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Transcriptomic and Histological Analysis of the Greentail Prawn (*Metapenaeus bennettae*) Following Light Crude Oil Exposure

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Abstract: Oil spills pose a significant threat to marine biodiversity. Crude oil can partition into sediments where it may be persistent, placing benthic species such as decapods at particular risk of exposure. Transcriptomic and histological tools are often used to investigate the effects of hydrocarbon exposure on marine organisms following oil spill events, allowing for the identification of metabolic pathways impacted by oil exposure. However, there is limited information available for decapod crustaceans, many of which carry significant economic value. In the present study, we assess the sublethal impacts of crude oil exposure in the commercially important Australian greentail prawn (*Metapenaeus bennettae*) using transcriptomic and histological analyses. Prawns exposed to light, unweathered crude oil "spiked" sediments for 90 h were transferred to clean sediments for a further 72 h to assess recovery. Chemical analyses indicated that polycyclic aromatic hydrocarbons increased by approximately 65% and 91% in prawn muscle following 24 and 90 h of exposure, respectively, and significantly decreased during 24- and 72-h recovery periods. Transcriptomic responses followed an exposure and recovery pattern with innate immunity and nutrient metabolism transcripts significantly lowered in abundance after 24 h of exposure and were higher in abundance after 72 h of recovery. In addition, transcription/translation, cellular responses, and DNA repair pathways were significantly impacted after 24 h of exposure and recovered after 72 h of recovery. However, histological alterations such as tubule atrophy indicated an increase in severity after 24 and 72 h of recovery. The present study provides new insights into the sublethal impacts of crude oil exposure in greentail prawns and identifies molecular pathways altered by exposure. We expect these findings to inform future management associated with oil extraction activity and spills. *Environ Toxicol Chem* 2022;41:2162–2180. © 2022 John Wiley & Sons Ltd. This article has been contributed to by U.S. Government employees and their work is in the public domain in the USA.

Keywords: Shrimp; RNA; Tubule atrophy; Decapods; Immune response; KEGG pathway

INTRODUCTION

Marine ecosystems are under threat of oils spills through exploration and transportation of hydrocarbons such as crude oil (Barron et al., 2020; Luís & Guilhermino, 2012; Martínez-Gómez et al., 2010). Although oil spills only account for approximately 10% of crude oil entering the marine environment (Farrington, 2013), they can have significant localized impacts on marine ecosystems (Almeda, Wambaugh, Chai, et al., 2013; Da Silva et al., 1997) through physical smothering of biota and habitats, and chemical toxicity (Almeda, Wambaugh, Wang, et al., 2013; Hook, 2020; National Research Council, 2003). Crude oil is a complex mixture composed of thousands of compounds (Meador & Nahrgang, 2019), including polycyclic aromatic hydrocarbons (PAHs), which are most frequently associated with toxicity. However, Meador and Nahrgang (2019) argue a combination of hydrocarbon compounds causes common toxicological effects often attributed to PAHs alone, including narcosis, reduced membrane integrity and function, and inhibited larval development (Di Toro et al., 2000; Sørhus et al., 2015). Hydrocarbon toxicity is well documented in many marine organisms, including fish, molluscs, copepods, and

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zooplankton (Almeda, Wambaugh, Wang, et al., 2013; Brown-Peterson et al., 2017; Lavarías et al., 2011; Osman et al., 2017). Although there have been numerous studies assessing the risks of hydrocarbons to marine biota, there is a paucity of information for many ecologically and commercially important invertebrate species such as decapods, which hinders effective management.

Prawn (or shrimp) species have substantial economic value, supporting commercial and recreational fisheries around the world (Kenny et al., 2014; Zeng et al., 2011). In Australia alone, commercial prawn fisheries have been valued at approximately $396 million annually (Australian Bureau of Agricultural and Resource Economics and Sciences, 2018). The greentail prawn, *Metapenaeus bennettae*, is primarily benthic and inhabits shallow, coastal marine and estuarine environments from Rockhampton, Queensland to the southern coast of South Australia (Edgar, 2008; Kirkegaard & Walker, 1970; Young et al., 2013). Commercial fisheries operate largely from southeast Queensland in Moreton Bay and Brisbane River (annual catch >500 t) and the Wallis Lake-Hunter region in New South Wales (annual catch 20–50 t; Taylor, 2015). During the day greentail prawns take refuge in soft sediments and feed in the water column at night (Kirkegaard & Walker, 1970), making them susceptible to both water-soluble and sediment-bound contaminants such as hydrocarbons (Carvalho Neta et al., 2019; Lewtas et al., 2014; Pasquevich et al., 2013). Exposure to hydrocarbons can have toxic effects on prawn species and decapods generally, impairing swimming velocity (Silva et al., 2013), eliciting oxidative stress (Lavarías et al., 2011; Ren et al., 2015), initiating DNA damage (Ren et al., 2015; Vijayavel & Balasubramanian, 2008; Wen & Pan, 2016), and causing histopathological alterations in ovaries (Wen & Pan, 2016). However, only a few studies have investigated the molecular responses of decapods to hydrocarbon exposure and therefore the molecular mechanisms involved in exposure responses remain unclear (Pasquevich et al., 2013; Yednock et al., 2015; Yu et al., 2018).

To date, decapod responses to hydrocarbon exposure has been determined using multiple analytical approaches: cytochrome P450s (CYP) and other stress–response transcript expression (Dam et al., 2008; Lavarías et al., 2011; Ren et al., 2015), oxidative stress and xenobiotic metabolizing enzyme activities (Gravato et al., 2014; Luís & Guilhermino, 2012; X. Y. Ren et al., 2014b), and PAH metabolite concentrations (Douglas et al., 2018; Nudi et al., 2010; Silva et al., 2013). To a lesser extent, assessments of DNA damage (Ren et al., 2015; Vijayavel & Balasubramanian, 2008), oxidative damage (Lavarías et al., 2011; Ren et al., 2015), and immunotoxic effects (X. Ren et al., 2014a) have also been used to determine the effects of petroleum hydrocarbon exposure. However, PAH exposure biomarkers do not always predict ecologically relevant impacts of crude oil exposure, including changes in survival, behavior, growth, or reproduction (reviewed in Hook, Mondon, Revill, Greenfield, Stephenson, et al., 2018).

Modern transcriptomic approaches utilized by ecotoxicological studies are overcoming these limitations. By using next-generation sequencing technologies combined with advanced bioinformatic tools, researchers are able to analyze transcriptome-wide responses to environmental stressors in both model and non-model species (Feldmeyer et al., 2011; Lenz et al., 2014; Richardson & Sherman, 2015; Riesgo et al., 2012). The transcriptomic literature investigating decapod stressors has increased in recent years, from response to pathogens (He et al., 2021; Jin & Zhu, 2019), hypoxia (Sun et al., 2014, 2020), aquaculture waste products (Liu et al., 2020; Yu et al., 2019), and heavy metals (Liu et al., 2021; Zhang et al., 2019), to adaptation to extreme deep-sea environments (J. Cheng, Hui, et al., 2019; Hui et al., 2018). Following the *Deepwater Horizon* oil spill in the Gulf of Mexico in 2010, several studies used transcriptomic profiling in field and laboratory settings to assess the impacts of crude oil exposure on native marine fish species (Pilcher et al., 2014; Whitehead et al., 2012). For example, killifish collected from oil-affected and -unaffected locations differed in CYP1A protein expression profiles in the tissues of adult and larval fish, with an up-regulation of CYP1A protein in the gills, liver, intestine, and head kidney indicating the metabolism of PAHs corresponded with the arrival of the *Deepwater Horizon* spilled oil (Dubansky et al., 2013). Such studies have been pivotal in improving our understanding of physiological and molecular responses triggered by environmental changes and the pathways underpinning stress responses in nonmodel species (Gomiero et al., 2006; Hook et al., 2010; Jiang et al., 2017).

Ecotoxicological studies have used alterations in transcript abundances as evidence of altered cellular mechanisms and potential higher organism impacts in response to contaminant exposure (Hook, Mondon, Revill, Greenfield, Smith, et al., 2018; Mehinto et al., 2012). In addition, studies have successfully integrated transcriptomics and histopathology to observe "higher" level organism damage that aligns with changes in related transcript abundances (Hook, Mondon, Revill, Greenfield, Smith, et al., 2018; Hook, Mondon, Revill, Greenfield, Stephenson, et al., 2018; Zhang et al., 2019), a common practice known as "phenotypic anchoring" (Waters & Fostel, 2004). In prawns exposed to hypoxic conditions, the down-regulation of oxidative stress gene expression and changes in fatty acid metabolism were related to the vacuolation of R-cells in the hepatopancreas (Sun et al., 2014). Similarly, in fish, changes in inflammatory response and cell death-related transcripts correlated with necrosis observed in liver and gill tissues (Hook, Mondon, Revill, Greenfield, Stephenson, et al., 2018). Transcriptomic studies with the incorporation of histology allow for assessments of transcriptional changes and impacted physiological mechanisms in nonmodel species like decapods. Importantly, these studies can point to alternative biomarkers of hydrocarbon exposure/effect, because responses often tend to be taxon- and contaminant-specific (Gravato et al., 2014).

In the present study, we conducted transcriptomic and histological analysis of greentail prawns (*Metapenaeus bennettae*) following controlled crude oil exposure. Our experiments involved exposing greentail prawns to sediments contaminated with varying concentrations of light, unweathered Australian crude oil. The experimental crude oil concentrations used are environmentally relevant and similar to those recorded in Louisiana salt marshes following the *Deepwater Horizon*

wellhead blowout (Turner et al., 2014). Our goals were to (1) determine the sublethal impacts of crude oil exposure on greentail prawns by assessing histological alterations, the tissue PAH concentrations at which this occurred, and differentially abundant transcripts, (2) identify potential biomarkers of crude oil exposure in decapod species, (3) identify response and/or recovery cellular pathways after crude oil exposure in decapods species, and (4) inform monitoring targets to be used in the event of future oil extractions or spills. We expect the findings from the present study will provide novel insights into molecular and physiological impacts after hydrocarbon exposure in decapod crustaceans and form a resource for guiding future management associated with oil extraction activity and spills.

