

## ARTICLE

## Macrosystems Ecology

# Are all copepods the same? Variation in copepod stoichiometry with taxonomy, ontogeny, latitude, and habitat

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**Abstract**

Copepods are the most abundant metazoans on Earth, driving cycles of key elements in aquatic systems, most prominently carbon (C), nitrogen (N), and phosphorus (P). One key factor determining nutrient cycling is copepod somatic stoichiometry, which can reflect ecological strategy. We conducted a systematic review that updates the seminal work of Båmstedt (1986) by summarizing the effects of latitude, habitat, life history stage, and taxonomy on C:N, C:P, and N:P ratios of field-collected copepods. We found that differences among copepod families accounted for the greatest variation, with the Rhincalanidae and Diaptomidae being particularly C-rich, while the Calanidae were more N- and P-rich. Copepod C:N was higher in inland waters compared with animals from marine environments in both copepodites and adult females, matching the higher C content of seston in many inland freshwaters. For both copepodites and adult females, mid-latitude animals had higher C:N and C:P than high-latitude animals, which matched predictions based on the availability of nutrients or adaptation to cold environments. More data must be gathered to fill gaps in our knowledge of copepod stoichiometry, focusing particularly on younger life stages, non-calanoids, low and high latitudes, the southern hemisphere, and estuarine and some inland water habitats, including large lakes. Such information will help better parameterize models of aquatic ecosystems and improve our understanding of how copepods influence consumer-driven nutrient cycling and food web dynamics.

**KEYWORDS**

ecological stoichiometry, habitat, latitude, ontogeny, seasonality

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## INTRODUCTION

Copepods are Earth's most abundant metazoans and the dominant metazoan primary consumer within the oceans and in many lakes (Andrady et al., 2012; Turner, 2004). They are a key trophic link between phytoplankton and consumers like fish (Lavigne, 2003; Wishner et al., 1988). Because sinking copepod fecal pellets are an important part of the biological carbon pump (Fowler & Knauer, 1986), copepods also strongly influence nutrient cycling and carbon sequestration (Turner, 2004; Verity & Smetacek, 1996). Yet, despite their importance, copepods are often modeled as a single functional group with respect to variables like behavior, food preference, and elemental content (Mitra et al., 2014), even though there are strong ecological and evolutionary reasons to suspect there are stoichiometric differences between copepods based on environment characteristics, including habitat type (inland waters, estuarine, marine), latitude (low, mid-, and high latitude), taxonomy, and ontogeny. To improve models and better understand the influence of copepods on food webs and consumer-driven nutrient cycling, we need to assess systematic variation in key functional traits among copepod species and environments (Hipsey et al., 2020; Litchman et al., 2013; Mitra et al., 2014).

One key functional trait is an organism's elemental composition (Elser et al., 1996; Sterner & Elser, 2002). Elemental content is related to several important ecological characteristics of copepods, including motility, reproductive rate, and ability to survive long periods without food (Sterner & Elser, 2002). Because dominant consumers influence the availability of nutrients like N and P in aquatic ecosystems (Elser et al., 1988; Shapiro & Wright, 1984), patterns in copepod stoichiometry could influence key ecosystem feedbacks. For example, copepods can affect the abundance of ammonia-oxidizing bacteria by altering N availability through the excretion of ammonia (Valdés et al., 2018). Furthermore, as copepods dominate the grazing performed by metazoans within many aquatic ecosystems (Andrady et al., 2012; Turner, 2004), copepod-driven nutrient recycling of elements like N may influence elemental availability within ecosystems, and thus influence stoichiometric adaptations of organisms in those habitats, including for other copepods. Previous studies attempting to summarize variation in copepod stoichiometry are lacking in differing ways, such as assessing variation in nitrogen and carbon content only (Båmstedt, 1986), or leaning on indirect estimates of elemental content based on body size rather than direct measurements of somatic composition (Ventura, 2006).

Although prior work has included taxa-specific data (Båmstedt, 1986), it has not generally focused on how

relatedness influences differences in stoichiometry among taxa. Much prior work has instead focused on understanding the causes of variation within taxa, such as the response of stoichiometry to variable respiration and excretion rates (Hébert et al., 2016; Meunier et al., 2016; Persson et al., 2010). For example, elemental composition within the copepod *Parvocalanus crassirostris* differs on physiological timescales in response to variation in temperature and the elemental composition of resources (Mathews et al., 2018). However, such plasticity in response to differences in environmental conditions is typically small compared with variation among taxonomic units (Båmstedt, 1986; Corner, 1973; Mauchline, 1998; Mayzaud & Martin, 1975; Saiz et al., 2020). We expect differences in copepod stoichiometry between contrasting physical, chemical, or food conditions will primarily reflect the fitness of the predominant copepod taxa to those conditions rather than physiological plasticity. Here, we characterize differences in C, N, and P composition among copepod families in the extant literature. We then also assess differences in copepod stoichiometry across latitudes and inland to oceanic habitats.

