RESEARCH ARTICLE

Nutritional condition drives spatial variation in physiology of Antarctic lipid-storing copepods

Cory A. Berger[1,2] 🟢 | Deborah K. Steinberg[3] | Ann M. Tarrant[1] 🟢

[1]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA

[2]MIT/WHOI Joint Program in Oceanography/Applied Ocean Science & Engineering, Cambridge and Woods Hole, Cambridge, Massachusetts, USA

[3]Virginia Institute of Marine Science, William & Mary, Gloucester Point, Virginia, USA

Correspondence
Cory A. Berger, Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA, USA.

Ann M. Tarrant, Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.
Email: cberger@ucsb.edu and atarrant@whoi.edu

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Abstract

Lipid-rich copepods form an essential link between primary producers and higher trophic levels in high-latitude oceans. These zooplankton can take advantage of ephemeral phytoplankton blooms to fuel development and reproduction. However, we have limited understanding of how the physiological condition of these animals varies in relation to environmental factors such as food availability. Due to high advection, it is likely that physiological plasticity, rather than local adaptation, is primarily responsible for physiological differences within a region. We use transcriptomics and other physiological metrics to understand how two species of copepods (*Calanoides acutus* and *Calanus propinquus*) vary across environmental gradients along the West Antarctic Peninsula. For the primarily herbivorous *C. acutus*, physiological separation between sampling locations appears to be driven by feeding status, and gene expression differences indicate differential expression of genes regulating lipid metabolism, reproduction, aerobic metabolism, and protein translation. For the more omnivorous *C. propinquus*, physiology and gene expression did not segregate as clearly by location, showed minimal signs of food deprivation at any location, and had a weaker relationship with chlorophyll compared to *C. acutus*. By comparing these results with concurrent starvation experiments, we find that spatial variation in gene expression reflects short-term differences in food availability (particularly for *C. acutus*), and we identify genes whose expression indicates recent feeding status. Further examination of the relationships between food availability, copepod physiology, and population dynamics will ultimately improve our capacity to predict how copepod populations will respond to rapidly changing environmental conditions in the West Antarctic Peninsula ecosystem.

KEYWORDS
Antarctic, copepod, metabolism, Southern Ocean, transcriptomics, zooplankton

TAXONOMY CLASSIFICATION
Ecophysiology

Cory A. Berger and Ann M. Tarrant contributed equally to this article.

1 | INTRODUCTION

In temperate and polar marine ecosystems, copepods in the family Calanidae are key consumers of phytoplankton and microzooplankton and often comprise the dominant component of the mesozooplankton biomass (Aarflot et al., 2018; Campbell et al., 2009; Froneman et al., 2000; Thibault et al., 1999). Copepods within this group can store large amounts of lipid in a specialized organ called the oil sac and can overwinter in a dormant state called diapause. These traits enable them to optimize feeding, growth, and reproduction during times of high food availability and to persist during times of low food availability (Baumgartner & Tarrant, 2017; Hirche, 1983; Sainmont et al., 2014). Due to this strategy of seasonal lipid accumulation, dormancy, and reproduction, these species can utilize transient phytoplankton blooms as a primary food source and are themselves a rich food source for predators, such as fish, seabirds, and baleen whales. High-latitude marine ecosystems are experiencing reduced sea ice cover, rising temperatures, and altered circulation patterns, which in turn affect the abundance, spatial distribution, taxonomic, composition, and phenology of the phytoplankton and zooplankton communities (Ardyna & Arrigo, 2020; Cimino et al., 2023; Dalpadado et al., 2020; Rogers et al., 2020). While some changes in copepod distributions have already been documented (Dalpadado et al., 2020; Johnston et al., 2022; Montero et al., 2021), understanding and predicting future changes will require an improved understanding of the metabolic strategies of individual species and their responses to changes in food availability on varied spatial and temporal scales.

Antarctic and sub-Antarctic calanid copepods exhibit particularly diverse strategies to optimize their use of highly variable food supplies in seasonal environments. *Calanoides acutus* relies heavily on large phytoplankton as a dietary source, and most individuals overwinter in diapause at depth (Schnack-Schiel et al., 1991). In contrast, *Calanus propinquus* has a comparatively omnivorous diet, can overwinter in surface waters, and is only weakly dependent on diapause (Bathmann et al., 1993; Pasternak et al., 2001; Schnack-Schiel et al., 1991). This opportunistic strategy enables *C. propinquus* to be abundant under sea ice during late winter, where it can obtain about half of its dietary carbon from ice algae-derived prey over short time scales (Kohlbach et al., 2016, 2018). *C. acutus* and *C. propinquus* also differ in their form of lipid storage (Hagen et al., 1993; Kattner et al., 1994; Ward et al., 1996). *C. acutus* stores lipids primarily in the form of wax esters, similar to the Arctic *Calanus* species. In contrast, *C. propinquus* (and the sub-Antarctic *Calanus simillimus*) primarily stores triglycerides. Despite these differences, *C. acutus* and *C. propinquus* are both broadly distributed throughout the Southern Ocean and have similar spatio-temporal patterns of abundance at large scales (Marin, 1988). Within the surface waters of the West Antarctic Peninsula (WAP), the focal region for this study, copepods comprise the dominant component of the surface (0–300 m) mesozooplankton during summer. *C. acutus* is among the most abundant large copepods in this region, and *C. propinquus* is much less abundant but comprises

a substantial portion of the mesozooplankton biomass due to its large size (Gleiber, 2014).

The Southern Ocean and Antarctic coastal seas have been experiencing pervasive changes in physical conditions, including temperature, circulation, and ice cover. The WAP is among the most rapidly warming regions on Earth, experiencing a 2.8°C increase in average annual air temperature from the 1950s to 2000 (Turner et al., 2016) and a decrease in the extent and seasonal duration of sea ice comparable to the greatest rates observed in the Arctic (Stammerjohn et al., 2012; Stammerjohn & Maksym, 2017). Corresponding changes in the distribution and abundance of Antarctic organisms have been documented at all trophic levels (reviewed by Constable et al., 2014; Rogers et al., 2020; Turner et al., 2014). Phytoplankton productivity and community composition have shown large interannual and regional variability (Schofield et al., 2017; Venables et al., 2013). The southern region has shown a trend toward increasing frequency of conditions favoring a shallow seasonal mixed layer depth, which is associated with large diatom blooms and increased primary production, whereas no trends are discernable in the northern region (Schofield et al., 2017, 2018). The abundance of copepods has increased in association with sea ice retreat and elevated chlorophyll *a* (chl *a*; Gleiber, 2014). However, it is unknown how lipid-storing copepods will respond to predicted reductions in the abundance of large phytoplankton, their major food source. Predicting responses of copepods to future environmental changes, including changes in phytoplankton abundance, can be informed by examining physiological variations in relation to current variations in environmental conditions.

Gene expression profiling is increasingly being used to infer physiological conditions within natural populations. Among pre-adults of the copepod *Neocalanus flemigeri* in the Gulf of Alaska, correlations of metabolic gene expression with food availability suggested a substantial capacity for physiological plasticity in response to patchy food supply (Roncalli et al., 2019). In contrast, *Calanus glacialis* pre-adults in the eastern Bering Sea showed relatively uniform expression of metabolic biomarkers, suggesting that they had experienced favorable growth conditions throughout the study area (Tarrant, Eisner, & Kimmel, 2021). While comparable molecular studies have not yet been conducted for Antarctic lipid-storing copepods, observations of egg production, lipid content and composition, and metabolic enzyme activity provide insight into their physiological condition. For example, within the marginal ice zone of the Weddell Sea, lipid content and citrate synthase activity in female *C. acutus* and *C. propinquus* were low at ice-covered stations and higher at ice-edge and ice-free stations experiencing phytoplankton bloom or post-bloom conditions (Geiger et al., 2001).

Although previous studies document the abundance of copepods within regions of the Southern Ocean (Conroy et al., 2023; Gleiber, 2014; Kasyan et al., 2022; Ward et al., 2018), less is known regarding how their physiological condition varies spatially and what environmental factors may drive these differences. These patterns may differ among species with different metabolic and life

history strategies, and physiological responses to environmental variation can inform predictions of how populations and species will respond to future environmental changes. We used transcriptomics, biochemical measurements, and visual observations to understand if and how spatial variation in food availability affects the physiology of adult females of two key copepod species in the Southern Ocean across environmental gradients within the WAP. We predicted that aspects of their metabolic and reproductive condition, particularly of the more herbivorous *C. acutus*, would correlate with environmental chlorophyll concentration and that aspects of condition would also correlate with local abundance of these species. In addition, we compared the observed physiological variation with responses to experimental feeding manipulations conducted at the same time (Berger et al., 2024) to more directly link gene expression patterns observed in the field with responses to food availability and to identify putative molecular markers of metabolic status in these species.

2 | METHODS

2.1 | Sampling

Zooplankton were sampled during austral summer 2019 aboard the ARSV *Laurence M. Gould* in association with sampling conducted by the Palmer Antarctica Long-Term Ecological Research (PAL-LTER) program. Samples were collected within the PAL-LTER sampling grid, which is positioned along the WAP and across the continental shelf (Figure 1a, Waters & Smith, 1992). Stations within the grid are designated by a pair of numbers indicating the distance along the shelf followed by the distance across the shelf (e.g., 600.000 is a northern, inshore station; 100.200 is a southern, offshore station; Figure 1). At each station, hydrographic properties were profiled using a CTD (SeaBird Electronics Seacat SBE 19plus sensor), and chl *a* concentration was profiled using a WET Labs ECO-FL fluorometer.

As part of the long-term monitoring of community composition, zooplankton were sampled via double oblique net tows with rectangular frame nets: macrozooplankton were sampled from 0 to 120 m using a 2 × 2 m net with 700-μm mesh, and mesozooplankton were sampled from 0 to 300 m using a 1 × 1 m net with 333-μm mesh. Aboard the ship, macrozooplankton samples were sorted taxonomically, the biovolume and numbers of copepods and other taxa were quantified, and whole or quantitative subsamples were preserved in buffered formalin (Ross et al., 2008; Steinberg et al., 2015).

To sample copepods for physiological and transcriptomic studies, tows were conducted to a depth of either 120 or 200 m using the 700-μm mesh net. Sampled copepods were predominantly adult females, and therefore only females were analyzed. Female copepods were contained in an ice-chilled bucket during sorting and individually photographed under a stereomicroscope. For transcriptional profiling, copepods were preserved in RNAlater at −20°C. For

citrate synthase activity, copepods were stored at −80°C. Samples were shipped to Woods Hole Oceanographic Institution on dry ice. Because the distributions of *C. propinquus* and *C. acutus* differed and *C. propinquus* was less abundant, the numbers of stations analyzed and samples per station differed between species for each measurement type, as subsequently described.