METHODS

Wild collections and controlled laboratory exposures

Sixty greentail prawns were collected via netting from St Vincent Gulf, near Adelaide in South Australia on February 9, 2016. The prawns were transported to the South Australian Research and Development Institute (SARDI) laboratories in Adelaide, where they were acclimatized for 2 weeks in 2250 L circulating tanks and fed diced pipis (clams; *Donax deltoids*), with salinity, temperature, and dissolved oxygen monitored routinely prior to the trial's commencement.

An 8-day exposure/recovery experiment to determine the effects of sublethal crude oil exposure in greentail prawns was conducted at SARDI in Adelaide. Four prawns were assigned to five 25-L tanks for each of the control and treatment (low and high PAH concentration exposure) groups (Figure 1). The substrate of each tank consisted of 13 kg of sand sediment, collected from North Haven and Henley Beaches (Adelaide, South Australia). Treatment tank sediments were contaminated with low (nominal concentration of 0.5 mg/kg) and high (nominal concentration 2 mg/kg) concentrations of crude oil (Northwest Shelf 2). To achieve this, 1 kg of clean sand (~1 mm grain size), collected from the same source, was placed in an amber jar and was contaminated with 4.8 and 19.2 ml of crude oil (Northwest Shelf 2, with a density of 0.81 g/L) for low and high treatment group sediments, respectively. Excess water was removed, but the sand was not dried. The jars were filled with seawater to eliminate headspace, sealed, and put on sediment rollers for 7 days. The seawater was then decanted, reducing the water-soluble (and potentially narcotic) PAHs present, and the contaminated sediment was then added to 12 kg of clean sediment and mixed by hand. Again, the excess water was removed from the sand but the sediment was not dried. Homogenization of sediments was clear as contaminated sediments were significantly darker than the clean sediment. The tanks were assigned letters (A–E) for each treatment replicate and filled with filtered seawater, then arranged by replicate rather than treatment (e.g., control A, 0.5A, 2A, control B, 0.5B, 2B, etc.), avoiding placement bias.

Four prawns were randomly assigned to each of the five replicate tanks for each treatment (control, low, and high; Figure 1) and monitored to ensure they buried into the sediments. Animals were fed diced pipis (*Piebidonax deltoids*) periodically throughout the experiment (once or twice daily), water temperature (21.1 ± 0.1 °C), salinity (38‰), and dissolved oxygen (92.0% ± 0.4%) concentrations were monitored daily, and animals were checked twice daily for mortality. Uneaten food and other particulate matter was also removed daily from the tank via siphoning. Prawns were exposed to their treatment sediments for a total of 90 h, due to the limited persistence of light crude oil in the sand (Hook, Mondon, Revill, Greenfield, Stephenson, et al., 2018), before being carefully transferred to clean sediment tanks for the remaining 72 h of the experiment (Figure 1). One prawn from each tank was euthanized at uptake time points 24 and 90 h, and recovery time points 24 and 72 h by submerging the prawn in an ice-water slurry to anesthetize it, followed by cervical dislocation. Length (average 25.1 ± 0.4 mm) and weight (average 11.4 ± 0.4 g) measurements were recorded, followed by tissue dissection for subsequent analysis. A section of hepatopancreas was removed and submerged in RNA later© (Ambion), stored at 4 °C overnight then transferred to −20 °C for RNA analysis. A muscle section was taken and stored at −20 °C for hydrocarbon analysis. Sections of gill, hepatopancreas, muscle, digestive tissue (if identifiable), and ovary (if identifiable) were fixed in 10% seawater-buffered formalin at room temperature for histological analysis.

Analysis of exposure sediment

Sediments collected at the beginning ($t = 0$ h) and end ($t = 90$ h) of the uptake period were analyzed for PAH concentrations following the protocols specified in Hook, Mondon, Revill, Greenfield, Stephenson, et al. (2018). Briefly, sediments were initially spiked with a known mixture of denatured PAH standards and extracted following a modified method from Bligh and Dyer (1959), where PAHs were extracted three times with a one-phase dichloromethane–methanol–water mixture (1:2:0.8 v/v/v). Sediment samples were ultrasonicated for 10 min during each extraction, centrifuged, and the supernatant extracts combined. Lipids were recovered in the lower organic layer and the solvent removed in vacuo after phase separation. Samples were analyzed as total extracts; PAH concentration was determined by gas chromatography–mass spectrometry (GC/MS) using a ThermoScientific 1310 GC coupled with a TSQ triple quadrupole. Samples were then injected using a Triple-plus RSH auto sampler with a nonpolar HP-5 Ultra 2 bonded-phase column (50 m × 0.32 mm i.d. × 0.17 μm film thickness). The initial oven temperature of 45 °C was held for 1 min, followed by temperature programming at 6 °C min to 180 °C then at 3 °C min to 315 °C, where it was held for 15 min. Helium was used as the carrier gas. Mass spectrometer operating conditions were electron impact energy 70 eV, emission current 250 μA, transfer line 310 °C, and source temperature 240 °C. The instrument was operated in selected ion monitoring (SIM) mode to detect each compound group. Data were acquired and processed with Thermo Scientific Xcalibur software and analytes quantified by comparison of peak areas with the relevant deuterated standard.

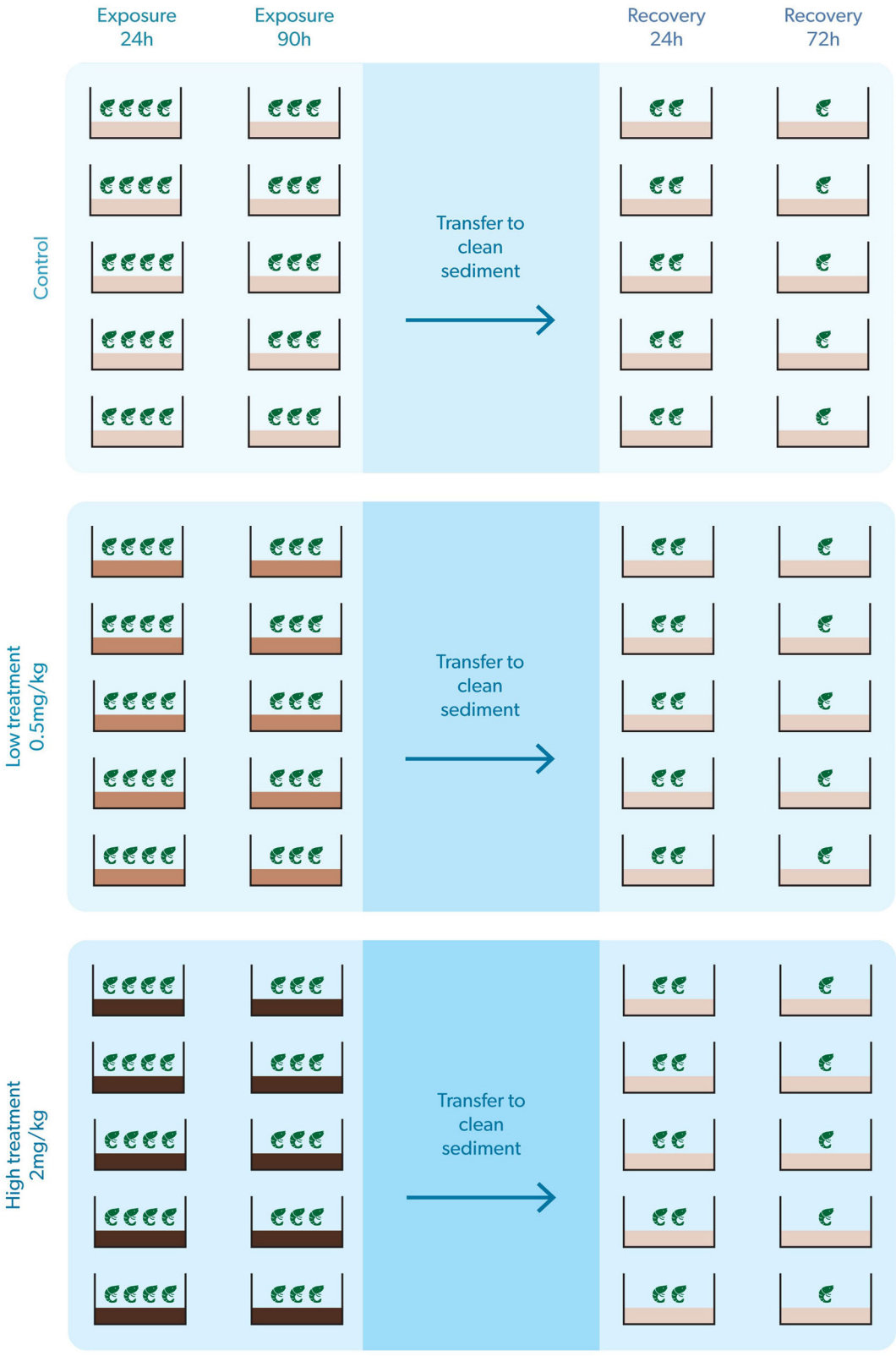

FIGURE 1: Flow chart depicting the outline of the laboratory trials undertaken at the South Australian Research and Development Institute in Adelaide. Sixty prawns were separated into three treatment groups, control, low (sediment spiked with 0.5 mg/kg of crude oil), and high (sediment spiked with 2 mg/kg of crude oil). One prawn from each of the five replicate tanks was taken for subsequent analysis after each exposure time points (24 and 90 h of exposure). Remaining prawns were then transferred to tanks with clean sediment, and one prawn was collected after each recovery time point (24 and 72 h of recovery).

Hydrocarbon concentrations in muscle

Muscle samples were placed in 10-ml test tubes, spiked with a mixed standard containing d8-naphthalene and d10-phenanthrene, and 5 ml of dichloromethane added. Tubes were sonicated in a water bath for 2 × 5 min with stirring in between, centrifuged, and the solvent transferred to a clean tube. This was repeated twice. The combined solvent extracts were reduced gently under a stream of N_2 before being transferred to small vials. Dry weights were determined after drying the extracted material in an oven at 45 °C for 48 h.