Taxonomy can influence copepod stoichiometry, particularly if correlated with differences in copepod size, feeding, and trophic mode, which are themselves associated with traits like motility and defense. For example, Brun et al. (2017) showed that evolutionarily divergent groups of copepods differ in key functional traits like feeding mode. Most calanoid copepods are active feeders that generate feeding currents, or cruise through the water searching for prey, whereas cyclopoid copepods are either sit-and-wait ambush predators or particle feeders that colonize marine snow aggregates for long feeding periods. For example, because active feeding requires more movement and thus more N-rich musculature (Kjørboe et al., 2010), one might expect that calanoid copepods will have lower C:N than cyclopoid copepods. More broadly, we might hypothesize that such associations of traits with elemental composition should be shared among more phylogenetically related species, resulting in strong stoichiometric differences among different families.

Another potentially important determinant of copepod stoichiometry is the developmental stage. We know stoichiometry changes through ontogeny, leading to differences in elemental composition among life stages (Meunier et al., 2016; Tanioka & Matsumoto, 2018). The growth rate hypothesis states that rapidly growing organisms and life stages require more P to produce the RNA and ribosomes required for fast growth (Carrillo et al., 2001; Elser et al., 1996; Mathews et al., 2018; Sterner & Elser, 2002). Laboratory studies showed that copepod N:P shifts during development, with younger, rapidly growing stages having lower N:P than older stages (Meunier et al.,

2016). Older copepods have more N-rich protein and muscle, and require more N for reproduction or greater motility (Augustin & Boersma, 2006; Elser et al., 1996; Sterner & Elser, 2002). We hypothesize that younger life history stages collected from the field will have lower C:P and N:P ratios than older copepods, and that older copepods will have lower C:N ratios than younger copepods. While the growth rate hypothesis has been supported in laboratory studies of specific species (e.g., Meunier et al., 2016), comparing field-based data on copepod stoichiometry throughout ontogeny would test the generality of the growth rate hypothesis.

Next, we hypothesize that the stoichiometry of resident species will vary among environments to match differences in supply of nutrient resources, as would result from competitive exclusion and natural selection. In North America, whole ecosystem experiments and comparative studies indicate that net primary production (NPP) in pelagic inland waters is most often limited by the supply of P over longer timescales (Schindler et al., 2008). However, the prevalence of N fixation within inland waters coupled with variable rates of N fixation and denitrification can cause N limitation, P limitation, or colimitation at the more immediate timescales relevant to organismal responses (Elser et al., 2007; Wurtsbaugh et al., 2019). In the pelagic ocean, the limitation of NPP by the supply of N is commonly indicated by micro- and mesocosm studies (Elser et al., 2007; Oviatt et al., 1995) as well as by the greater depletion of N relative to P progressing from deep to surface waters (Tyrell, 1999). The limitation of NPP by P supply is implicated only at millennial timescales in the ocean as a whole (Falkowski et al., 1998; Redfield, 1958; Smith, 1984). Stoichiometric plasticity may account for some differences in consumer stoichiometry among these gradients (Meunier et al., 2014; Persson et al., 2010), but intrinsic differences in dietary elemental requirements among taxa could play an even larger role. Because of the relationship between somatic and dietary elemental requirements, we predict tissue C:N will be higher in marine than in inland water copepods, and C:P and N:P will be lower in marine than in inland freshwater copepods.

Lastly, we hypothesize that latitudinal differences in temperature and the strength of seasonality will influence copepod stoichiometry. By having more ribosomal RNA, nondiapausing copepods at higher latitudes may compensate for slower biochemical rates of transcription per enzyme under colder temperatures. Such compensation could lead to increased somatic P (Woods et al., 2003). In diapausing copepod species, the content of C-rich lipids can also vary widely in response to food availability and seasonality (reviewed in Båmstedt, 1986; Mauchline, 1998). We predict that high-latitude copepods sampled during the growing season, which are probably