2.2 | Copepod abundance and environmental correlations

C. acutus and *C. propinquus* were enumerated within mesozooplankton samples from 11 stations, including all the stations at which gene expression was characterized. Samples were split using a Folsom Plankton splitter. For each species, a fraction ranging from 1/16 to the entire sample was counted, to either count at least 100 individuals or enumerate the entire sample. For these stations, correlations were examined among *C. acutus* and *C. propinquus* abundance, copepod abundance in the macrozooplankton samples, average temperature and average chl *a* concentration in the upper 200 m (averaged from discrete measurements at each meter of depth), latitude, and bottom depth. A matrix of Spearman's rank correlation coefficients was created using the rcorr function in the "hmisc" R package (Harrell Jr & Dupont, 2023). The strength and significance of correlations were visualized using the "corrplot" R package (Wei & Simko, 2021).

2.3 | Citrate synthase activity

Copepods were thawed on ice, blotted on a lint-free tissue, and weighed on a Cahn C-33 microbalance. Groups of 2–5 copepods were pooled into 300 μL of ice-cold buffer (25 mM Tris, pH 7.8, 1 mM EDTA, 10% glycerol) in a 5-mL Potter-Elvehjem homogenizer. Tissue was homogenized using a motorized PTFE pestle for two 30-s bursts with 30 s of ice cooling between bursts. Homogenates were centrifuged at 14,000 g for 20 min at 4°C, and the supernatant was retained. Citrate synthase activity was measured with a modification to the protocol of Hawkins et al. (2016) and normalized to wet weight, as described previously (Tarrant, McNamara-Bordewick, et al., 2021). Normality of the residuals and equality of variance were verified using the Shapiro–Wilk's test and Levene's tests, respectively. Differences among stations were tested using a t-test or one-way ANOVA; post-hoc pairwise comparisons were tested using the holm method, as implemented in the "multcomp" package (Hothorn et al., 2023).

2.4 | Photo analysis

Photographs were scored by two independent observers for the presence/absence of food in the gut and the degree of egg development. Egg development was scored in three categories ranging
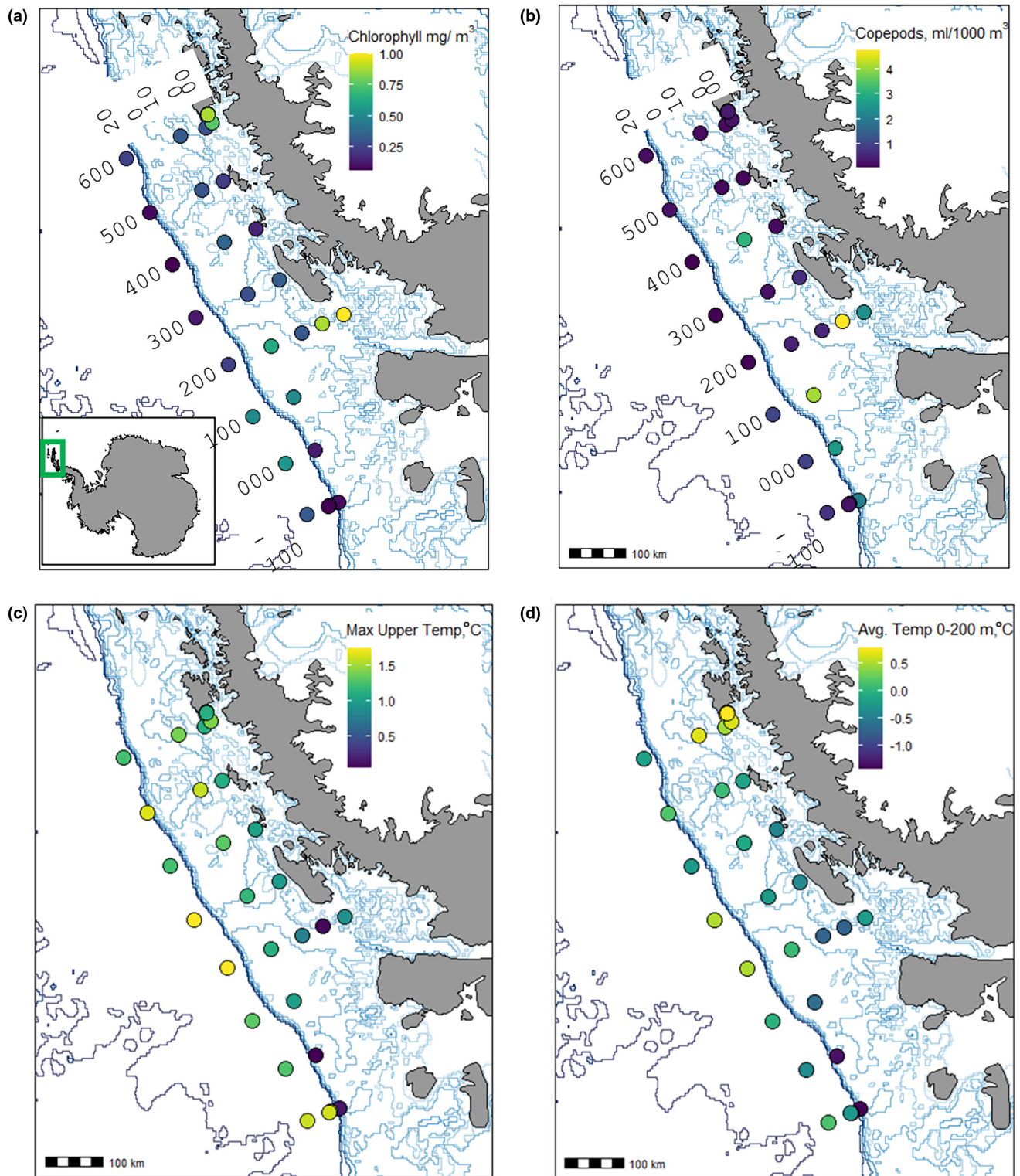
FIGURE 1 Spatial patterns in chlorophyll *a* concentration, copepod abundance, and temperature along the Western Antarctic Peninsula in January 2019. Numbers indicate grid of station names (see Section 2.1). Light blue lines indicate bathymetric contours, and heavy blue line indicates continental shelf break. (a) Mean chlorophyll *a* in the upper 200 m of the water column; green box in inset shows study area. (b) Total copepod biovolume (mL) per 1000 m³ seawater (sampled with 700 μm mesh net). (c) Maximum temperature (°C) within the upper 200 m depth. (d) Average temperature (°C) within the upper 200 m depth.

from 1 (no discernable eggs) to 3 (well-developed), using only photos in dorsal orientation for *C. acutus* and lateral orientation for *C. propinquus*. Photographs included copepods used for RNA-seq analysis

and other copepods from the same tow. All photographs of sufficient quality were included in the analysis (for *C. acutus*, n = 78/123 for food and reproduction, respectively; for *C. propinquus*, n = 55/49).

2.5 | RNA extraction, library construction, and sequencing

Total RNA was extracted from individual copepods using the Aurum Fatty and Fibrous Tissue Kit (Bio-Rad) without DNase treatment (because we have observed that omitting this step does not result in any observable DNA contamination; see also Berger et al., 2021). RNA yield and purity were measured using a Nanodrop Spectrophotometer. RNA was submitted to Arraystar Inc (Rockville, MD) for library construction and sequencing. Libraries were constructed using the KAPA Stranded RNA-seq Library Preparation Kit, and bar-coded libraries were sequenced with 150 base pair paired-end reads to a depth of 40M reads on an Illumina HiSeq 4000. The number of stations represented, number of libraries constructed per station, and number of individuals per library differed between species due to differences in their distribution and abundance, as well as financial constraints. For *C. acutus*, libraries were constructed from individual copepods; five libraries were sequenced from each of six stations. For *C. propinquus*, equivalent amounts of RNA from two individuals from the same station were pooled to create a sample for library construction, and RNA-seq was conducted on four pooled libraries from each of four stations. In addition, feeding experiments were performed for each species (detailed methods and results described in Berger et al., 2024). Experimental animals were collected from the field and either fed or starved onboard the ship, and they were sampled after 5 and 9 days for RNA-seq ($n = 4$ libraries per time point and treatment). Sequence quality was assessed using FASTQC (v0.11.7, Babraham Bioinformatics).

2.6 | Gene expression

Trimmed reads were mapped to the reference transcriptome for the appropriate species (Berger et al., 2024) using Salmon v1.1.0 (Patro et al., 2017) with the "gcBias," "seqBias," and "validateMappings" flags and summarized to the gene level using the R package "tximport" v1.20.0 (Soneson et al., 2015) with the "countsFromAbundance = lengthScaledTPM" option. The gene expression matrix of the field samples (i.e., excluding the experimental samples from Berger et al., 2024) was filtered to retain clusters with at least 15 counts in at least four libraries. This retained 49,025/110,415 clusters for *C. acutus* and 53,379/143,667 for *C. propinquus* (Files S1 and S2).

Sample distances were visualized using principal component analysis (PCA) with the "prcomp" R function. The PCA was performed on log-scaled counts per million (CPM) of all genes expressed above the threshold cutoff. Differential expression (DE) analysis was performed using "limma" v3.48.3 (Phipson et al., 2016) with quality weights (Liu et al., 2015). For *C. acutus*, contrasts were performed between two "station groups" with similar overall gene expression patterns: stations 200.000 and 200.040 formed the first group, and all other stations formed the second group (described further in Section 3.2). For *C. propinquus*, contrasts were performed between

each station and the mean of the other three stations. Genes were considered DE with an adjusted *p*-value below .05. Weighted gene co-expression network analysis (WGCNA) was conducted using the combined set of field-collected and experimental samples, as described in Berger et al. (2024). For *C. acutus*, a linear model ("lm" function in R) was used to compare module expression between station groups, and Pearson correlations were calculated between eigengene expression and environmental variables (log10 chl, temperature, and log10 abundance of *C. acutus*). For *C. propinquus*, module expression was compared across stations as in the DE analysis, but not with environmental variables because we judged that four field stations were too few to observe meaningful associations.

2.7 | GO enrichment

Gene ontology (GO) terms that were enriched among DE genes (DEGs) or WGCNA modules were identified using GO_MWU (Wright et al., 2015) with genes ranked by $\log_2$(fold change). Semantically similar terms were summarized using Revigo (Supek et al., 2011) with the "Small" setting (0.5 SimRel similarity threshold). In addition to exploratory analysis of all enriched GO terms, we also explicitly examined terms associated with *lipid metabolism* (GO:0006629), *protein metabolism* (GO:0019538), *reproduction* (GO:0000003), and *response to stress* (GO:0006950); descendant terms were retrieved using the R package GO.db v3.14.0 (Carlson, 2019).

