Bernhard *et al.*, 2020). Ultimately, our study highlights that each developmental stage of *H. americanus* exhibits innate molecular differences and will respond to the varied climate-driven stressors that result from stage-specific distribution differently (Fig. 4).

After molt to stage II, transcripts related to shell-building processes were largely downregulated compared with stage I. This suggests that priorities shift from shell-building in stage I, when there is limited time to develop an exoskeleton while the organism is most vulnerable to predation, to other processes such as growth in stage II. Stage II is distinguished from stage I by the presence of pleopods and is also the stage at which uropods are developing internally. Further, differentially expressed transcripts within the KEGG pathway "amino sugar and nucleotide sugar metabolism" were largely downregulated after molt to stage two. In the crayfish *Procambarus clarkii* (Girard, 1852), downregulation of amino acid and nucleotide metabolism has been shown to result from the silencing of crustacean hyperglycemic hormone (CHH) (Li *et al.*, 2019), which is responsible for regulating metabolism, osmoregulation, molting, and reproduction (Fanjul-Moles, 2006; Webster *et al.*, 2012). It is thus possible that this pattern can be attributed to lowered CHH as a result of the point in the molt cycle these lobsters are in 48–60 h after molt to stage II. This pathway has also been implicated in chitin synthesis, and its varied expression may also signify varied shell-building processes at different points in the molt cycle (Bulik *et al.*, 2003).

We sampled each stage 48–60 h post-molt to ensure as much development time in each stage as possible while keeping sampling time points consistent. Due to variation in the duration of each molt cycle from stage to stage, however, the lobsters we sampled were likely at different points in their respective molt cycles (Sasaki, 1984). The four stages of the molt cycle include anecdysis, in which the exoskeleton is calcified and tissue growth occurs; proecdysis, in which the claw muscle atrophies and any limb regeneration occurs, the epidermis is separated from the exoskeleton, and the lobster ingests water, swelling its tissues to prepare for molt; ecdysis, in which the exoskeleton is actually shed; and metecdysis, in which continued ingestion of water enlarges the new exoskeleton, endocuticle synthesis begins, and calcification of the exoskeleton is underway (Mykles, 2024). As such, certain changes in gene expression, especially those related to shell-building processes, may be attributable to these respective points in the molt cycle. The expression of key genes throughout the molting process has been characterized in other crustaceans (Benrabaa *et al.*, 2023), none of which were the focus of our study. Future studies should investigate the transcriptomic changes within the molt stages of each instar for *H. americanus*.

We found the fewest number of transcriptional changes between stages II and III, which corresponds with the similar, difficult to capture, distribution of these two stages in the water column (Harding *et al.*, 1987), as well as similar morphology and physiology (Annis *et al.*, 2022). In a separate study, our research group employed respirometry and found stages II and III both reach zero scope for activity after acute exposure to 8–10 °C and 32 °C, and both experienced reduced survivorship after chronic exposure to 8 °C compared with stage I (Annis *et al.*, 2022; unpublished data). This is of interest because many previous studies have targeted only one or two early life stages. As gaps in the knowledge of stage-specific biology for this species are addressed, it is useful to be aware of the similarity in the molecular biology of these two stages, as they may be grouped together with less consequence for our understanding than grouping stages I-IV or I-III together, as is often done.

After molt to stage III, transcripts related to proteolysis and transferase activity were upregulated, which may be a result of