Sample extracts were analyzed by GC/MS operating in SIM mode (Thermo Scientific TSQ8000 with Trace 1310 GC). Then 0.5 µl was injected from a total volume of 200 µl onto a DB-5 column using a PTV injector at 45 °C, which was ballistically heated postinjection to 305 °C at 3 °C/s. The GC oven was initially held at 45 °C for 1 min and then ramped to 180 °C for 6 min, then to 250 °C for 3 min, and finally to 315 °C at 10 min with a final hold time of 15 min. The MS was operated in SIM mode with target ions in groups according to retention time with no more than three ions per group. Each mass had a dwell time of 0.2 s. The target compounds were calculated by comparison to the relevant internal standard via peak area.

Body burdens between treatments were compared using the nonparametric Kruskal–Wallis test in conjunction with a Wilcoxon rank sum test, in RStudio (v3.6.0), as a high proportion of zeros prevented assumptions being met for analysis of variance (ANOVA).

RNA extractions, sequencing, and quality control

Hepatopancreas from control and high treatment groups for the two exposure times (24 and 90 h) and the 72-h recovery period were used for transcriptomic analysis, and RNA was extracted following a method previously described by Hook et al. (2017). Briefly, RNA was extracted from individual prawns using approximately 10 mg of hepatopancreas. The tissue was added to 1 ml of TRIzol reagent (Invitrogen) and homogenized using an MP Biomedical bead beater at maximum speed (6.5 m/s) using lysing matrix D (MP Biosciences). RNA extraction followed the TRIzol protocol until the aqueous phase was separated, then the Qiagen RNeasy kit was used to purify the aqueous phase. The RNA quantity and purity was established by a nanodrop spectrometer (Fisher Scientific) using a minimum 260/280 ratio of 2.0, with quality determined using an Agilent bioanalyzer (minimum RIN of 8); three (two from the control 24-h exposure group and one from the control 72-h recovery group) hepatopancreas samples were found to have low-quality RNA and therefore were not sequenced (Supporting Information, Table S8). Because of the cost of RNASeq experiments, only the high treatment, which was hypothesized to exhibit the greatest changes, was submitted for sequencing. Ideally, five replicates would have been used, but three replicates are routinely used in transcriptomic studies (Jenny et al., 2016; Qiao et al., 2018; Zhang et al., 2019). The RNA libraries were prepared and sequenced by the Ramaciotti Centre using 1 µg of RNA from 27 individuals following the TruSeq Standard mRNA prep and dual indexing Illumina protocols. Libraries were subsequently sequenced on two HiSeq 2500 150 bp ER Rapid v2 single-end lanes in accordance with the manufacturer's protocols. Sequencing data were uploaded to the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) database (https://www.ncbi.nlm.nih.gov/sra) under the project name PRJNA509986 and assigned accession numbers SAMN10591652—SAMN10591660. Sequencing data from 27 samples were mapped to the greentail prawn de novo transcriptome published previously in Armstrong et al. (2019), using RSEM (Li & Dewey, 2011) and Bowtie Ver. 1.2.0 (Langmead, 2010) with default parameters.

Differential gene expression and enrichment analysis

Differentially abundant transcripts were identified using BioConductor's DESeq2 (Ver. 1.30.1; Love et al., 2014) in RStudio (Ver. 4.0.5; Team, 2020). Pairwise comparisons were made between control ($n = 3$) and high ($n = 5$) treatment groups at 24 h of exposure, between control ($n = 5$) and high ($n = 5$) treatment groups at 90 h of exposure, and between control ($n = 4$) and high ($n = 5$) treatment groups at 72 h of recovery. Differentially abundant transcripts were determined using a false discovery rate (FDR) <0.05 and a log-fold change (LFC) >2.

Gene ontology enrichment analysis was performed using the R package GoSeq (Ver. 1.36.0) on the differentially abundant transcripts, while controlling for length bias (Young et al., 2010). Differentially abundant transcripts were annotated with gene ontology terms and lengths obtained from the greentail prawn transcriptome previously described by Armstrong et al. (2019). Gene ontology enrichment analysis was performed following Young et al.'s (2010) standard pipeline. Briefly, length bias was accounted for using the probability weighting function, which uses the length data of each transcript to estimate the probability that a transcript will be differentially expressed based solely on its length (Young et al., 2010). The probability weighting value instructs the Wallenius approximation test used to determine enriched or under-represented gene ontology terms with an FDR value <0.05.

The pathway analysis database Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to assign KO IDs to transcripts, providing a biological function to transcripts. The R packages gage (Ver. 2.40.2), gageData (Ver. 2.28.0), and pathview (Ver. 1.30.1) were used to analyze altered pathways for 24- and 90-h exposure and 72-h recovery treatments, and significance was determined using a q-value <0.1.

Histology

Histological preparation and evaluation followed a standard paraffin procedure described in Mondon et al. (2001). Briefly, gill, hepatopancreas, muscle, and gonad tissue was fixed in 10% buffered formalin, with delicate tissue (e.g., gills) placed between biopsy pads inside histology cassettes for protection. Tissue processing involved automated dehydration of fixed

TABLE 1: Semiquantitative scale for epithelial lifting on gill lamellae in the greentail prawn

Score	Description
0	No lifting of the majority of lamellae
1	Low-level lifting, significantly less than one half of lamellae have lifting
2	Moderate-level lifting, approximately half of lamellae have lifting
3	High-level lifting, significantly more than one half of lamellae have lifting
4	Severe-level lifting, all lamellae have lifting

tissue in ascending concentrations of ethanol (30%, 50%, 70%, 90%, 95%, 100%), which were then cleared in Histolene (Leica ASP 300S vacuum infiltration processor), followed by embedding in paraffin (Leica EG1150 H manual embedding station). Embedded tissue was sectioned to 4 μm using a HM 325 μm microtome, mounted on glass slides, stained using the standard hematoxylin and eosin (H&E) protocol, and cover slipped using Entallin®.

Each slide was observed using a Zeiss Axiovert Universal Microscope and picto-micrographs were taken using a Zeiss Axiocam HRc micro imaging GmbH camera. Micrographs of muscle tissue captured at ×100 magnification and gonad tissue captured at ×200 magnification were examined for pathologies with presence/absence recorded. Micrographs of gill lamellae were captured at ×100 magnification and examined for presence/absence of pathologies, and epithelial lifting was ranked against a semiquantitative scale that was designed specifically for the present study (Table 1).

Hepatopancreas micrographs taken at ×100 magnification were examined for pathologies with presence/absence recorded. Hepatopancreas tubule atrophy was categorized based on the semiquantitative scale developed by the National Oceanic and Atmospheric Administration (NOAA) for bivalves (Kim et al., 2006) with a score of 0–4 (Supporting Information, Table S1). Micrographs of hepatopancreas captured at ×400 magnification were imported into the software "ImageJ" and external and internal (lumen) tubule areas were measured. The lumen percentage area was calculated by dividing the internal lumen area with the total tubule area and multiplying by 100. Tissue degeneration through necrosis was ranked against a semiquantitative scale that was designed specifically for the present study (Supporting Information, Table S2). Tubules were also ranked based on the R-cell impairment scale used by Mazurová et al. (2010). Epithelial lifting, tubule atrophy, lumen percentage area, and R-cell impairment comparisons were analyzed for statistical significance using a one-way ANOVA and Tukey's honest significant difference test in RStudio (Ver. 3.6.0). Assumptions of equal variances and normality were checked using Levene's and Shapiro–Wilk normality tests and residual plots.

RESULTS

Hydrocarbon analysis

The Northwest Shelf 2 (NWS-2) crude oil used in this experiment is a light oil which is normal for Australian Northwest shelf crude oils (Qi et al., 2011), with a density of 0.81 mg/L and viscosity of 3.45 cP at 20 °C. Polycyclic aromatic hydrocarbon concentrations measured in source oil and sediments before and after exposure are presented in Figure 2. The treatments, nominal control, low (0.5 mg/kg), and high (2 mg/kg) sediments had respective total PAH concentrations of 10.9 ± 0.3, 165 ± 1.7, and 519 ± 5.5 ng/g before exposure and 12.6 ± 0.3, 196 ± 3.4, and 192 ± 2.4 ng/g after exposure. Naphthalene and phenanthrene were measured in control sediments pre- and post-exposure, possibly due to pre-existing contamination in the sediments collected from an urban environment. The lower proportion of naphthalenes measured in sediments pre- and

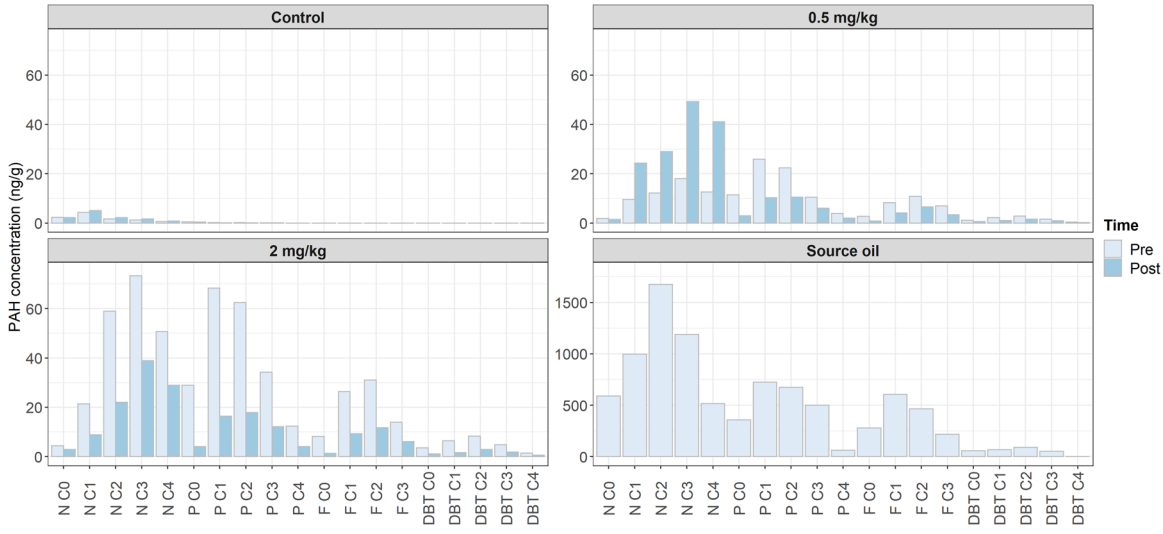

FIGURE 2: Polycyclic aromatic hydrocarbon (PAH) concentrations (ng/g) measured in the control, low (nominal concentration of 0.5 mg/kg), high (nominal concentration of 2 mg/kg), and higher concentrations (scale from 0 to 1800 ng/g) of source oil (Northwest Shelf 2). N = naphthalene; P = phenanthrene; F = fluorene; DBT = dibenzothiophene; C1–C4 indicate the alkyl congeners for each PAH.

post-exposure compared with the source oil is attributed to the volatile and water-soluble nature of this PAH. We would hypothesize that these compounds were poured off with the overlying water or lost in the flow-through systems after dissolving in the water column, rather than adhering to the sediments. Crude oil was lost from tanks over the 4-day exposure period (Figure 2), indicating exposure to PAHs could not have been sustained for a longer experimental time. Naphthalene, phenanthrene, fluorene, and dibenzothiophene sediment concentrations decreased postexposure, with the exception of an increase in naphthalene concentration in the low treatment sediments after 90 h of exposure (Figure 2). A reduction in total PAH concentrations could be caused by a number of factors, including accumulation, and metabolism by prawns, sorption to the tank's glass walls, and/or adsorbtion to the prawns' carapace, degradation by microbial communities, and/or desorbtion and loss to overlying water from the sediments by burrowing prawns.