2.8 | Comparison with experimental feeding manipulations

Gene expression patterns in field-collected animals were compared with animals that were incubated aboard the ship for up to 9 days in fed or starved conditions (Berger et al., 2024). $\log_2$(fold change) associated with field chl *a* measurements was correlated with $\log_2$(fold change) in the experimental response to starvation. We used discriminant analysis of principal components (DAPC) in the "adegenet" package v2.1.8 (Jombart, 2008) to test whether the discriminant axis between fed and starved animals also separated field samples. To identify possible biomarkers of feeding status, we selected genes with the most significant changes of expression associated with experimental starvation and apparently favorable feeding conditions in the field (stations 200.000 and 200.040; see Section 3.2). We selected biomarkers based on both experimental feeding and field data because useful biomarkers should be statistically variable (differentially expressed) among field samples. Putative biomarkers were required to have a minimum mean expression of 5 TPM10K, a $\log_2$(fold change) of at least 1 in the starvation experiment and the field comparison, and an unadjusted *p*-value of .001 in both tests. TPM10K is an expression measure similar to transcripts-per-million (TPM) except it also normalizes for the size of the transcriptome and is thus more comparable between species (Munro et al., 2022).

3 | RESULTS

3.1 | Environmental measurements and species distributions

Hydrographic profiling and zooplankton sampling were conducted at a grid of stations along the WAP in January 2019 (Table 1). Temperatures within the upper 200 m ranged from −1.84°C to 1.76°C, with the warmest maximum values in the outer slope stations and the warmest average temperatures in the northern inshore stations (Figure 1c,d). Chl *a* was highest at inshore stations along the 600 and 200 lines (Figure 1a). Moderately high chl *a* was observed in the southern half of the grid along the shelf and in slope waters. Physiological and transcriptomic measurements were made using *C. acutus* and/or *C. propinquus* sampled from a total of seven focal stations (6 stations *C. acutus*, 4 *C. propinquus*, 3 shared; Table 1). Among these stations, a northern inshore station (616.040) was characterized by warm water (>0.5°C) throughout the upper 200 m

and a strong surface chl *a* signal (Figure 2). Three stations (200.000, 100.180, and 000.100) had shallow (<50 m) mixed layers and pronounced thermoclines, with the warmest surface water at the slope station (100.180). The other stations were more deeply mixed and had cold surface water. Substantial subsurface chl *a* maxima (peak concentration > 4 mg/m^3) were present at 200.000 and 100.180, while 200.040 had moderately high chlorophyll (peak concentration 2.4 mg/m^3), and the other stations had lower chl *a* concentrations throughout the water column.

Overall abundance of large copepods was greatest at inshore, southern stations (Figure 1b). *C. acutus* and *C. propinquus* abundance was determined at 11 stations, including all the focal stations (Figure 3; Table 1). As expected, *C. acutus* was much more abundant than *C. propinquus*. Both species were more abundant at the southern stations, and the highest abundance for both was observed at a far southern station (−100.100) that was near the shelf break and partially covered with ice. Abundances of *C. acutus* and *C. propinquus* were positively correlated with one another (ρ = .62, *p* = .04;

TABLE 1 Locations, hydrographic properties, and copepod abundances at stations sampled for copepod physiological measurements (transcriptomics = T; citrate synthase = CS, neither = X) and/or species abundance.

Station	Latitude S	Longitude W	Date	Depth (m)	Max temp (°C)	Avg temp (°C)	Max Chl*a* (mg/m^3)	Avg Chl*a* (mg/m^3)
−100.100	68°49.171′	77°05.016′	01/23/19	637	0.15	−1.44	1.66	0.09
−100.160	68°22.875′	77°55.286′	01/25/19	3352	1.62	0.15	1.89	0.32
000.100	68°16.583′	75°07.077′	01/22/19	438	0.05	−1.34	1.09	0.13
100.180	67°09.441′	74°28.573′	01/21/19	3026	1.32	−0.02	3.81	0.51
200.-040	68°2.271′	69°18.376′	01/17/19	714	0.90	−0.21	7.71	1.00
200.000	67°46.319′	69°56.667′	01/17/19	750	0.08	−0.74	6.05	0.88
200.040	67°30.664′	70°35.377′	01/14/19	773	0.78	−0.73	2.36	0.32
400.040	66°15.237′	67°20.194′	01/10/19	270	1.03	−0.44	1.07	0.15
500.100	65°14.029′	66°46.558′	01/31/19	441	1.61	0.07	1.75	0.31
600.100	64°34.516′	65°20.483′	01/05/19	615	1.40	0.67	1.84	0.33
616.040	64°50.101′	64°09.763′	02/04/19	587	1.12	0.78	12.26	0.88

Station	Physiology		Abundance (individuals m^3)			Copepod biovolume (mL/1000 m^3)
	C. acutus	*C. propinquus*	*C. acutus*	*C. propinquus*	*R. gigas*	
−100.100	T, CS	T, CS	4516	30	133	2.10
−100.160	X	X	1568	6.7	628	0.76
000.100	T, CS	T. CS	1201	23	45	2.57
100.180	X	T	962	28	203	1.04
200.-040	X	X	532	1.1	24	2.41
200.000	T, CS	T	295	9.0	164	4.74
200.040	T, CS	X	1051	12	83	0.56
400.040	T	X	358	9.5	42	0.18
500.100	X	X	235	7.7	51	0.17
600.100	X	X	81	4.2	33	0.20
616.040	T	X	15	3.2	46	0.39

Note: Hydrographic measurements: Water column depth, maximum and average temperature in the upper 200 m, maximum and average chlorophyll concentration in the upper 200 m. Copepod biovolume and species abundance from 700- and 333-μm mesh nets, respectively. Species names: *Calanoides acutus*, *Calanus propinquus*, *Rhincalanus gigas*.

FIGURE 2 Depth profiles of (a) temperature and (b) chlorophyll _a_ for stations sampled for physiological measurements. Copepods were collected from the top 200 m of the water column.
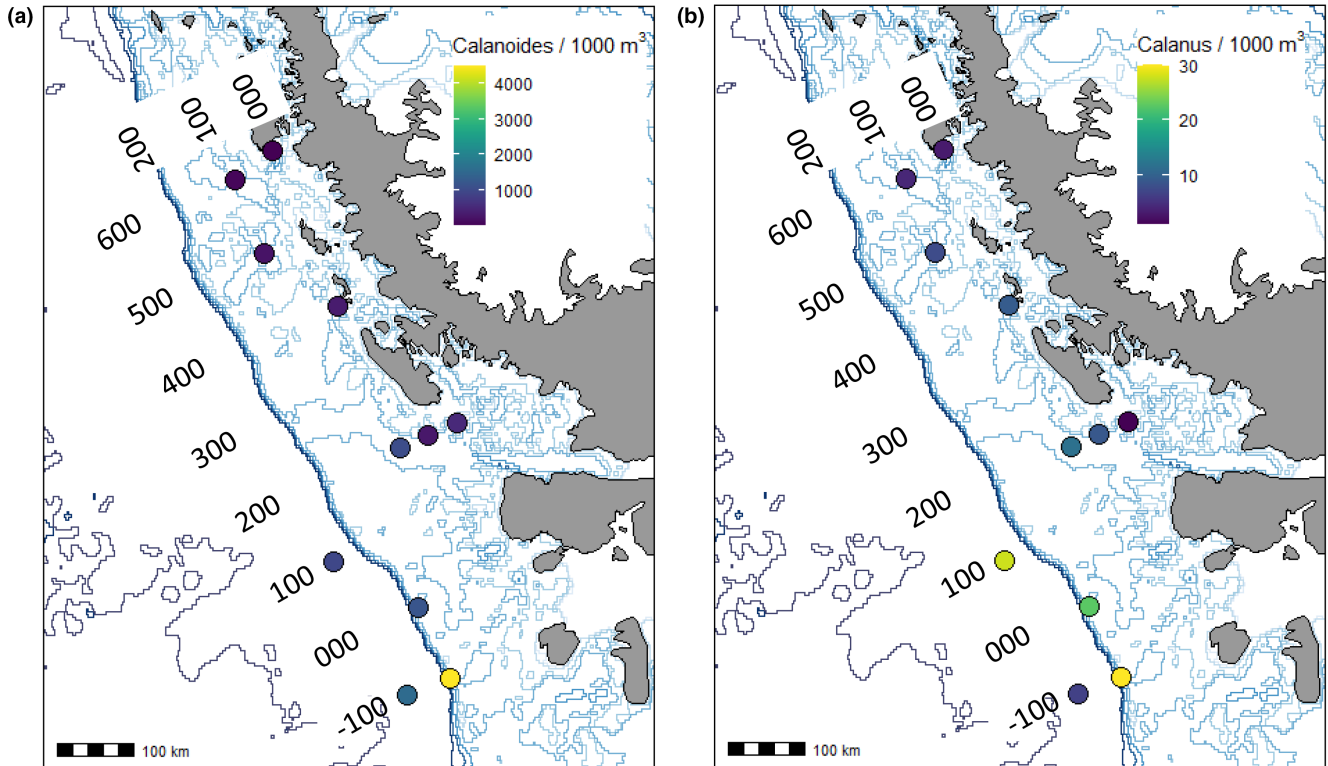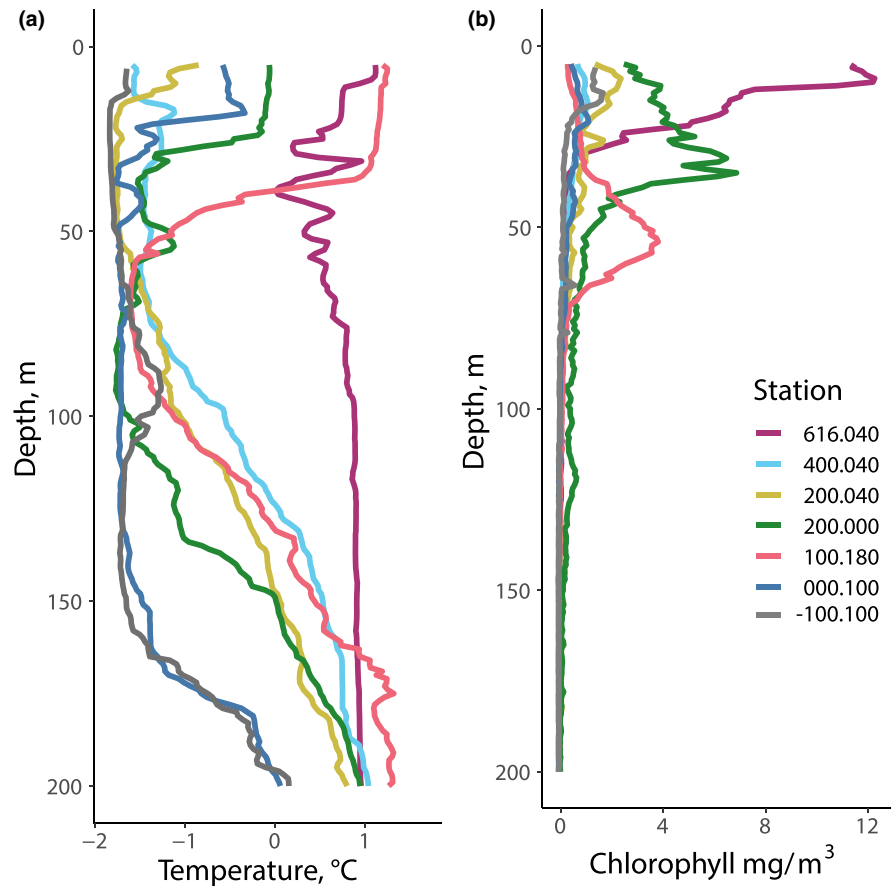

FIGURE 3 Spatial patterns in abundance of focal species along the Western Antarctic Peninsula in January 2019. Numbers indicate grid of station names (see Section 2.1). Light blue lines indicate bathymetric contours, and heavy blue line indicates continental shelf break. Abundance of _Calanoides acutus_ (a) and _Calanus propinquus_ (b) from a subset of stations sampled (with 333 μm mesh net), note difference in abundance scales.