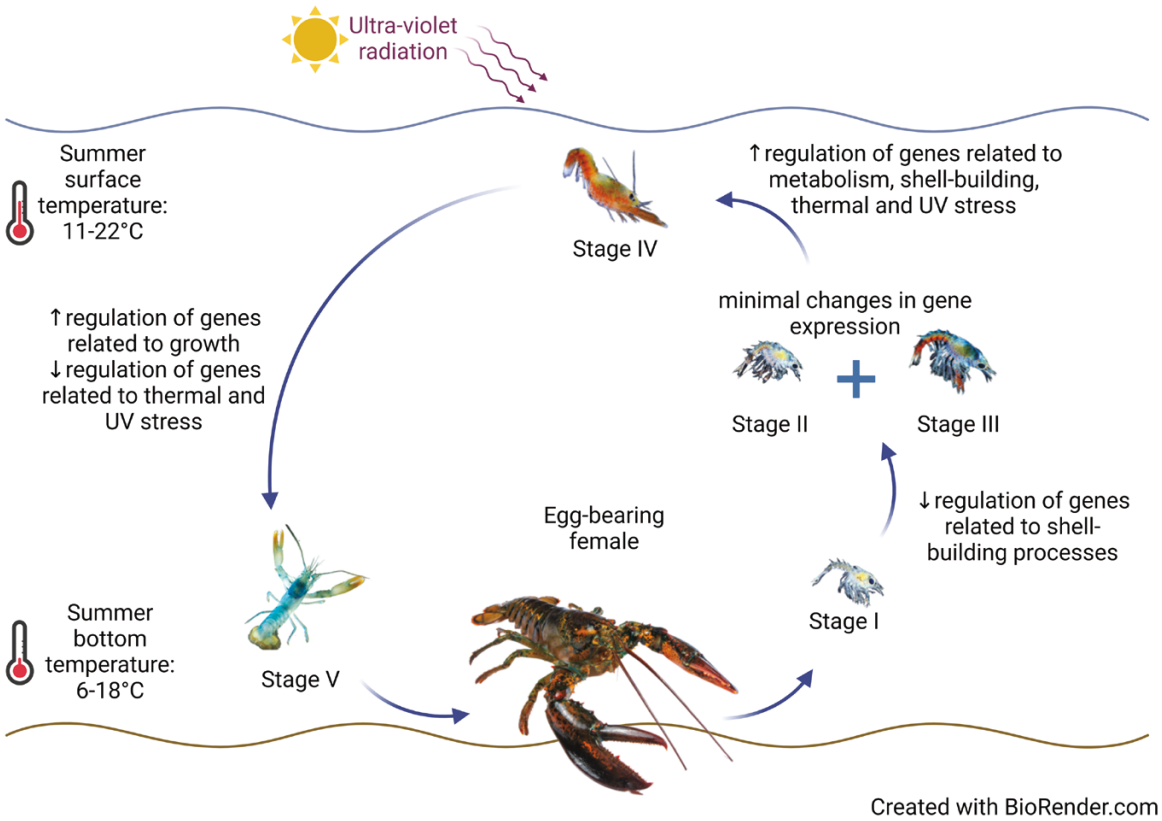

Figure 4. Conceptual illustration of shifts in gene expression with development in American lobster stages I through V. Stage I larvae hatch off at the bottom. Stage II and III are similarly distributed throughout the mid water column. Stage IV post larvae are the most abundant of the stages in the upper water column and are more exposed to higher temperature and UV radiation than other stages. Stage V post-larvae settle at the benthos again. Changes in gene expression in GO terms and KEGG pathways are matched to the ontogenetic changes as noted.

the recycling and reorganizing of proteins in preparation for molt to the first post-larval stage, while the upregulation of transcripts related to structural constituents of the cuticle is likely for similar reasons. As such, it is likely that shell-building processes are the most likely to be subject to energetic trade-offs when environmental stressors are present. Alternatively, it is possible that the increased expression of transcripts related to proteolysis and transferase activity can be attributed to the hepatopancreas being larger in stage IV compared to stage III, and thus making up a greater proportion of our RNA sample. Further study is needed to better understand the internal anatomy of each larval stage. No significantly enriched KEGG pathways between stages II and III were observed, providing more evidence that these two stages are relatively comparable in terms of their molecular biology.

After metamorphosis into postlarvae, GO terms for metabolic function and shell-building were significantly enriched, similar to gene expression patterns found in a previous study of *H. americanus* using microarray technology (Hines *et al.,* 2014). Specifically, transcripts related to the carbohydrate metabolic process, proteolysis, and structural constituent of cuticle were largely upregulated. The carbohydrate metabolic process was also identified as a significantly enriched GO term in an analysis of the transition to the post-larval stage in *Litopenaeus vannamei* and could be related to the increased energy demands of the most active planktonic stages of these crustaceans (Wei *et al.,* 2014). The same study also found significant enrichment of GO terms related to shell building (i.e., "structural constituent of cuticle" identified in our samples) and likened this to preparation for benthic life. In our samples, differentially expressed transcripts within the KEGG pathway for amino sugar and nucleotide sugar metabolism were largely upregulated in stage IV, rising again after downregulation in stage II. This result may also be due to increased energy demands in this highly active stage, which spends most of its time in the turbulent surface waters or sounding for the seafloor (Annis, 2005), or the increased production of chitin (Bulik *et al.,* 2003). Further, altered exoskeleton quality in stage IV lobsters results in greater negative buoyancy and thus can be linked to higher energy demands, with stage IV larvae having to swim continuously to maintain their vertical position in the water column (Capuzzo & Lancaster, 1979; Sasaki, 1984).