Polycyclic aromatic hydrocarbons with molecular weights greater than that of phenanthrene are not abundant in light crude oils such as the NWS-2 oil used in our experiment (Figure 2). We focused the tissue body burden analyses on sensitive detection of the low molecular weight PAHs instead of measuring the full spectrum of compounds, as we expected very low concentrations of higher molecular weight PAHs in the prawn muscle, to allow for lower detection limits and greater accuracy in these measurements. Uptake of naphthalenes and phenanthrenes was measured after 24 and 90 h of exposure, while a rapid depuration was observed after transfer to clean sediment for 24 and 72 h (Figure 3). Naphthalene uptake was highly variable, probably due to the water-soluble nature of these hydrocarbons and the uneven exposure from sediment. However, naphthalene was accumulated in proportion with treatment: the low treatment measured 3.8 ± 2.0 and 17.1 ± 1.8 ng/g of naphthalene after 24 and 90 h, respectively, and the high treatment measured 4.9 ± 1.9 and 34.9 ± 12.9 ng/g of naphthalene after 24 and 90 h, respectively (Figure 3). Accumulation of phenanthrene in muscle tissue was similar regardless of dose after 24 h, with concentrations of 1.9 ± 0.9 and

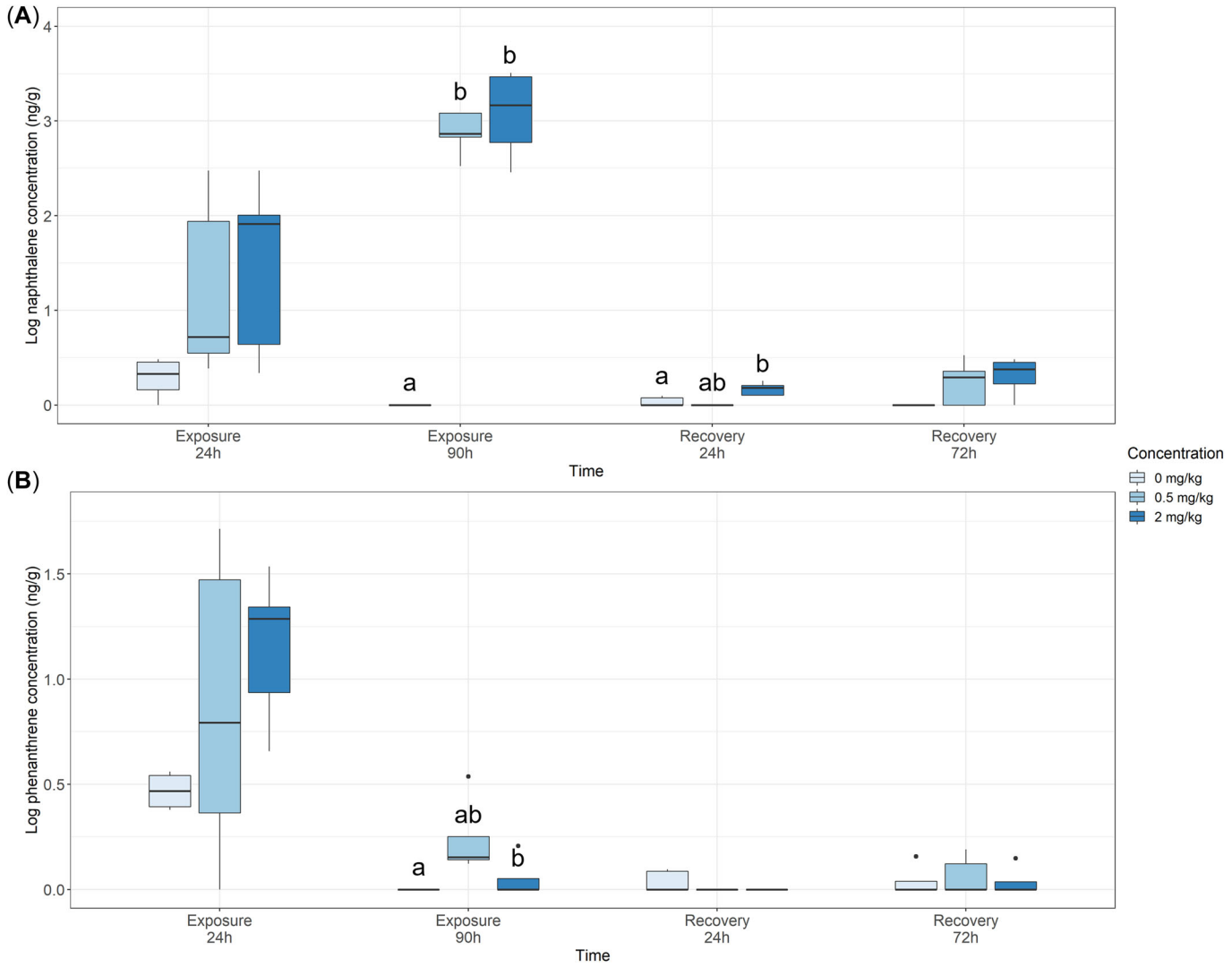

FIGURE 3: Naphthalene and phenanthrene concentrations (ng/g) measured in prawn muscle tissues after both exposure and recovery periods. Significant differences ($p > 0.05$) between treatments for individual exposure and recovery time points are indicated by letters.

2.3 ± 0.5 ng/g after exposure to the low and high treatments, respectively (Figure 3). After 90 h of exposure, the concentration of phenanthrene decreased in muscle tissues in both treatments to 0.3 ± 0.1 and 0.3 ± 0.2 ng/g, still significantly higher ($p = 0.048$) than the control treatment at 0.02 ± 0.02 ng/g of phenanthrene (Figure 3). Naphthalene and phenanthrene concentrations in the muscle tissue of exposed prawns reduced dramatically after 24 and 72 h of exposure to clean sediments (Figure 3). After a 24-h recovery, naphthalene muscle concentrations reduced to 0.45 ± 0.45 and 0.19 ± 0.03 ng/g in the low and high treatments, respectively (Figure 3). After a 72-h recovery, naphthalene muscle concentrations reduced to 0.29 ± 0.1 ng/g in the low treatment, whereas the high treatment slightly increased to 0.38 ± 0.1 ng/g (Figure 3). After a 24-h recovery, phenanthrene muscle concentrations reduced to 0 ± 0.0 ng/g in the low and high treatments (Figure 3). After a 72-h recovery, phenanthrene muscle concentrations reduced to 0.07 ± 0.04 and 0.12 ± 0.09 ng/g in the low and high treatments, respectively (Figure 3).

Differential transcript abundance

Pairwise differential abundance analysis between 24-h exposure control and high treatment prawns identified 47 (six higher and 41 lower) transcripts that were differentially abundant (Supporting Information, Table S2). Financial constraints prohibited the sequencing of the low exposure concentration. Of these transcripts, 34 were annotated with a known function, three had no annotation in the databases, and 10 were annotated as an "uncharacterized protein." Crustacean innate immunity transcripts reduced in abundance after 24 h of exposure compared to the control, including the transcripts *c-type lectin 2*, *glucan pattern-recognition lipoprotein*, and *cathepsin L* (Figure 4A). In addition, proteolytic transcripts including