Figure 4). *C.acutus* was more abundant at cooler, high-latitude stations, and *C.propinquus* was more abundant at cooler, low-chl *a* stations. Total copepod abundance within the mesozooplankton sample was positively correlated with latitude ($\rho = .71$, $p = .02$) but not with either of the focal species or any environmental factors (temperature, chl *a*, or depth; Figure 4).

3.2 | Physiological variation of *Calanoides acutus*

Principal component analysis (PCA) of *C.acutus* transcriptomic data revealed a pattern separating two mid-latitude inshore
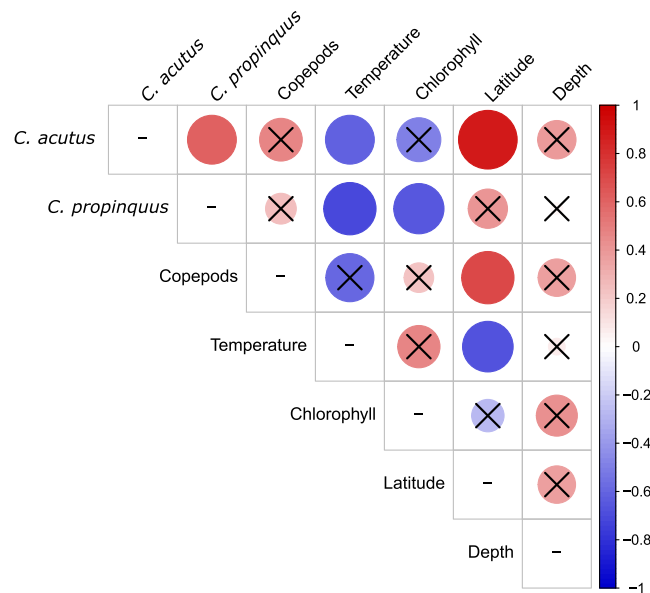
stations in Marguerite Bay (200.040 and 200.000, subsequently "200.xxx") from all others (Figure 5a), referred to as "station groups" hereafter. The 200.xxx stations exhibited intermediate temperatures and moderate-to-high chl *a* levels when averaged over the upper 200 m, from which the copepods were sampled. Station 200.000 had a warm (~0°C) surface layer, a pronounced thermocline around 25 m, and a strong chl *a* peak below the thermocline. Station 200.040 was partially ice-covered, had colder and less stratified surface water, and moderately high chl *a* that peaked near the surface. *C.acutus* abundance at these stations was low relative to the southern stations. Among the other stations, 000.180 and 400.040 also formed distinct clusters. While both of these stations had low chlorophyll levels, 000.180 was a well-mixed offshore site with relatively high *C.acutus* abundance, whereas 400.040 was a stratified inshore site with lower *C.acutus* abundance.

Food was visible within the guts of 35% of copepods from 200.xxx (15 of 43) but not in copepods from any of the other stations (0 of 35; X^2, $p < .01$; Figure 6a; Table 2). A higher proportion of copepods had well-developed eggs within 200.xxx (25%) versus other stations (15%), but this difference was not significant (Figure 6b; Table 2). Citrate synthase activity was higher in copepods from 200.xxx than in the two other stations for which measurements are available (ANOVA with Holm post-hoc test, $p < .05$; Figures 6c and 7; Table 3).

Because of the pronounced differences in physiology (Figure 6a–c) and gene expression (Figure 5a) between station groups, we focus on this contrast for the remainder of the manuscript. However, there may also be gene expression variation among sites within each station group (e.g., between 000.180 and 400.040). A pairwise contrast between station groups identified 12,053 differentially expressed genes (DEGs, adj.*p*-value <.05; Table 4), with 8712 genes upregulated at stations 200.xxx and 3341 downregulated. Genes with higher expression at stations 200.xxx were enriched for gene ontology (GO) terms related to lipid metabolism, protein metabolism, reproduction, and response to stress (Table 5, File S1). Upregulated genes within *regulation of lipid metabolic process* included genes with conserved roles in regulating lipid homeostasis, such as an E75-like nuclear receptor, and NF2L1 homolog (Cáceres et al., 2011;

FIGURE 4 Heatmap showing Spearman's correlations between pairs of measurements of copepod abundance and environmental parameters. From left to right: *Calanoides acutus* abundance, *Calanus propinquus* abundance, total copepod biovolume, average upper water column (0–200 m) temperature, average (0–200 m) chlorophyll *a* concentration, latitude, and water depth. Black crosses indicate non-significant comparisons ($p > .05$). Color scale and relative size both indicate correlation coefficient sign and magnitude.
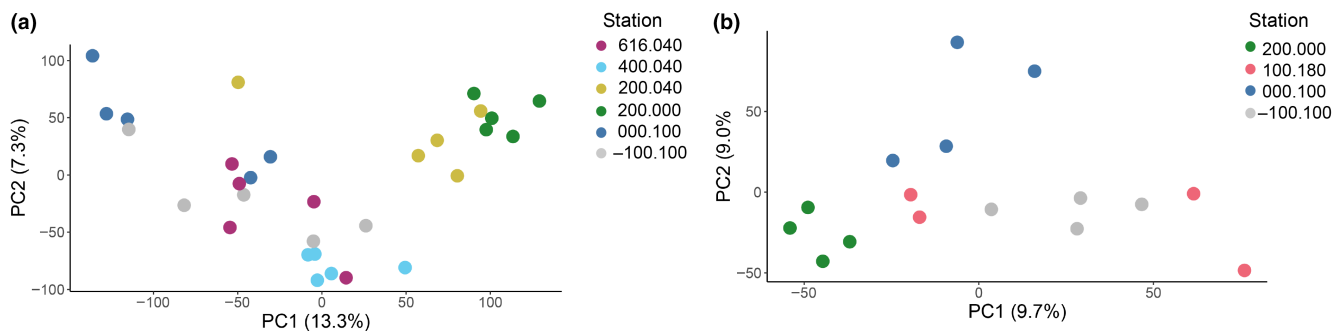

FIGURE 5 Overview of *Calanoides acutus* and *Calanus propinquus* gene expression patterns by station. Principal component analysis of (a) *C.acutus* and (b) *C.propinquus* gene expression. Axis labels show percent variation in gene expression explained by each principal component. Each point indicates a single library. Colors indicate field stations.

FIGURE 6 Physiological characteristics of *Calanoides acutus* (left) and *Calanus propinquus* (right). *C. acutus* data pooled by station group (see text). (a, d) Number of individuals having or lacking food in gut (in (a) no animals from "Other" stations had food in gut); (b, e) Relative egg development (see Figure 7 for examples); (c, f) Citrate synthase activity, Units per mg wet weight. The line in the box indicates the median, and the box encompasses the 25th through 75th percentiles of the data.
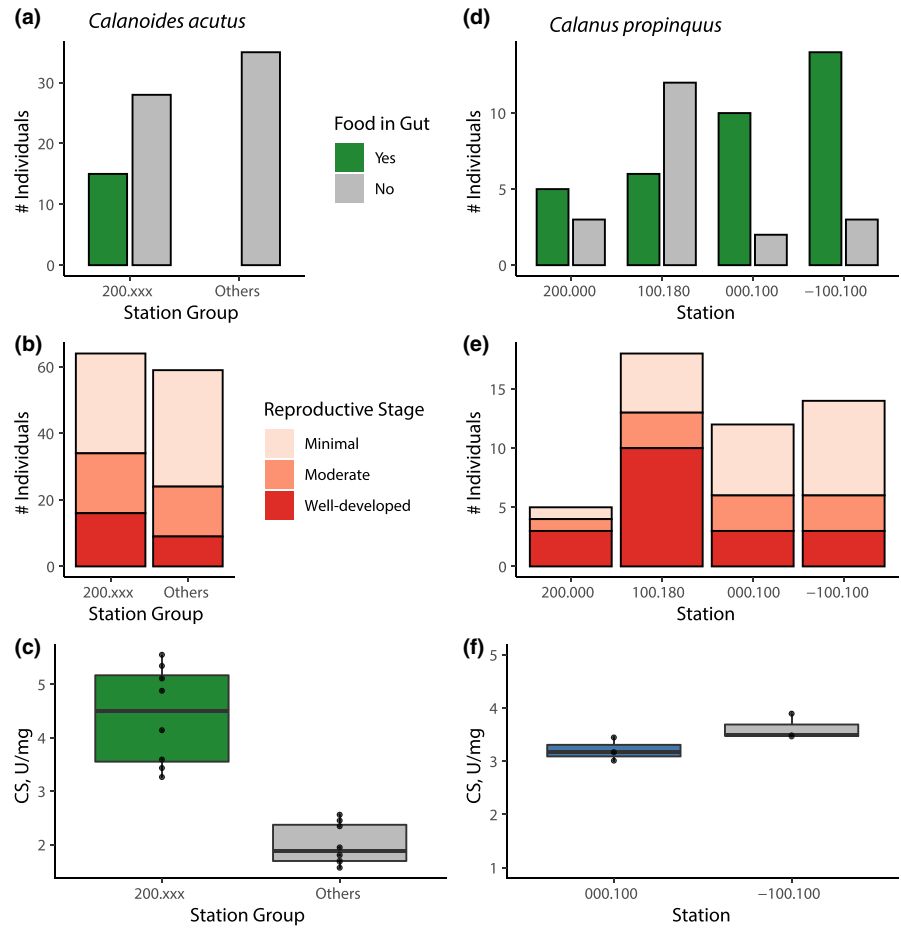
Hirotsu et al., 2012), but also a zinc finger protein, prosaposin and alkaline ceramidase, which have less certain roles in metabolic regulation. Among the stress-related genes, *response to hypoxia* was enriched. Among the 104 upregulated genes associated with this term were hypoxia inducible factor 1A, catalase, soluble guanylate cyclases, and 85 genes annotated as "REI-silencing transcription factor." Genes with lower expression at stations 200.xxx were enriched for GO terms associated with protein metabolism (*cytoplasmic translation*, *regulation of translation*, *proteolysis*, *protein ubiquitination*, and *mitochondrial translation*) and stress response (*DNA repair* and *cellular response to stress*). Examples include small ribosomal subunit proteins, DNAJ chaperone proteins, and various protein kinases. No terms enriched among downregulated genes were associated with lipid metabolism or reproduction.