The molt to the first fully benthic stage (stage V) yielded the greatest differential gene expression, with a focus on metabolism and growth. Midway through stage IV, larvae start sounding for the seafloor, beginning their transition to the benthos (Cobb *et al.,* 1989). Molt to the first benthic stage was marked by an upregulation of transcripts related to carbohydrate metabolism and amino sugar and nucleotide sugar metabolism, signaling a further increase in energy demands and chitin-building as growth processes are prioritized. Differentially expressed transcripts within the GO term "hydrolase activity, hydrolyzing O-glycosyl compounds" were also upregulated, along with genes within KEGG pathways for "glycosaminoglycan biosynthesis" (i.e., biosynthesis of a glycoconjugate known to regulate cell growth and

proliferation (Casale & Crane, 2023)) and "glycosphingolipid biosynthesis" (i.e., biosynthesis of a glycoconjugate known to be involved in cell-to-cell recognition and signal transduction (Schnarr *et al.*, 2022)). Additionally, transcripts annotated to the GO term for "oxidation-reduction process" were largely upregulated, which may also be the result of the prioritization of growth processes in this stage. Lipid catabolism and mitochondrial fatty acid β-oxidation are both processes that are essential in driving growth and molting in crustaceans and are both associated with the production of reactive oxygen species (ROS) and thus oxidative stress (Wang *et al.*, 2014). Another driving factor behind the production of ROS in crustaceans is acclimation to colder temperatures (Meng *et al.*, 2014). Given that this is the first benthic stage and thus is the first stage to acclimate to colder bottom temperatures (Annis *et al.*, 2022), the upregulation of genes related to oxidation-reduction processes may be another innate defense mechanism against environmental stress.

Our analysis of GO:0031072 revealed differential expression patterns of several genes related to thermal and UV tolerance in stages IV and V, despite the fact that our lobsters were laboratory-reared and never exposed to these stressors. Of particular interest was the increased expression of one DnaJ homolog in stage IV, compared to all the other stages, and the decreased expression of other DnaJ homologs in stage V. DnaJ proteins are molecular co-chaperones that recruit other chaperones in the heat shock family to perform functions such as protein folding and protein transport (UniProt, 2023). Of these, member 1-like homologs function as a co-chaperone for heat shock proteins and promote apoptosis in response to cellular stress in response to UV (UniProt, 2023). Member 2-like homologs are also co-chaperones of heat shock proteins, facilitating the folding of unfolded proteins (UniProt, 2023). Specifically in crustaceans, DnaJ has been implicated in responses to heat shock (Chen *et al.*, 2018), infection (Jaree & Somboonwiwat, 2023), pollutant exposure (Zhu *et al.*, 2018), and low pH (Chen *et al.*, 2018). DnaJ has also been implicated in ovarian development in shrimps (Chen *et al.*, 2018), but the expression pattern that we observed does not line up with the pattern described in that study nor what is known about the timing of ovarian development in American lobster, which occurs much later than in the stages we saw elevated expression in (Aiken & Waddy, 1982). DnaJ homologs are relatively conserved and also have an application for use as an effective index for evaluating drought resistance in maize cultivars (Dong *et al.*, 2022). In our samples, the member 1-like homolog is elevated in stage IV, the stage which spends approximately 65% of its time at the surface and is most likely to experience UV stress in the wild (Annis, 2005). All DnaJ homologs are downregulated in stage V, the only benthic stage tested which is less likely to experience heat and UV stress in the wild. Given what is known about the function of DnaJ and the conditions in which our lobsters were reared, we believe this molecular marker has potential as a marker for heat resistance in crustaceans and warrants further investigation.

CONCLUSION

While many studies have investigated temperature tolerance in adult *H. americanus*, and some that have targeted stage IV postlarvae, our data show that there are varied processes within each larval stage that may be subject to energetic trade-offs. We identified potentially innate responses to stressors related to climate change, suggesting stage-specific resilience. Further investigation of these stage-specific trade-offs and levels of resiliency using the molecular markers outlined in this study will elucidate potential environmentally driven bottlenecks in the pre-settlement of *H. americanus*. As such, our findings should facilitate future studies that enhance predictive models for this financially, ecologically, and culturally important species.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. List of significantly enriched GO terms in stage-wise comparison of *Homarus americanus.*

S2 Table. List of significantly enriched KEGG pathways in stage-wise comparison of *Homarus americanus.*

S3 Table. FPKM values of transcripts in GO:0031072 from *Homarus americanus* larvae and postlarvae stages I-V.

S4 Table. Transcript sequences of *Homarus americanus* from transcripts discussed in GO:0031072.

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