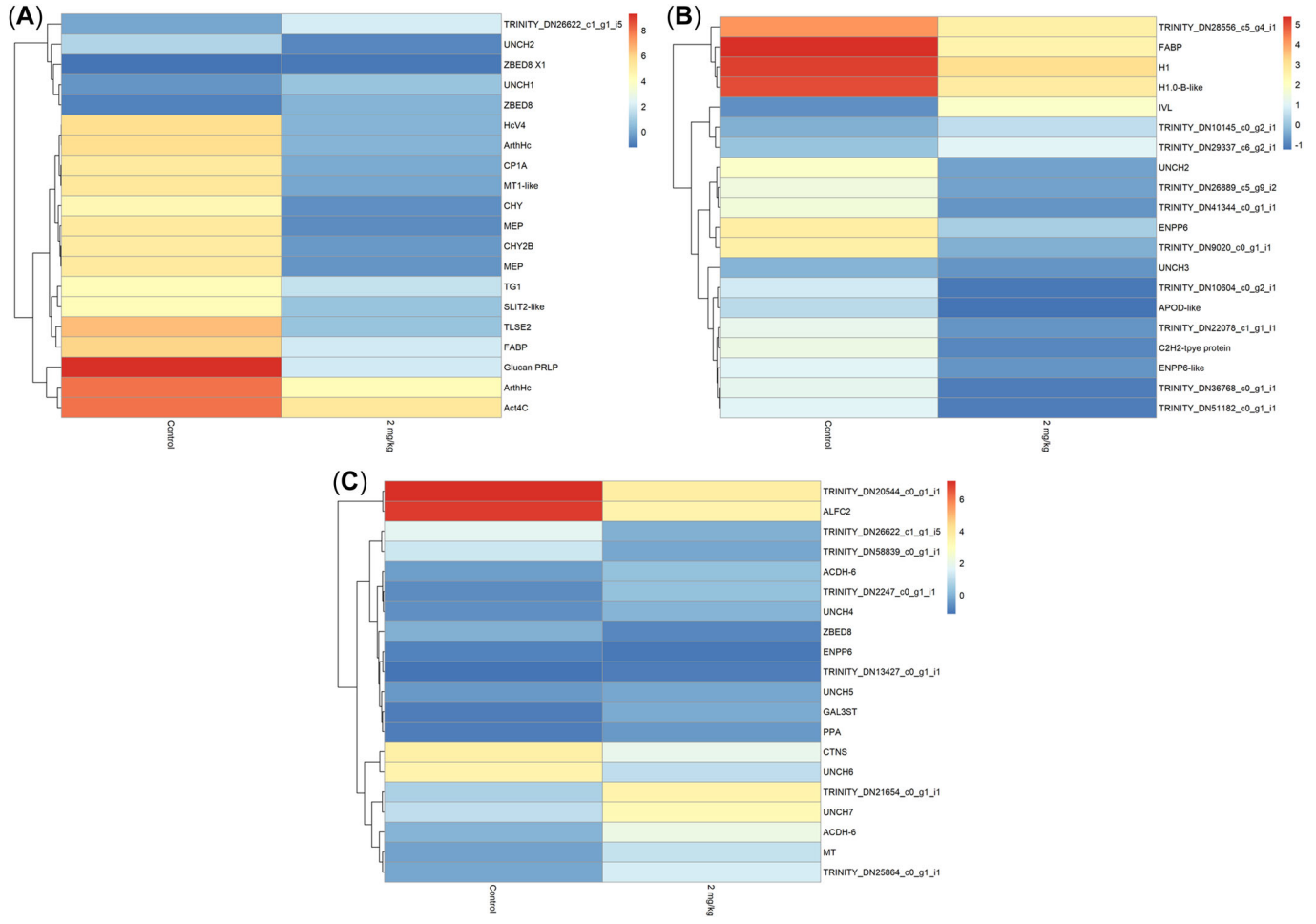

FIGURE 4: Top 20 differentially abundant transcripts for each pairwise comparison. Colour bar indicates the abundances of the transcripts, red = high and blue = low; (**A**) 24 h of exposure control versus 2 mg/kg, (**B**) 90 h of exposure control versus 2 mg/kg, and (**C**) 72 h of recovery control versus 2 mg/kg. ACDH-6 = acyl-CoA dehydrogenase 6; Act4C = cytoplasmic-type actin 4; ALFC2 = anti-lipopolysaccharide factor 2; APOD-like = apolipoprotein D-like; ArthHc = athropod hemocyanin; C2H2-type protein = C2H2-type domain-containing protein; CHY = chymotrypsin; CHY2B = chymotrypsin BII; CP1A = carboxypeptidase A; CTNS = cystinosin homolog; ENPP6 = ectonucleotide pyrophosphatase/phosphodiesterase family member 6; ENPP6-like = glycerophosphocholine cholinephosphodiesterase; FABP = fatty acids binding protein; GAL3ST = putative galactose-3-O-sulfotransferase; glucan PRLP = glucan pattern-recognition lipoprotein; H1 = Histone 1; H1.0-B-like = putative histone H1.0-B-like; HcV4 = hemocyanin V4; IVL = involucrin; MEP = metalloendopeptidase; MT = metallothionein; MT1 = metallothionein 1-like; PPA = inorganic pyrophosphatase; SLIT2-like = slit homolog 2 protein-like; TG1 = thyroglobulin type-1; TLSP2 = trypsin-like serine proteinase 2; UNCH = uncharacterized protein; ZBED8 = ZBED8 protein; ZBED8 X1 = ZBED8 isoform X1 protein (note transcripts with an unknown function are annotated with their Trinity contig ID).

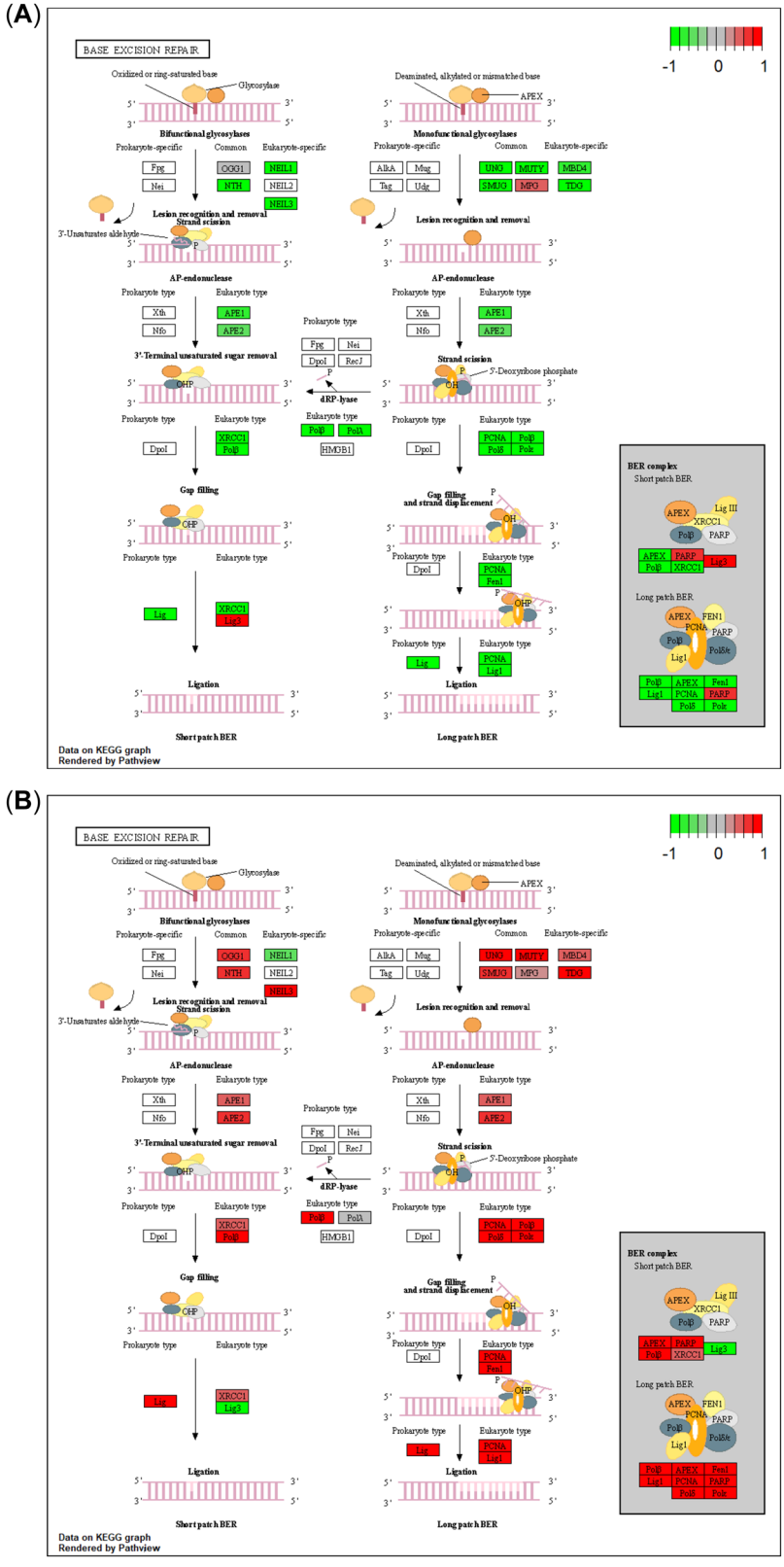

FIGURE 5 Continued.

Chymotrypsin and *carboxypeptidase B-like*, and the detoxification transcript *metallothionein 1-like* reduced in abundance (Figure 4A). Several other detoxification transcripts, including *cytochrome P450 4* (*CYP4*) and *thymidine phosphorylase* (*TP*) also reduced in abundance, but were not significant following FDR correction (FDR = 0.06).

Pairwise differential abundance analysis between 90-h exposure control and high treatment prawns identified 14 transcripts that were differentially abundant (two higher and 12 lower; Supporting Information, Table S3 and Figure 4B). Of these transcripts, three were annotated with a known function, eight had no annotation in the databases, and three were annotated as an "uncharacterized protein." Of the three annotated transcripts identified, *Ectonucleotide pyrophosphatase/phosphodiesterase family member 6* (*ENPP6*) is known to hydrolyze phospholipids (Zimmermann, 2021), *apolipoprotein D-like* (*APOD-like*) is believed to be an important invertebrate coloration protein in decapod crustaceans (Zhao et al., 2020), and *involucrin* (*IVL*) is a transglutaminase substrate protein, found initially in cell cytosol, which becomes cross-linked at sites in the plasma membrane, forming an insoluble layer beneath (Adhikary et al., 2004).

Pairwise differential abundance analysis between 72-h recovery control and high treatment prawns identified a total of 13 differentially abundant transcripts (seven higher and six lower in abundance; Supporting Information, Table S4). Of these transcripts, seven had known functional annotations, one was annotated with "uncharacterized protein," and five had no annotation in the databases. The detoxification transcript *metallothionein* was higher in abundance in the recovery period compared to the control, in addition two transcripts annotated *probable acyl-CoA dehydrogenase 6* (*ACDH-6*) and *putative ACDH-6* were also higher in abundance (Figure 4C). *Probable acyl-CoA dehydrogenases* are mitochondrial flavoenzymes responsible for catabolizing fatty acids and amino acids (Swigonová et al., 2009). The lysosomal transcript *cystinosin homolog* was lower in abundance and the antimicrobial peptide transcript *antilipopolysaccharide factor* (*ALF2*) was higher in abundance in the 72-h recovery period (Figure 4C), both of which are involved in crustacean innate immunity.

Functional annotation

Gene ontology enrichment analysis of these differentially abundant transcripts identified 22 significantly enriched gene ontology terms after 24 h of exposure to oil "spiked" sediments, including "serine-type endopeptidase activity," "proteolysis," and "lipid binding" (Supporting Information, Figure S6a). Only one gene ontology term, "pigment binding," was significantly enriched in the 90-h exposure period. Six enriched gene ontology terms were identified in the 72-h recovery period, including "glycolipid biosynthetic process" and "acyl-CoA dehydrogenase" (Supporting Information, Figure S6b).