The list of DEGs was inspected for genes previously associated with lipid synthesis and reproduction in *Calanus* spp., as well as the DEGs with the largest fold-changes. Very-long chain fatty acid elongases (ELOV/ELVL), fatty acid desaturases (also called acyl-CoA desaturases, "desaturases" hereafter), and fatty acid binding proteins (FABPs) are involved in the synthesis and transport of storage lipids; these genes are subsequently referred to as "lipid-associated genes." Many of these genes were upregulated at stations 200.xxx (12 ELOV/ELVLs, 13 desaturases, and 17 FABPs), and only a single FABP was downregulated at these stations (Table 6). Three desaturases were among the most strongly upregulated genes, and the

upregulation of desaturases and other putative lipid synthesis genes is consistent with elevated lipid synthesis by copepods in better feeding conditions at these stations. Genes annotated as digestive lipases (gastric or pancreatic lipase) showed both directions of regulation, but one "gastric triacylglycerol lipase" was strongly upregulated. Vitellogenins are lipoproteins that are major components of egg yolk. Six vitellogenins were upregulated at stations 200.xxx; none were downregulated. Genes that were strongly upregulated at 200.xxx relative to other stations included five ubiquitin oxidoreductases, a cytochrome c oxidase, and ATP synthase. Genes that were strongly downregulated at 200.xxx included FTZ-F1, Fem1, arginine kinase, a DNAJ family member, 2 phenoloxidase activating factors, a cytochrome P450 family 4c3 homolog, and thioredoxin.

Weighted gene co-expression network analysis identified 25 groups of genes (modules) with highly correlated expression across a combined dataset of the field samples described here and samples from an associated shipboard experiment (Berger et al., 2024). Module eigengenes were associated with environmental conditions through Pearson correlations, and with station groups with a linear model (Figure 8). As described below, we identified modules for which expression was correlated with station group and/or chl *a* concentration. No modules were correlated with either temperature or *C. acutus* abundance.

Eigengenes of eight modules had higher expression at stations 200.xxx relative to the remaining stations ($p < .05$). Of the 4051

upregulated and annotated genes, 85% were assigned to one of three modules: Turquoise, Greenyellow, or Red; the Turquoise and Greenyellow modules were also positively correlated with chl _a_. The Turquoise module was the largest module; in the station-group comparison, it included 2938 upregulated annotated genes, 11 lipid-associated genes, and all the differentially expressed vitellogenins. GO terms enriched in the Turquoise module corresponded to diverse processes, including DNA metabolism, regulation, and methylation; regulation of reproductive processes; cellular responses to stimuli; and muscle structure development (see Berger et al., 2024, for full GO enrichment results of WGCNA modules). The Greenyellow module included two lipid-associated genes and was enriched for GO terms related to translation and biosynthesis of amides/organo-nitrogen compounds. The Red module included 14 lipid-associated genes and was enriched GO for terms related to lipid and fatty acid synthesis, ion transmembrane transport, and amino acid transport and metabolism.

Eigengenes of seven modules were downregulated in stations 200.xxx relative to the other stations. Of the 2507 downregulated and annotated genes, 74% were assigned to one of three modules: Yellow, Black, or Magenta; the Yellow module was also negatively correlated with chl _a_. The Yellow module contained over half of the downregulated genes with annotation (1309 genes) and was associated with enriched GO terms related to RNA processing and metabolism, synthesis and catabolism of proteins and other macromolecules, mitochondrial respiratory chain assembly, and aerobic respiration. The Black module was enriched with terms related to cell division, RNA processing, and protein ubiquitination. The Magenta module was enriched for terms related to DNA replication and repair, cellular response to stress, intracellular transport, and RNA metabolism.

TABLE 2 _Calanoides acutus_ observations of food in gut and reproductive condition. Note that sample sizes reflect the photographs of sufficient quality to evaluate, which differed depending on the observation type.

Station	Food in gut	Reproduction[a]
−100.100	Yes: 0, No: 10, _N_: 10	Min: 12, Mod: 8, WD: 2, _N_: 22
000.100	Yes: 0, No: 12, _N_: 12	Min: 18, Mod: 3, WD: 4, _N_: 25
200.000	Yes: 7, No: 5, _N_: 12	Min: 16, Mod: 13, WD: 6, _N_: 35
200.040	Yes: 8, No: 23, _N_: 31	Min: 14, Mod: 5, WD: 10, _N_: 29
400.040	Yes: 0, No: 4, _N_: 4	Min: 1, Mod: 0, WD: 1, _N_: 2
616.040	Yes: 0, No: 9, _N_: 9	Min: 4, Mod: 4, WD: 4, _N_: 10

Station Group	Food in Gut	Reproduction[a]
200.000 & 200.040	Yes: 15, No: 28, _N_: 43	Min: 30, Mod: 18, WD: 16, _N_: 64
Others	Yes: 0, No: 35, _N_: 35	Min: 35, Mod: 15, WD: 9, _N_: 59

[a]Reproductive categories: Min = Minimal egg development, Moderate egg development = Moderate, WD = Well-developed eggs ("_N_" denotes sample size).

3.3 | Physiological variation of _Calanus propinquus_

For _C. propinquus_, transcriptomic data from the four stations sampled clustered into three groups: one with samples from 200.000, a second with samples from 000.100, and a third with samples from both 100.180 and −100.100 (Figure 5b). Stations 100.180 and −100.100 had the highest _C. propinquus_ abundances (Figure 3b), but they did not share other similar environmental characteristics. Station
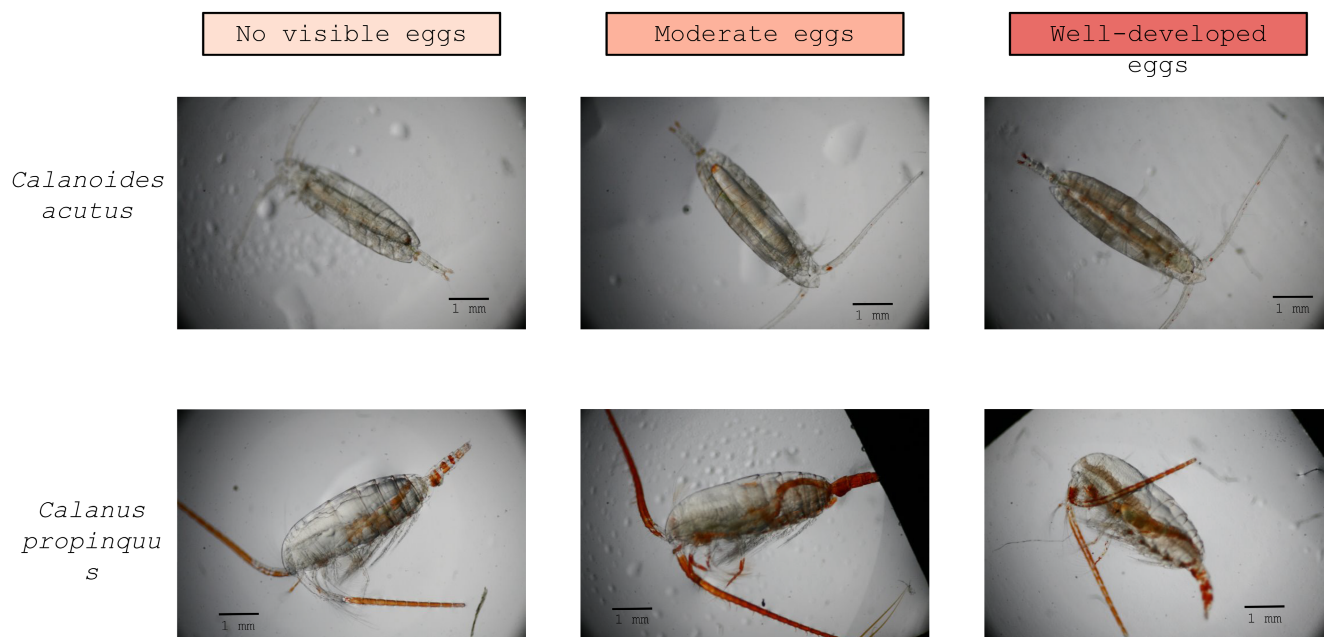

FIGURE 7 Representative images showing relative stages of egg development for _Calanoides acutus_ (top) and _Calanus propinquus_ (bottom).

100.180 had warm surface water, a pronounced thermocline, and a strong subsurface chl *a* peak. In contrast, Station −100.100 was cold and well-mixed with relatively low chl *a* (Figure 2).

Physiological observations did not provide any consistent basis for grouping stations. Food was visible within the guts of most individuals from three of the four stations (29 of 37; 63%–83% per

TABLE 3 ANOVA and post-hoc statistics for *Calanoides acutus* citrate synthase measurements.

Measurement	df	F	p-value
Citrate synthase	3, 12	8.07	<.001
Contrast station pair	**T**	**df**	**p-value**
200.000 vs. 000.100	5.242	6	.001
200.000 vs. −100.100	5.750	6	.001
200.040 vs. 000.100	3.920	6	.006
200.040 vs. −100.100	4.427	6	.003

TABLE 4 Differentially expressed genes between *Calanoides acutus* station groups (200.000 and 200.040 = 200.xxx vs. all other stations).

	All	Annotated	Up Starve[a]	Up Fed[a]
Up 200.xxx	8712	4051	4	1674
Down 200.xxx	3341	2507	846	3
Total	12,045	6558	850	1677

[a]Genes that were also differentially expressed in an associated feeding experiment (Berger et al., 2024).

station), but a minority of individuals from 000.100 (6 of 18, 33%; Figure 6; Table 7). Station 100.180 had the highest proportion of copepods with well-developed eggs (56% vs. 20%–25% in other stations; Figure 6e; Table 7). Due to limited specimen availability, citrate synthase activity was only measured at two stations; though the mean value was higher at −100.100 than at 000.100, this difference was not significant (*t*-test, $t = -2.1547$, df = 4, *p*-value = .09747; Figure 6f).