The KEGG pathway enrichment analysis of the 24-h exposure period showed a significant reduction in 15 pathways (Supporting Information, Table S7). These included DNA repair pathways such as "nucleotide excision repair," "Fanconi anemia pathway," and "base excision repair" (Figure 5), translation pathways such as "RNA transport" and "ribosome," and cell growth and death pathways such as "cell cycle" and "meiosis—yeast." No pathways were found to be enriched in the 90-h exposure period. Eleven similar pathways that showed a significant reduction in the 24-h exposure period were found to significantly increase in the 72-h recovery period, including the DNA repair, RNA translation, and cell growth and death pathways (Supporting Information, Table S7).

Histology

Hepatopancreas and gill pathologies in prawns exposed to low and high treatments indicated a trend of increasing severity after 24 h of exposure compared to the time matched controls (Table 2). In addition, after transfer to clean sediments (recovery) for 24 and 72 h, the severity of pathologies across both treatments, including percentage lumen area, tissue degeneration, and R-cell changes, continued to increase (Table 2). However, the gill lamellae epithelial lifting scores of 2.1 ± 0.5 and 2.4 ± 0.2 in the low and high treatment prawns after 72 h of recovery were similar to the time-matched control score of 2.6 ± 0.4, indicating possible recovery (Table 2 and Supporting Information, Figure S3).

Percentage lumen area provided a quantitative measure of tubule atrophy. A nonstatistically significant trend in increased percentage lumen area was evident after 24 h of oil exposure, where percentage lumen area increased from $24.0\% \pm 5.9\%$ to $30.1\% \pm 2.8\%$, and $31.1\% \pm 4.0\%$ in low and high treatment prawns, respectively. However, no difference was observed between the time-matched control and low and high treatments after 90 h of oil exposure (Table 2 and Figure 6). Following 24 h of recovery, a significant increase in lumen area was present in exposed prawns ($40.1\% \pm 5.6\%$ and $41.7\% \pm 3.8\%$ in

FIGURE 5: Base excision repair pathway (ko03410; which repairs DNA damage caused by oxidation and alkylation from endo- and exogenous sources), highlighting differentially abundant transcripts (green indicates a decrease in abundance and red indicates an increase). (**A**) The 24-h exposure period saw a significant decrease in pathway transcript abundance, compared to (**B**) the 72-h recovery period, which saw a significant increase. AlkA = DNA-3-methyladenine glycosylase II; APE1/APEX = AP endonuclease 1; APE2 = AP endonuclease 2; DpoI = DNA polymerase I; Fen1 = flapendonuclease-1; Fpg = formamidopyrimidine-DNA glycosylase; HMGB1 = high mobility group protein B1; Lig = DNA ligase; Lig1 = DNA ligase 1; Lig3 = DNA ligase 3; MBD4 = methyl-CpG-binding domain protein 4; MPG = DNA-3-methyladenine glycosylase; Mug = double-stranded uracil-DNA glycosylase; MUTY = A/G-specific adenine glycosylase; Nei = endonuclease VIII; NEIL1 = endonuclease VIII-like 1; NEIL2 = endonuclease VIII-like 2; NEIL3 = endonuclease VIII-like 3; Nfo = deoxyribonuclease IV; NTH = endonuclease III; OGG1 = N-glycosylase/DNA lyase; PARP = poly [ADP-ribose] polymerase 1; PCNA = proliferating cell nuclear antigen; Polβ = DNA polymerase beta; Polλ = DNA polymerase lamba; Polδ = DNA polymerase delta subunit 1; Polε = DNA polymerase epsilon subunit 1; RecJ = single-stranded-DNA-specfic exonuclease; SMU = single-strand selective monofunctional uracil DNA glycosylase; Tag = DNA-3-methyladenine glycosylase I; TDG = thymine-DNA glycosylase; Udg = uracil-DNA glycosylase; UNG = uracil-DNA glycosylase; XRCC1 = DNA-repair protein XRCC1; Xth = exodeoxyribonuclease III.

TABLE 2: Quantitative and semiquantitative scores for multiple pathologies observed in hepatopancreas and gill tissues

		Hepatopancreas					Gill
				R-Cell			
Time (h)	Nominal concentration (mg/kg)	Lumen area (% ± SE)[a]	Tissue degeneration (±SE)[b]	General integrity (±SE)[c]	Cellular integrity (±SE)[d]	Nuclear pleomorphism (±SE)[e]	Epithelial lifting (±SE)[f]
Exposure (24)	Control	24.0 ± 5.9	1.9 ± 0.3	2.7 ± 0.3	2.7 ± 0.2	3.0 ± 0.2	1.2 ± 0.2
Exposure (24)	0.5	30.1 ± 2.8 ↑	2.3 ± 0.5 ↑	2.8 ± 0.1 ↑	3.1 ± 0.04 ↑	3.1 ± 0.1 ↑	1.5 ± 0.3 ↑
Exposure (24)	2	31.1 ± 4.0 ↑	2.3 ± 0.2 ↑	2.8 ± 0.03 ↑	3.0 ± 0.2 ↑	3.1 ± 0.1 ↑	2.3 ± 0.7 ↑
Exposure (90)	Control	40.9 ± 9.4	3.5 ± 0.2	3.2 ± 0.2	3.3 ± 0.1	3.2 ± 0.2	2.5 ± 0.6
Exposure (90)	0.5	33.4 ± 5.6	2.9 ± 0.4	3.0 ± 0.2	3.3 ± 0.1	3.3 ± 0.1 ↑	1.6 ± 0.1
Exposure (90)	2	38.7 ± 2.9	3.3 ± 0.2	3.2 ± 0.1	3.4 ± 0.1	3.3 ± 0.1 ↑	2.7 ± 0.5 ↑
Recovery (24)	Control	28.4 ± 0.8[a]	2.5 ± 0.5	2.7 ± 0.2	3.0 ± 0.1	3.1 ± 0.1	2.5 ± 0.4
Recovery (24)	0.5	40.1 ± 5.6 ↑[ab]	3.1 ± 0.3 ↑	3.2 ± 0.2 ↑	3.4 ± 0.1 ↑	3.3 ± 0.1 ↑	2.7 ± 0.4 ↑
Recovery (24)	2	41.7 ± 3.8 ↑[b]	3.2 ± 0.2 ↑	3.0 ± 0.1 ↑	3.3 ± 0.1 ↑	3.4 ± 0.04 ↑	2.6 ± 0.4 ↑
Recovery (72)	Control	34.4 ± 3.4	3.1 ± 0.2	2.9 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	2.6 ± 0.4
Recovery (72)	0.5	41.4 ± 6.8 ↑	3.4 ± 0.2 ↑	3.1 ± 0.2 ↑	3.4 ± 0.1 ↑	3.2 ± 0.1 ↑	2.1 ± 0.5
Recovery (72)	2	40.7 ± 3.0 ↑	3.5 ± 0.04 ↑	3.1 ± 0.1 ↑	3.4 ± 0.1 ↑	3.2 ± 0.1 ↑	2.4 ± 0.2

Arrows indicate a potential trend in increase of pathology relative to 0 mg/kg control values.
[a]Internal lumen area as percentage of the total digestive tubule area.
[b]Semiquantitative scale for digestive cell degeneration tissue loss (Supporting Information).
[c]Semiquantitative R-cell general integrity scale (Mazurová et al., 2010).
[d]Semiquantitative R-cell cellular integrity scale (Mazurová et al., 2010).
[e]Semiquantitative R-cell nuclear pleomorphism scale (Mazurová et al., 2010).
[f]Semiquantitative scale for gill epithelial lifting (Supporting Information).
Significant differences ($p > 0.05$) are indicated by letters.