Because gene expression and physiological observations did not provide a consistent basis for grouping stations, gene expression from each station was compared with the mean expression from the other three stations (Table 8; File S2). Relatively few DEGs were identified in any comparison, with the largest sets associated with 000.100 (282 upregulated, 94 downregulated) and 200.000 (137 upregulated, 35 downregulated). Among these, differential expression of lipid-associated genes and vitellogenins was only observed at 200.000, where we observed upregulation of four elongases, a desaturase, and two vitellogenins (Table 6). This result is qualitatively similar to *C. acutus*, for which we observed upregulation of several lipid-associated genes and vitellogenins at Stations 200.xxx.

As with *C. acutus*, we identified enriched GO terms related to lipid metabolism, protein metabolism, reproduction, and response to stress (Table 9). GO terms associated with higher gene expression at 200.000 included those related to dietary lipid metabolism, regulation of histone methylation, and meiosis. These processes were similarly regulated in *C. acutus* at Stations 200.xxx, though the specific enriched GO terms were different. These gene expression patterns may suggest favorable feeding conditions at 200.000. Other patterns in *C. propinquus* included an apparent upregulation of translation-related genes at 000.100 and downregulation at

TABLE 5 *Calanoides acutus* Gene Ontology terms enriched in the gene expression contrast between stations 200.040 and 200.000 (200.xxx) and all other stations.

Category	Up in 200.Xxx	Down in 200.Xxx
Lipid metabolism	Regulation of lipid metabolic process, regulation of lipid biosynthetic process	None
Protein metabolism	Histone modification, histone deacetylation, regulation of histone methylation, positive regulation of cysteine-type endopeptidase activity involved in apoptotic process	Cytoplasmic translation, regulation of translation, proteolysis, protein ubiquitination, mitochondrial translation
Response to stress	Negative regulation of oxidative stress-induced cell death	DNA repair, *cellular response to stress*
Reproduction	Meiotic gene conversion, mating behavior, *ovarian follicle cell development*, *positive regulation of reproductive process*	None

Note: Selected metabolic categories are shown; for full results see File S1. Italicized terms were collapsed during REVIGO summarization.

TABLE 6 Differentially expressed lipid-associated genes and vitellogenins in the copepods *Calanoides acutus* and *Calanus propinquus*.

Species	Calanoides acutus		Calanus propinquus	
Contrast/gene category	Up 200.xxx	Down 200.xxx	Up 200.000	Down 200.000
Elongase (ELOV or ELVL)	12	0	4	0
Desaturase (Acyl-CoA or fatty acid)	13	0	1	0
Fatty acid-binding protein (FABP)	15	3	0	0
Vitellogenin or Vitellogenin receptor	6	0	2	0

200.000, as well as downregulation of reproductive processes and several stress-related processes at −100.000.

Linear models were used to associate WGCNA eigengenes with each site compared to the mean of all other sites (Figure 9). Consistent with the relatively large number of DEGs, the largest number of modules (8) was associated with 000.100. Upregulated modules were enriched for GO terms related to nucleolus/RNP complexes (Lightgreen module), translation (Darkturquoise), and RNA processing and protein folding (Salmon). Downregulated modules were enriched for terms related to ribosomes and mitochondria

(Greenyellow), chromatin replication (Lightyellow), triglyceride metabolism and ion transport (Midnightblue), and carboxylic acid, amino acid, and carbohydrate metabolism (Royalblue).

3.4 | Correlation of field chlorophyll _a_ measurements and experimental starvation response

In an accompanying paper, we measured gene expression changes in _C. acutus_ and _C. propinquus_ in response to short-term (9 days) starvation
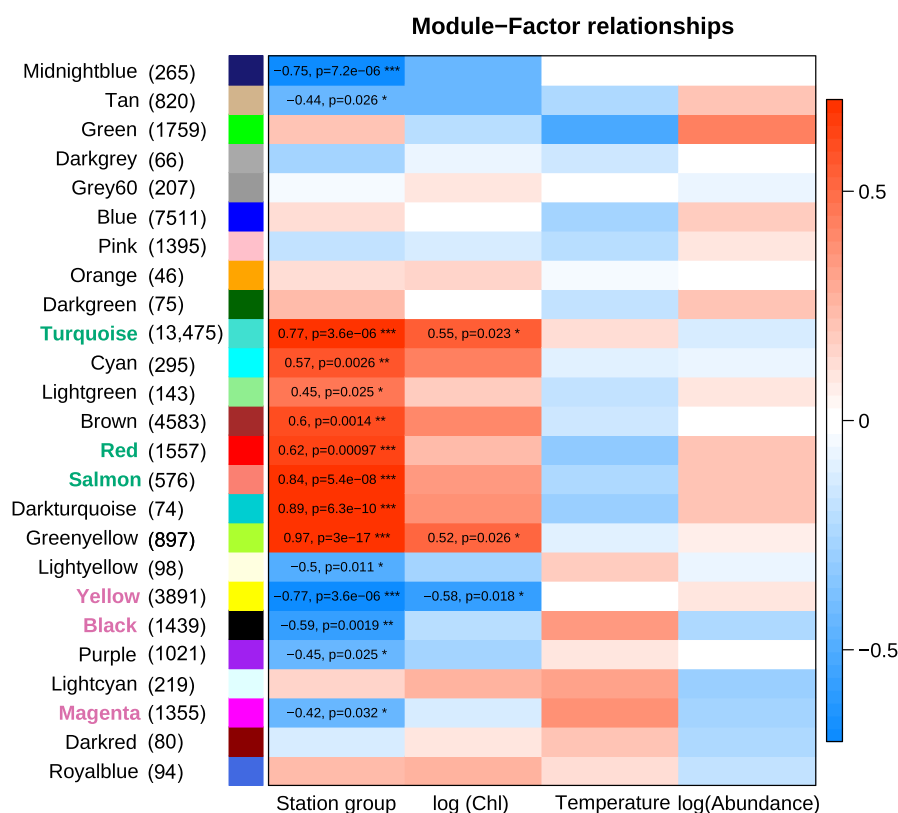

FIGURE 8 Heatmap showing _Calanoides acutus_ WGCNA module eigengene expression associated with collection station (station group) and ecological factors (log average chl _a_ in upper 200 m, average water temperature upper 200 m, log _C. acutus_ abundance). Module labels are colored to indicate comparison with results from a shipboard feeding experiment; modules with green labels were upregulated in fed animals, and modules with pink labels were upregulated in starved animals (see also Berger et al., 2024). Numbers indicate the number of genes within each module. Eigengene expression was regressed against factor values. Colors represent the correlation coefficient and direction of regulation: Red indicates positive correlations or upregulation at station group 200.xxx, blue negative correlations or downregulation at station group 200.xxx. Values of significant correlation coefficients are shown, along with the adjusted _p_-value from the linear model (*_p_ < .05; **_p_ < .01; ***_p_ < .001).

TABLE 7 _Calanus propinquus_ observations of food in gut and reproductive condition. Note that sample sizes reflect the photographs of sufficient quality to evaluate, which differed depending on the observation type.

Station	Food in gut	Reproduction[a]
−100.100	Yes: 14, No: 3, _N_: 17	Min: 8, Mod: 3, WD: 3, _N_: 14
000.100	Yes: 10, No: 2, _N_: 12	Min: 6, Mod: 3, WD: 3, _N_: 12
100.180	Yes: 6, No: 12, _N_: 18	Min: 5, Mod: 3 WD: 10, _N_: 18
200.000	Yes: 5, No: 3, _N_: 8	Min: 1, Mod: 1, WD: 3, _N_: 5

[a]Reproductive categories: Min = Minimal egg development, Moderate egg development = Moderate, WD = Well-developed eggs ("_N_" denotes sample size).

(Berger et al., 2024). In both species, the fold-change of genes that were DE in starved animals was correlated with the fold-change associated with field chl *a* in the present study (Spearman's $\rho = .73$ in *C. acutus*, $\rho = .44$ in *C. propinquus*; Figure 10), suggesting that transcriptomic differences between high- and low-chl *a* stations might recapitulate the starvation response. To investigate this, we used discriminant analysis of principal components (DAPC) to examine whether field populations separated along the first discriminant axis between fed and starved experimental samples. This was the case for *C. acutus*, as low-chl *a* stations overlapped with or were close to the starved samples, and high-chl *a* sites overlapped with fed samples (Figure 11a; the exception being Site 616.040; see Section 4). However, all field *C. propinquus* samples were close to the fed samples along the first discriminant axis, consistent with our observations that most *C. propinquus* had food in the gut (Figure 11b).

For *C. acutus*, we noted that gene expression changes between station groups broadly reflected the experimental starvation response. The correlation between $\log_2$(fold change) of DE genes in the starvation experiment and $\log_2$(fold change) between station groups was $\rho = .79$, slightly stronger than the correlation with chl *a* itself. Of 3687 DEGs in the starvation experiment, 2527 (69%) were also DE between station groups. Of these, >99% (2520/2527) were expressed in the direction consistent with their experimental starvation response (i.e., genes up-/downregulated at stations 200.xxx were also up-/downregulated in fed animals; Table 4). This overall similarity was also reflected in the WGCNA analysis, as the Red, Salmon, and Turquoise modules were upregulated in fed animals and at stations 200.xxx, and the Black, Magenta, and Yellow modules were downregulated in fed animals and at stations 200.xxx (Figure 8).

For *C. propinquus*, we identified fewer DEGs within both the field study and experiment. Of 1186 DEGs from the starvation experiment, only 27 were also DE in any comparison among stations. Of these, most (20) were upregulated at 200.000 and in the fed group. WGCNA showed some concordance between field chl *a* and experimental starvation, as the Ivory, Darkorange, and Purple modules were downregulated in fed animals and at station 200.000. The Royalblue module was upregulated in fed animals and at station 200.000 but downregulated at 000.100 (Figure 9). Thus, although *C. propinquus* had a weaker association between field chl and starvation overall, gene expression patterns suggest that animals at site 200.000 were somewhat better fed than other sites, because they have reduced expression of starvation-associated WGCNA modules and higher expression of some lipid-associated genes.
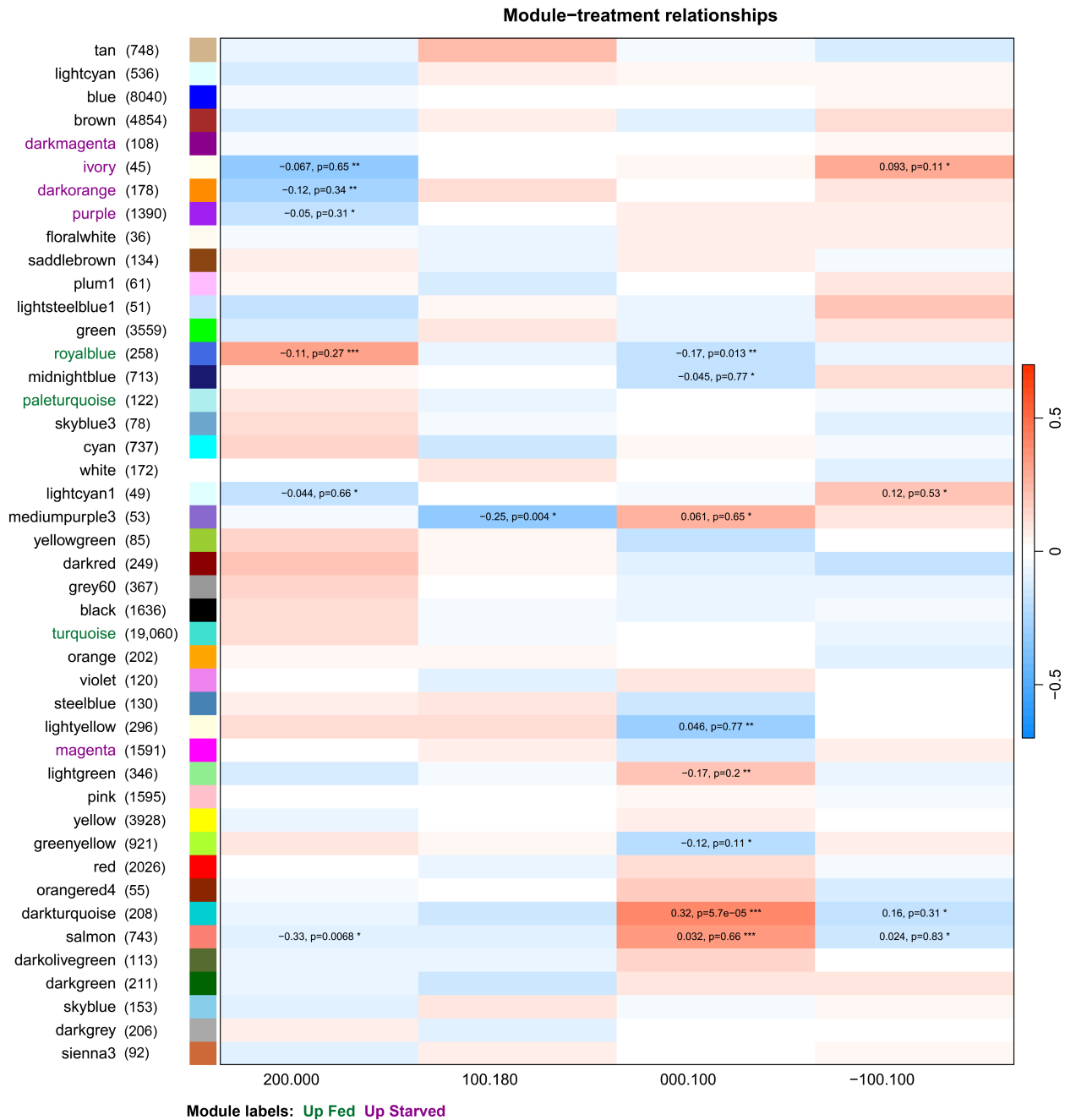
TABLE 8 Differentially expressed genes among *Calanus propinquus* stations.

Station	Up[a]	Down[a]
200.000	137	35
100.180	22	0
000.100	282	94
−100.100	36	4

[a]Expression at each station was compared with mean expression across all other stations.

TABLE 9 *Calanus propinquus* Gene Ontology terms enriched in the gene expression contrast between each station and the mean of all other stations.

Station	Upregulated	Downregulated
200.000	L: very long-chain fatty acid metabolic process, regulation of steroid metabolic process P: regulation of histone methylation, histone H3-K9 methylation S: response to ischemia, S/R: double-strand break repair involved in meiotic recombination R: positive regulation of meiotic cell cycle	P: negative regulation of histone H3-K3 methylation, translation S: positive regulation of double-strand break repair via nonhomologous end joining
100.180	R: border follicle cell migration	
000.100	L/P: dolichol-linked oligosaccharide biosynthetic process P: translation, regulation of translation, proteasomal ubiquitin-independent protein catabolism, protein modification by small protein conjugation or removal S: regulation of transcription from RNA polymerase II promoter	
−100.100	P: regulation of protein phosphorylation S: response to laminar fluid shear stress	L: regulation of steroid metabolic process P: translational initiation, histone deacetylation, regulation of histone methylation S: response to hypoxia, response to ischemia, cellular response to DNA damage stimulus, regulation of oxidative stress-induced cell death; R: multi-organism reproductive process

Note: Selected metabolic categories shown: Lipid- and reproduction-associated genes, and enriched GO terms associated with lipid metabolism (L), response to stress (S) and reproduction (R). For full results see File S2.

FIGURE 9 Associations of _Calanus propinquus_ gene expression with collection stations. Heatmap showing WGCNA module eigengene expression associated with collection station. Module labels are colored to indicate comparison with results from a shipboard feeding experiment modules with green labels were upregulated in fed animals, and modules with pink labels were upregulated in starved animals (see also Berger et al., 2024). Numbers indicate the number of genes within each module. Eigengene expression was regressed against factor values. The color represents the correlation coefficient and direction of regulation: Red indicates upregulation relative to the mean expression across all other stations, and blue indicates downregulation. For significant correlations, the value is shown along with the adjusted p-value from the linear model ($*p < .05$; $**p < .01$; $***p < .001$).

3.5 | Putative biomarkers of feeding status

We next sought to identify genes that might reliably indicate feeding status by selecting genes with the most significant changes in expression in both the starvation experiment and the field study. In the field study, we selected genes that were DE at stations with

signatures of favorable feeding conditions: 200.xxx for _C. acutus_ and 200.000 for _C. propinquus_. After applying filtering criteria based on expression, effect size, and significance in both studies (see Section 2), we identified 183 such genes in _C. acutus_ and 25 in _C. propinquus_. Two homologous genes were found in both lists: a vitellogenin, which had one copy in _C. propinquus_ and two paralogs in

FIGURE 10 Correlation (Spearman's) between the fold-change associated with chlorophyll a concentration in the field (*y*-axis) and the fold change associated with starvation in *Calanoides acutus* (left) and *Calanus propinquus* (right); starvation response taken from Berger et al. (2024).
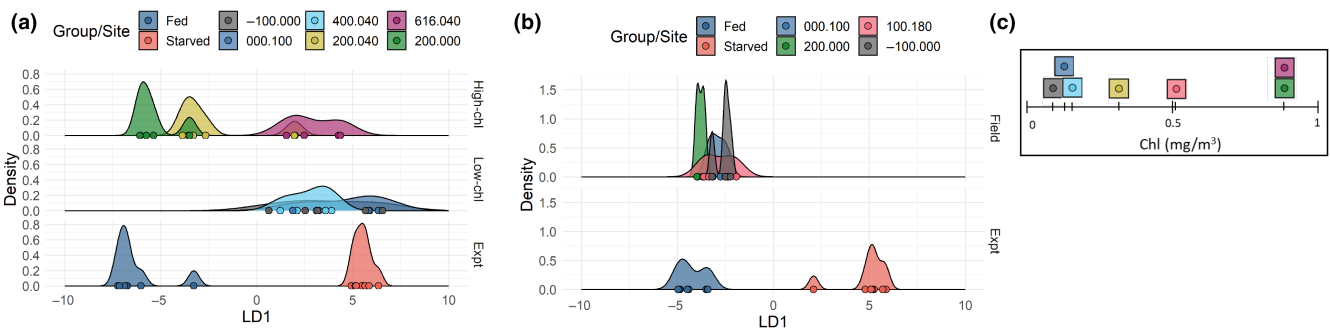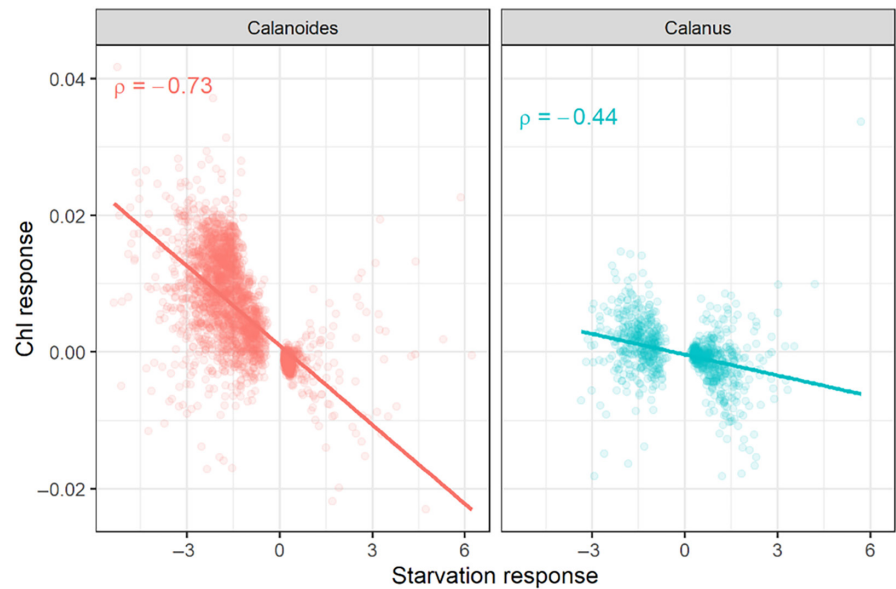

FIGURE 11 Comparison of transcriptomic patterns from field stations and starvation experiments using discriminant analysis of principal components for (a) *C. acutus* and (b) *C. propinquus*. LD1 is the linear discriminant axis that maximizes variation between Fed and Starved samples within an experiment ("Expt", bottom plot). (a) Stations 200.xxx overlap with Fed samples, and low-chlorophyll *a* stations overlap with Starved samples. The three highest-chl *a* (top) and three lowest-chl *a* stations (middle) are shown separately for clarity. (b) All *C. propinquus* field stations cluster with Fed samples. (c) Field stations ordered according chl *a* concentration (averaged over top 200 m).

C. acutus, and a methyltransferase, also with one copy in *C. propinquus* and two copies in *C. acutus*.

In *C. acutus*, 182 of 183 putative biomarkers were upregulated in fed animals; the downregulated biomarker was annotated as an enoyl-CoA hydratase, which is involved in fatty acid catabolism. Upregulated biomarkers included several genes with roles in amino acid catabolism (an L-threonine 3-dehydrogenase, a proline dehydrogenase, a phenylalanine/tryptophan hydroxylase), a gene that catalyzes a step in fatty acid degradation (inorganic pyrophosphatase; Chandel, 2021), and genes with key roles in lipid synthesis (desaturases and an elongase; these are among the "lipid-associated genes" in Table 6). In *C. propinquus*, 19 of 25 biomarkers were upregulated in fed animals, including several possible hemerythrins (oxygen-binding proteins) and a tricarboxylate transfer protein. Downregulated biomarkers included a CREB-like gene, triacylglycerol lipase, and phosphatidate phosphatase. This last gene catalyzes a rate-limiting step in triglyceride synthesis and also has roles in lipid signaling (Brindley, 1984; Chandel, 2021).