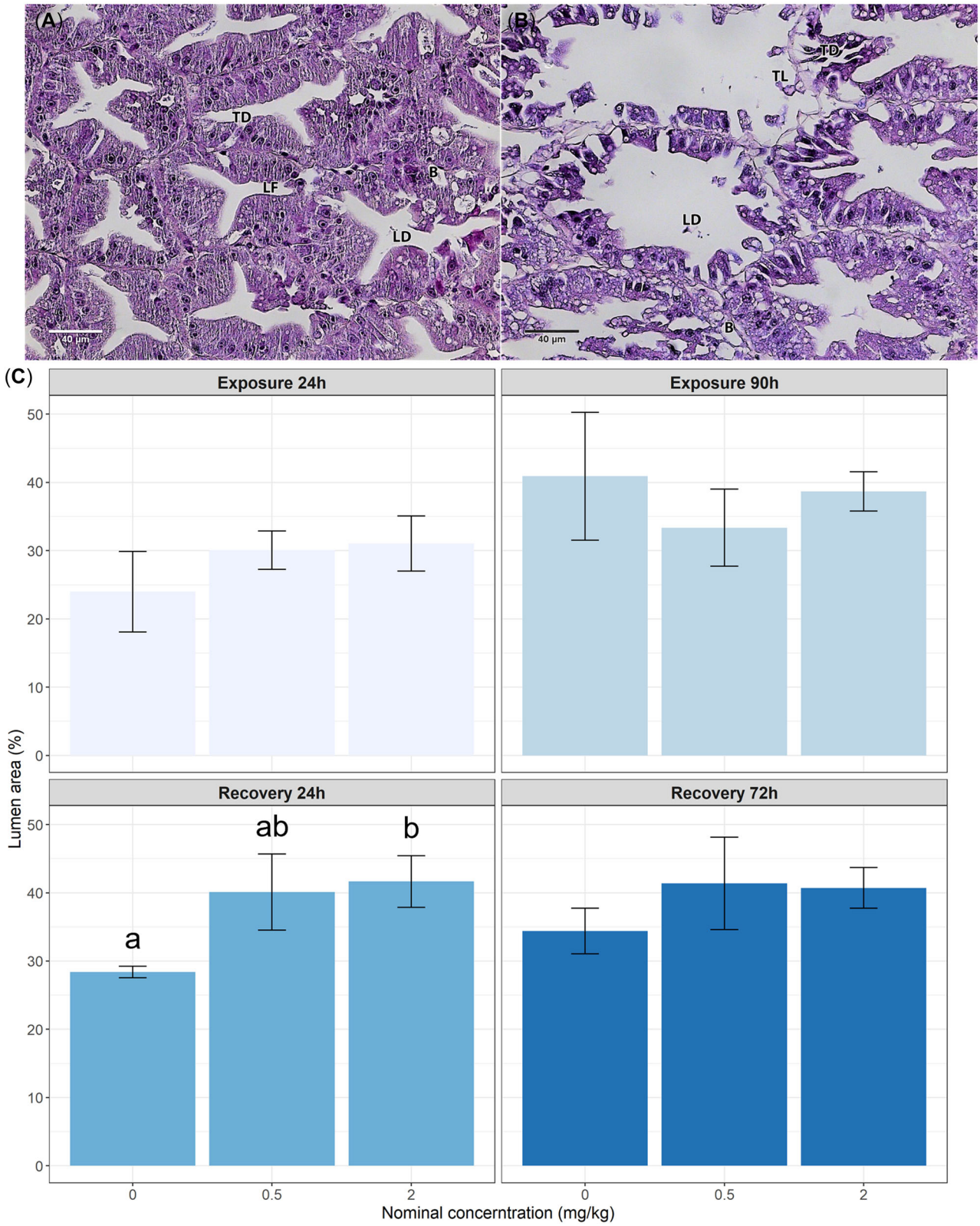

FIGURE 6: (**A**) Hepatopancreas (24 h of exposure control) showing small signs of lumen debris (LB), tissue degeneration (TD), lumen fracture (LF), and enlarged B cells. (**B**) Hepatopancreas (24 h of recovery low treatment) showing significant tissue loss (TL) and degeneration (TD), lumen debris (LB), and enlarged B cells. (**C**) Percentage lumen area of hepatopancreas tubules at three time points for different crude oil exposure treatments. Significant differences ($p > 0.05$) between treatments for individual exposure and recovery time points are indicated by letters.

low and high treatment prawns) relative to the controls (28.3 ± 0.8; $F_{2,12} = 4.26$, $P = 0.04$), and after 72 h of recovery a nonsignificant trend in increased percentage lumen area was still evident (Table 2 and Figure 6). The lack of statistically significant differences between treatments is likely to be due to the variability observed within individual prawns, for example one prawn's percentage lumen area ranged from 10.2% to 57.2% (Supporting Information, Figure S2).

DISCUSSION

In the present study, we identified transcriptomic and histological level changes in greentail prawns exposed to sublethal concentrations of crude oil. To our knowledge, our study is the first to employ the use of both transcriptomics and histological alteration to identify the effects of light, unweathered crude oil exposure on decapod crustaceans. Prawns exposed to sublethal concentrations of crude oil for 24 h exhibited a lower abundance of transcripts involved in innate immunity, detoxification, and proteolysis regulation. Furthermore, KEGG pathway analysis indicated a negative impact on nucleic acid repair, RNA transcription/translation, and cell growth and death pathways after the 24-h exposure period, with impacts easing during the 72-h recovery period. Conversely, histopathological alterations continued to increase in the digestive gland through the 24- and 72-h recovery periods, including an increase in tubule lumen area indicating tubule atrophy in the hepatopancreas. Overall, evidence of changes in transcript abundances and cellular degradation suggests that exposure to crude oil contaminated sediments can disrupt the innate immunity response, nutrient metabolism, and detoxication mechanisms employed by greentail prawns. These changes occurred after a single dose of crude oil exposure, where a rapid loss of PAHs in the higher concentration sediments was evident. Impacts may have been even greater if the concentrations had been constant. The findings from the present study are expected to provide insights into the potential risks of crude oil exposure in prawns to inform future oil extraction and spill management strategies.

Polycyclic aromatic hydrocarbons with molecular weights greater than that of phenanthrene are not abundant in light crude oils such as the NWS-2 oil used in our experiment (Figure 2). We focused the tissue body burden analyses on sensitive detection of the low molecular weight PAHs, instead of measuring the full spectrum of compounds, as we expected very low concentrations of higher molecular weight PAHs in the prawn muscle to allow for lower detection limits and greater accuracy in these measurements.

Low molecular weight PAHs, such as naphthalene and phenanthrene, are abundant in light crude oil and therefore bioavailable to the greentail prawns. We observed a clear uptake and assimilation of naphthalene and phenanthrene in prawns following exposure to crude oil "spiked" sediments for 24 and 90 h (Figure 3). Multiple studies on fish (Snyder et al., 2019; Struch et al., 2019), molluscs (Jenny et al., 2016), and decapods (Douglas et al., 2018; Sagerup et al., 2016) have also shown that low molecular weight PAHs such as naphthalene, phenanthrene, and fluorene, constitute a high proportion of total PAH concentrations measured in tissues after exposure to hydrocarbons. Specifically, naphthalene accumulated at higher concentrations than phenanthrene in prawn tissue, as seen in fishes exposed to hydrocarbons associated with the *Deepwater Horizon* oil spill (Snyder et al., 2019; Struch et al., 2019), and Arctic red king crab following exposure to marine diesel (Sagerup et al., 2016). Greentail prawns demonstrated an elimination of both naphthalene and phenanthrene from the body as body burden concentrations reduced after recovery, phenanthrene after only 24 h of recovery and naphthalene after 72 h of recovery (Figure 3). A 3-week recovery period also saw an elimination of PAHs from the Arctic red king crab's hepatopancreas (Sagerup et al., 2016). Elimination can be through biotransformation of PAHs, and diffusion and excretion of parent or metabolite PAHs (Meador, 2003), but metabolite analysis is needed to determine exactly how greentail prawns eliminate PAHs from their tissues. Nevertheless, transcriptomic analysis demonstrated a negative impact of PAH exposure on immune response, detoxification, energy metabolism, and essential physiological functions prior to elimination in the recovery period.

The immune system in aquatic species, including fish, crustaceans, and molluscs, is often sensitive to environmental contaminants including hydrocarbons (Ito et al., 2021; Nakayama et al., 2008; Su et al., 2017; Yu et al., 2018). Innate immune response transcripts, including pattern recognition receptors (PRRs), lysosomes, and hemocyanins lowered in abundance after 24 h of exposure to crude oil contaminated sediments compared to the control. C-type lectins (CTLs) are carbohydrate PRRs which play an important role in invertebrate innate immunity through the recognition of pathogens and initiation of subsequent immune responses (Jiang et al., 2016; Qin et al., 2019; Zhang et al., 2018). C-type lectin transcripts including *CTL*, *CTL2*, and *CTL4* were significantly lower in abundance after 24 h exposure to crude oil (Supporting Information, Table S2). A lower CLT expression was also seen in green mussel embryos after 24 h of exposure to benzo(a)pyrene (BaP; Jiang et al., 2016). In addition, altered expression of CLT transcripts has been detected in decapods after exposure to stressors such as ammonia (Si et al., 2020; Yu et al., 2019) and cadmium (Sun et al., 2016). *Glucan pattern-recognition lipoprotein* is another PRR transcript that was lower in abundance after 24 h of exposure and also plays an important role in activating immune responses, including phagocytosis, clotting cascade, and the phenoloxidase-activating system (Lai et al., 2011; Sritunyalucksana & Söderhäll, 2000; Wang & Wang, 2013). Lysosomes are also essential to crustaceans' innate immune system, providing protection against pathogens (Guo et al., 2019, 2021; Yu et al., 2018). In addition, lysosomal pathways and associated enzymes are sensitive to various environmental stressors (Guo et al., 2021; Jiao et al., 2020; Yu et al., 2018), as highlighted in the present study which showed that the lysosomal enzyme transcripts *cathepsin L* and *legumain* lowered in abundance after 24 h of exposure to

oil-contaminated sediments. Similarly, the lysosome pathway in Chinese mitten crabs and the phagosome pathway in green mussel embryos was altered following exposure to BaP (Jiang et al., 2016; Yu et al., 2018). In addition, *cathepsin L* was also lowered in expression after exposure to BaP in green mussels (Jiang et al., 2016) and to chromium in Pacific white shrimp (Jiao et al., 2020). Conversely, higher expression of *cathepsin L* was found in decapods after exposure to cadmium (Sun et al., 2016) and ammonia (Yu et al., 2019) when compared to the control. Overall, suppression of immune-related pathways and transcripts from PAH exposure demonstrated in our study, and in previous studies on fish (Nakayama et al., 2008), molluscs (Jiang et al., 2016), and other crustaceans (Yu et al., 2018), suggests greentail prawns could be susceptible to pathogens while exposed to crude oil. Indeed, previous studies have demonstrated increased susceptibility to infectious diseases and mortality in prawns when exposed to stressors, including PAHs (Ito et al., 2021; Kathyayani et al., 2019). Specifically, Ito et al. (2021) showed exposure to phenanthrene caused a proliferation of white spot syndrome virus in kuruma shrimp (*Penaeus japoncius*), resulting in increased mortality.

Nutrient and energy metabolism is centered in the hepatopancreas of decapods (Si et al., 2020; Wang et al., 2014) and is influenced by environmental stressors (Park et al., 2016; Si et al., 2020; Yu et al., 2018). In the present study, the biological function gene ontology term "proteolysis" was significantly enriched when comparing 24-h exposure control and high treatment prawns (Figure 5A). Proteolysis is the breakdown of proteins via proteolytic enzymes into smaller peptides or amino acids (Park et al., 2016), and is known to regulate other processes such as immunity, apoptosis, osmoregulation, and blood coagulation in crustaceans (Park et al., 2016; Russo et al., 2018). In the present study, several proteolytic enzyme transcripts lowered in abundance after exposure to crude oil contaminated sediments for 24 h. These included, *carboxypeptidase B-like*, *trypsin-like serine proteinase 2*, *Chymotrypsin, metalloendopeptidase*, *trypsin 3*, and *angiotensin-converting enzyme-like (ACE-like)* transcripts (Figure 4A and Supporting Information, Table S2). Transcriptional changes for *trypsin* and *carboxypeptidase B* were also observed in swimming crabs (*Portunus trituberculatus*) exposed to ammonia (Si et al., 2020). Conversely, proteolytic enzyme transcripts were higher in abundance after exposure to cadmium in freshwater crabs (*Sinopotamon henanese*; Sun et al., 2016), to chromium in the Pacific white shrimp (*Litopenaeus vannamei*), and to the antifouling biocide, Irgarol, in *Macrophthalmus japonicus* (Park et al., 2016). Many proteolytic enzymes, including *trypsin, chymotrypsin, carboxypeptidase A* isoforms, and *carboxypeptidase B* isoforms, are also essential to the function of the "pancreatic secretion" (ko04972) pathway and have been shown to be significantly enriched in Chinese mitten crabs (*Eriocheir sinensis*) and green mussel (*Perna viridis*) embryos exposed to BaP (Jiang et al., 2016; Yu et al., 2018). Although "pancreatic secretion" was not significantly enriched in the present study on greentail prawns, our analyses indicated that other pathways involved in genetic information processing and cellular processes were significantly enriched.

Fifteen KEGG pathways were significantly enriched, showing a lower abundance of pathway transcripts in greentail prawns after 24 h of exposure to crude oil contaminated sediments (Supporting Information, Table S7). Transcription and translation pathways such as "ribosome," "RNA transport," and "spliceosome" were significantly enriched in the present study (Supporting Information, Figure S4), as reported for other aquatic invertebrates following exposure to environmental contaminants. Previous studies demonstrated significant enrichment of the "ribosome" pathway following BaP and copper exposure (Guo et al., 2019; Jiang et al., 2016) and of the "spliceosome" pathway following cadmium exposure (Zhang et al., 2019). We also observed a significant enrichment in cell growth and death pathways, including "cell cycle" and "meiosis—yeast" in greentail prawns after 24 h of exposure (Supporting Information, Table S7). The "cell cycle" pathway was also significantly enriched in Pacific white shrimp after exposure to Rapamycin, a pharmaceutical drug (Wu et al., 2019). Of concern, DNA replication and repair pathways, including "base excision repair," "mismatch repair," and "Fanconi anemia pathway," which are the first line of defence against genotoxicants, were enriched in greentail prawns after 24 h of exposure to crude oil contaminated sediments (Figure 5 and Supporting Information, Table S7). Exposure to various stressors, including PAHs, causes genotoxic damage in decapods (C. H. Cheng, Ma, et al., 2019; Vijayavel & Balasubramanian, 2008; Wen & Pan, 2016), indicating the necessity of such pathways to be working effectively. The "base excision repair" pathway is of particular interest as it is the primary repair pathway utilized for small base lesions from oxidation and alkylation damage caused by a range of endogenous and exogeneous sources (Almeida & Sobol, 2007). The "base excision repair" pathway was significantly enriched in freshwater crabs exposed to cadmium and in Pacific white shrimp exposed to chromium (Jiao et al., 2020; Sun et al., 2016). However, after prawns were transferred to clean sediment tanks for a 72-h recovery period a reversal in genetic information processing and cellular processes pathways was observed.

The KEGG pathway analysis showed similar significantly enriched pathways with higher transcript abundances after a 72-h recovery period in greentail prawns (Supporting Information, Table S7). The change to these pathways indicates a recovery at the molecular level, as transcripts in significantly enriched pathways such as "cell cycle" (Supporting Information, Figure S8) and "base excision repair" increased in abundance (Figure 5B). In addition, innate immune response transcript, anti-lipopolysaccharide factor (Figure 5C), and nutrient metabolism transcripts, *ACDH-6* and *putative ACDH-6*, increased in abundance after a 72-h recovery period in greentail prawns (Figure 4C). These findings are consistent with other fish and crustacean examples. English sole (*Parophrys vetulus*) showed improvement in health indices, including reduction in CYP1A activities, levels of biliary FACs, DNA adduct formation, and histopathological lesions, after remediation of a PAH-contaminated site (Myers et al., 2008). Red king crabs (*Paralithodes camtschaticus*) showed a marked decrease in

PAH concentration in the hepatopancreas and no differences in biomarker responses between control and exposed crabs after a 3-week recovery period following marine diesel exposure (Sagerup et al., 2016). The reversal in transcript abundances of important pathways identified in the greentail prawn indicates the ability to recover from short-term exposure to light, unweathered crude oil after the cessation of exposure. However, further assessments are needed to determine the recovery capacity of prawns after chronic, long-term exposure to crude oil.

Sensitivity to low environmentally relevant PAH exposure concentrations, and subsequent onset of histological alteration responses, is likely to vary between individuals, more so than if exposed to high dose concentrations. Histological examination of gill and hepatopancreas pathologies indicated a trend of increased severity after 24 h of exposure to low concentration crude oil contaminated sediments. Interestingly, cellular damage and lumen area continued to increase after prawns were transferred to clean sediments, suggesting that although PAHs had been excreted, KEGG pathways reversed, and the number of significantly altered transcripts lowered from 47 to 13 after 72 h of recovery, a longer period of time is required for tissue recovery (Table 2 and Figure 6). A previous study on spotted dragonet (*Repomucenus calcaratus*, a flat fish) showed similar trends of tissue damage following controlled PAH exposure, where the presence of lesions continued to increase throughout the recovery period (90 h), which could be due to either a delayed onset of cellular damage or a delay in recovery after exposure to clean sediments (Hook, Mondon, Revill, Greenfield, Stephenson, et al., 2018). The significant tissue loss in the hepatopancreas after 24 h of recovery, indicated by an increase in the lumen area, could be related to the altered abundance of the lysosomal transcripts *cathepsin L* and *legumain*, and proteolytic enzyme transcripts, which are known to be involved in apoptosis regulation (Park et al., 2016; Russo et al., 2018). Similarly, the severity of necrosis measured in fish liver and gill tissues was connected to the altered abundance of inflammatory and apoptotic transcripts after exposure to hydrocarbons (Hook, Mondon, Revill, Greenfield, Stephenson, et al., 2018). Incorporating histological examination into transcriptomic studies supports a weight of evidence assessment of impacted physiological processes and transcriptional changes, particularly in non-model species.

Research effort increased significantly after the *Deepwater Horizon* oil spill, consequently much is now known about the health impacts of hydrocarbon exposure in fish species (Brown-Peterson et al., 2017; Pilcher et al., 2014; Whitehead et al., 2012). Yet despite the commercial and ecological importance of decapods, the number of studies investigating the molecular and physiological effects of crude oil exposure in decapods remains limited (Beyer et al., 2016). Impacts on innate immunity, nutrient and energy metabolism, and DNA repair pathways were evident in greentail prawn after short-term exposure to light, unweathered crude oil contaminated sediments. The findings in our study indicate a number of potential biomarkers in prawns suitable for future oil extraction and spill monitoring programs, including immune response and proteolytic enzyme gene expression assays, immunotoxic biomarkers such as ProPO and phenoloxidase activity, and phagocytic activity (X. Ren et al., 2014a). Furthermore, innate immunity and proteolytic genes are known to be conserved in decapods (Fang et al., 2013; Lai & Aboobaker, 2017), suggesting these biomarkers could also be used for other decapod species. Finally, the present study showed cellular damage to the hepatopancreas continued into the recovery phase of our study, highlighting the extended time needed for tissue recovery compared to the molecular response mechanisms.

CONCLUSIONS

The present study shows sublethal impacts in greentail prawns exposed to environmentally relevant concentrations of light, unweathered crude oil. Body burden analysis showed uptake of naphthalene and phenanthrene concentrations after 24 and 90 h of exposure to crude oil contaminated sediments, and rapid decrease in concentrations after 24 and 72 h of recovery in clean sediments, providing evidence of PAH elimination by the greentail prawn. Transcriptomic analysis indicated immune response and nutrient metabolism suppression after 24 h of exposure to crude oil contaminated sediments. Furthermore, KEGG analysis showed transcription and translation, cell growth and death, and DNA replication and repair pathways were negatively impacted after 24 h of exposure. In particular, the impact on DNA repair pathways is concerning as they protect the DNA from genotoxic substances, such as hydrocarbons, from causing damage. Encouragingly, after a 72-h recovery period, immune response and nutrient metabolism transcripts increased, and DNA repair and cellular processes pathways were enriched, indicating a possible recovery at the molecular level. However, digestive tubule atrophy, quantified by percentage lumen area, continued to increase in severity after 24 and 72 h of recovery, indicating the duration of impact on health is likely to exceed the oil exposure period. The lack of genomic research on economically and environmentally important decapods limits the understanding of transcriptomic change in response to hydrocarbon exposure, with some differentially abundant transcripts remaining unknown or uncharacterized. However, the information provided in the present study can be used to inform monitoring programs for future oil extraction activity and spills, and to add to the growing genetic resources available for the ecologically and economically important decapods.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5413.

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Disclaimer—The authors declare no conflict of interest.

Author Contributions Statement—**Emily K. Armstrong**: Conceptualization; Formal analysis; Writing—original draft; Writing—review & editing; Visualization. **Julie Mondon**: Conceptualization; Methodology; Writing—review & editing; Supervision. **Adam Miller**: Conceptualization; Writing—review & editing; Supervision. **Andrew T. Revill**: Investigation; Writing—review & editing. **Sarah A. Stephenson**: Investigation; Writing—review & editing. **Patricia Corbett**: Investigation; Writing—review & editing. **Mun Hua Tan**: Formal analysis; Writing—review & editing. **Paul Greenfield**: Formal analysis; Writing—review & editing. **Jared J. Tromp**: Formal analysis; Writing—review & editing; Visualization. **Sharon E. Hook**: Conceptualization; Methodology; Writing—review & editing; Supervision; Project administration; Funding acquisition.

Data Availability Statement—All data generated is publicly available as part of the Great Australian Bight Deepwater Marine Program. Additional data from the present study is available through the Supporting Information. The de novo transcriptome is available at DDBJ/EMBL and GenBank under the accession GHDJ00000000 (https://www.ncbi.nlm.nih.gov/nuccore/GHDJ00000000.1/), and raw sequencing data was uploaded to the NCBI SRA database under the project name PRJNA509986 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA509986) and has been assigned accession numbers SAMN10591652–SAMN10591660. Data, associated metadata, and calculation tools are also available from the corresponding author (sharon.hook@csiro.au).

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