4 | DISCUSSION

Field-based studies of gene expression have the potential to identify environmental drivers of physiological variation among marine organisms. However, field studies can be challenging to interpret because environmental factors are confounded with many other (possibly unobserved) variables. One promising approach is to pair field studies with experiments to determine how specific factors, such as food availability, contribute to physiological variation in situ. In this study, we assayed patterns of gene expression and other physiological metrics across stations within the PAL-LTER sampling grid for two key species of Southern Ocean zooplankton, *Calanoides acutus* and *Calanus propinquus*. By comparing field data with starvation experiments conducted on the same research cruise (Berger et al., 2024), we provide evidence that field chl *a* concentration generally reflects local food availability on a timescale of ~1 week, and that variation in food availability is a major factor associated with copepod physiological variation among sites, especially for the largely herbivorous *C. acutus*. We also identify candidate biomarker genes

whose expression reliably indicates feeding status. While we have identified some similarities in the physiological patterns between the two species, we found female _C. acutus_ to be more sensitive than _C. propinquus_ to short-term variation in food availability and to exhibit more spatial variation in physiological condition. These properties are consistent with a strong reliance of _C. acutus_ on continuous feeding on phytoplankton to support egg production.

4.1 | Strong separation based on feeding condition between sites for an herbivorous grazer

For _C. acutus_, we focused our analysis on two stations that formed a distinct cluster in the gene expression analysis and were differentiated from other sites physiologically. Stations 200.000 and 200.040 both had moderate-to-high chl _a_ in the upper 200 m, and copepods at these sites were actively feeding and had elevated citrate synthase activity, indicating elevated aerobic metabolism. The stations are both located within Marguerite Bay, an area of high productivity that sustains large zooplankton populations and is an important foraging ground for penguins, whales, and other large predators (Casanovas et al., 2015; Deibel & Daly, 2007; Rozema et al., 2017; Siegel et al., 2013). No _C. acutus_ from any other site was observed with food in its gut, despite one other site having comparably high _chl a_. There was strong concordance between starvation-response genes determined experimentally (Berger et al., 2024) and genes that were differentially expressed between station groups, indicating that separation in gene expression space was largely driven by differences in feeding status. High expression of lipid synthesis enzymes at these stations suggests active lipid synthesis in food-replete conditions, while upregulation of vitellogenins and other genes related to reproduction suggests partitioning of lipids into reproductive capacity.

These results are consistent with studies of _Neocalanus flemingeri_ in the Gulf of Alaska, which noted differences in metabolic gene expression associated with chlorophyll variation across sites (Roncalli et al., 2019) and between years (Roncalli et al., 2022). This suggests that chlorophyll levels are a primary factor contributing to gene expression differences among field sites for herbivorous copepods. Like _N. flemingeri_, _C. acutus_ relies on short phytoplankton blooms to mature, reproduce, and perform diapause.

Although there was a strong overall association between field chl _a_ and experimental starvation response-genes for _C. acutus_, one high-chl _a_ site (616.040) grouped with low-chl _a_ sites in gene expression analyses (Figures 2 and 11). This site had a uniformly warm water column in the upper 200 m and an intense, but shallow, surface chl _a_ maximum; chl _a_ maxima at other sites were deeper. No animals sampled from this site had food in their gut, samples were more similar to starved than fed experimental samples, and animals had low citrate synthase activity, indicating poor feeding conditions for _C. acutus_ despite the high chl _a_ concentration. One possible explanation is that this site had a distinct phytoplankton community: large calanoid copepods feed preferentially on diatoms compared to, for instance, the haptophyte _Phaeocystis_ spp., which is a lower-quality food source (Head & Harris, 1994; Turner et al., 2002). Although overall chl _a_ levels may be a useful proxy for food availability for grazers, the specific local abiotic and biotic factors, including phytoplankton community composition, determine feeding favorability.

4.2 | Lesser variation for an omnivore

C. propinquus physiology and gene expression exhibited less variation among sites than _C. acutus_. While this may be partially attributed to the smaller sample size for _C. propinquus_, the more omnivorous diet of _C. propinquus_ may also reduce its sensitivity to short-term variation in phytoplankton abundance. During summer _C. propinquus_ grazes heavily on diatoms, but microcrustaceans and protists can also comprise a substantial portion of the diet (Pasternak & Schnack-Schiel, 2001). Consistent with this, _C. propinquus_ had a weaker correlation between experimental starvation and field chl _a_ than _C. acutus_. Nonetheless, some starvation-response genes were associated with field chl _a_, and one high-chl _a_ site (200.000) had elevated expression of lipid synthesis genes (elongases and desaturases) and vitellogenins, suggesting favorable feeding conditions. This was also a "favorable" site for _C. acutus_, suggesting that the same favorable feeding conditions apply to both species. While most copepods at 200.000 had visible food within their guts, the proportion of copepods with food in gut were higher at two other sites (–100.000 and 000.000). This discrepancy may be due to the relatively short time window to visualize recently ingested food, and/or differences in opacity of various prey items. Overall, chl _a_ levels seem to drive some physiological variation in _C. propinquus_, but to a lesser extent than in _C. acutus_.

None of the _C. propinquus_ field samples grouped with starved experimental samples in our DAPC analysis, suggesting that none of the field samples had reached a comparable state of food deprivation. Indeed, animals were visibly feeding at all sites. In contrast, DAPC of _C. acutus_ suggests that the field samples spanned the entire range of nutritional conditions captured by the starvation experiment. Although _C. propinquus_ might genuinely exhibit less physiological variation within the region, sampling over a larger range of stations may uncover additional variation in this species.

4.3 | Chlorophyll _a_ is a predictive factor in copepod physiology as well as population dynamics

Long-term monitoring within the WAP region has shown that interannual variation in chl _a_ concentration is strongly associated with variation in _C. acutus_ abundance (Gleiber, 2014), as well as abundance of total copepods. Concordant with this, we observed a spatial correlation between chl _a_ and _C. acutus_ physiological condition. Gleiber (2014) also found an association between _C. propinquus_ abundance and chl _a_ lagged by 1 year. This lagged relationship may reflect the reduced or less direct dependence of _C. propinquus_ on phytoplankton; these traits may underlie our observations of a weaker

relationship between chl _a_ and _C. propinquus_ physiology. While chl _a_ is associated with interannual variation in the abundances of both species, and contributes to spatial variation in physiological conditions, we did not find a spatial relationship between chl _a_ and the abundance of either species. Despite the apparent favorability of site 200.000 for both species (and site 200.040 for _C. acutus_), _C. acutus_ and _C. propinquus_ were much more abundant farther south. This disconnect may be driven by small-scale mismatches between the phytoplankton and copepod populations, differential losses from predation, or reduced recruitment and retention due to currents (Jiang et al., 2007; Saunders et al., 2003; Sorochan et al., 2021).

From an evolutionary perspective, advection limits capacity of pelagic zooplankton for local adaptation, and many species have little population structure even on basin scales (Choquet et al., 2019). In such species, physiological plasticity may be more important than local adaptation for determining species distributions and population dynamics in the warming ocean. In the WAP, warming conditions and increased stratification are expected to lead to decreased production and biomass of large phytoplankton, especially in the northern region (Ferreira et al., 2020; Gleiber et al., 2016; Schofield et al., 2018; Venables et al., 2013). For _C. acutus_, a species that depends on seasonal phytoplankton blooms, these changes will certainly result in shifts in spatial patterns of habitat suitability, as well as possible phenological mismatches. _C. propinquus_ may be more robust to these changes due to its greater degree of omnivory, but nonetheless needs phytoplankton to feed and reproduce optimally (and the availability of other prey may depend on phytoplankton). The favorability of local feeding conditions affects the fitness and reproductive capacity of a population. Ultimately, spatial variation in fitness, together with advection, may contribute to regional differences in recruitment and source-sink dynamics and shape long-term population distributions.

4.4 | Feeding status biomarkers differ between species

Although many homologous genes respond similarly to starvation in both species (Berger et al., 2024), we identified only two homologous "biomarker" genes consistently associated with feeding status in both the field study and starvation experiment. Identification of such genes may have been hampered by the limited range of conditions captured in our sampling of _C. propinquus_. Nonetheless, vitellogenins were strongly downregulated in both species with starvation and at low-chl _a_ stations. Since vitellogenins are often highly expressed and sensitive to food availability, they may be particularly reliable indicators of recent feeding status (in the case of our starvation experiments, 5–9 days). Vitellogenin expression also has a clear biological interpretation—females increase their reproductive investment when they are better fed. Unfortunately, this biomarker is restricted to reproductively mature (or maturing) females. Methyltransferases, enzymes that catalyze transfer of methyl groups to many biological molecules (e.g., nucleic acids, histone, non-histone proteins), showed a similar expression

pattern, and further studies will be needed to determine whether methyltransferases could also serve as biomarkers within other developmental stages. Considering each species separately, genes involved in lipid metabolism appear to be reasonable indicators of metabolic condition, including desaturases and elongases (_C. acutus_) and lipases (_C. propinquus_).

Overall, our results lend confidence to interpretations of field-based RNA-seq studies of pelagic copepods. Much of the spatial variability in _C. acutus_ gene expression could be attributed to differences in feeding status, and even the omnivorous _C. propinquus_ exhibited some variation attributable to food availability. We identified highly expressed genes associated with both field chl _a_ and starvation responses, which may be used to infer relative differences in recent feeding status of these species in future field studies. In future studies, researchers should consider not only gross measures of abundance but also spatial variation in gene expression and other physiological metrics. These integrated studies will ultimately improve our capacity to understand and predict how copepod populations respond to changing conditions in the WAP ecosystem and other regions.

AUTHOR CONTRIBUTIONS

Cory A. Berger: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Deborah K. Steinberg:** Conceptualization (supporting); data curation (supporting); investigation (supporting); methodology (supporting); resources (supporting); writing – review and editing (supporting). **Ann M. Tarrant:** Conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); resources (lead); supervision (lead); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

OPEN RESEARCH BADGES

This article has earned the Open Data badge Data are available at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA757455/, https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA669816, and https://github.com/caberger1/Antarctic_copepod_scripts.

DATA AVAILABILITY STATEMENT

ORCID

Cory A. Berger https://orcid.org/0000-0002-6003-1955
Ann M. Tarrant https://orcid.org/0000-0002-1909-7899

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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