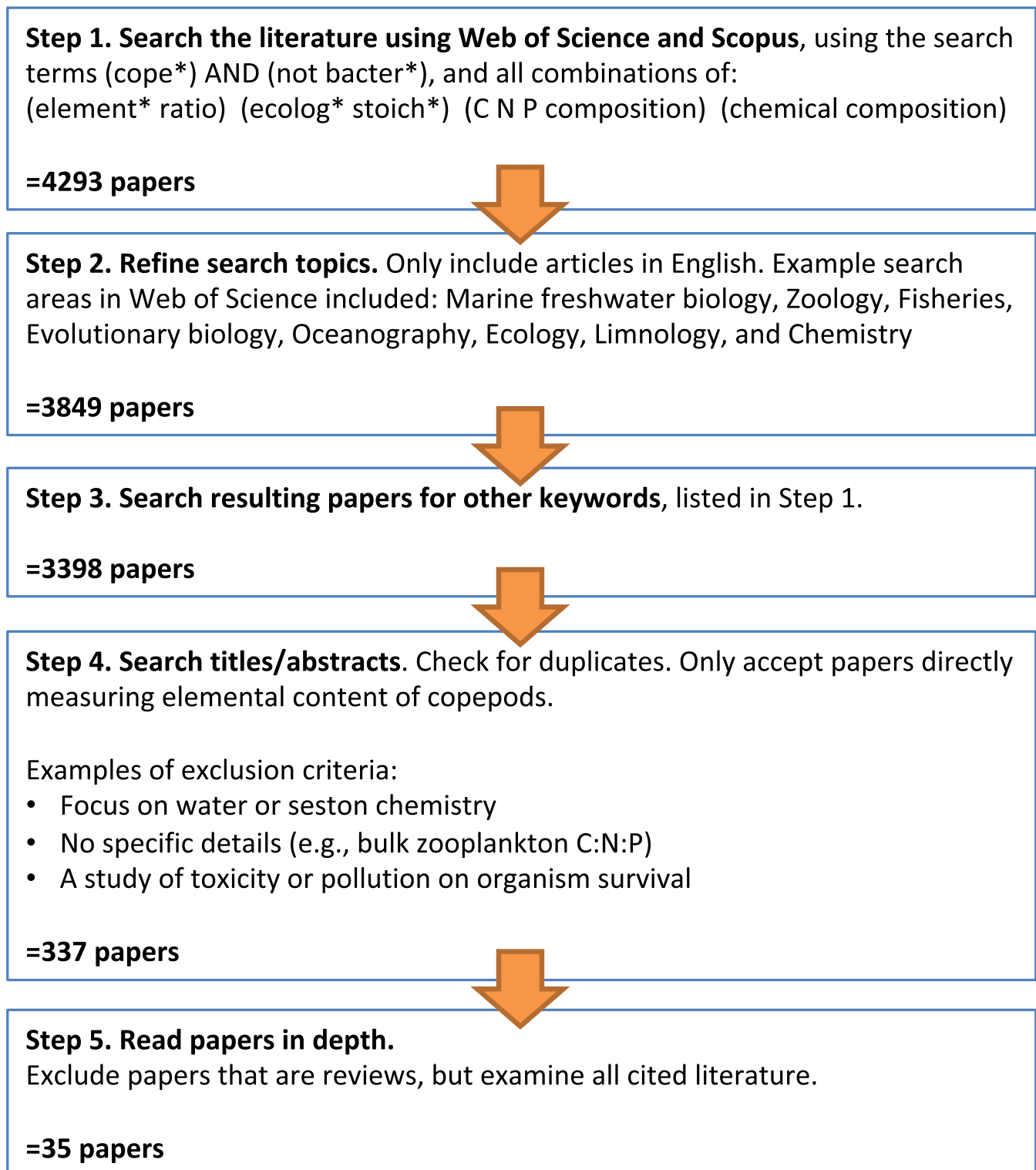
nondiapausing, will have a greater P content, and thus have lower C:P and N:P, relative to copepods from habitats with less pronounced seasonality. As important previous work by Båmstedt (1986) largely gathered data from mid-latitude regions, we tried to gather more data from high- and low-latitude copepods to expand upon our current understanding of copepod stoichiometry by latitude.

We test our hypotheses by conducting a systematic search of the literature on copepod stoichiometry and determining how copepod stoichiometry depends on (1) taxonomy, (2) ontogeny (3) habitat type, and (4) latitude. In this analysis, we take account of body size because it has been linked to ecologically relevant characteristics related to elemental composition, such as metabolic rate, population growth rate, and life span (Gillooly et al., 2004). Body size may therefore represent a way of explaining differences in elemental composition among taxa or between environments. We discuss the many remaining gaps in the extant scientific literature. We propose that gathering data for understudied taxa and age classes, for copepods from nonmarine habitats, and for copepods from high and low latitudes, would help illuminate how copepod stoichiometry influences food webs and consumer-driven nutrient cycling, and could ultimately inform aquatic ecosystem models.

## MATERIALS AND METHODS

We performed a systematic search of the literature from January 1 of the earliest year available through the Stony Brook University library database collection through October 2017. We used the following digital databases: Scopus (from 1966), Web of Science Core Collection (from 1900), Science Citation Index Expanded (from 1900), Social Sciences Citation Index (from 1956), Arts & Humanities Citation Index (from 1975), Conference Proceedings Citation Index–Science (from 1991), Conference Proceedings Citation Index–Social Science and Humanities (from 1991), Book Citation Index–Science (from 2005), Book Citation Index–Social Science & Humanities (from 2005), Emerging Courses Citation Index (from 2015), Current Chemical Reactions (from 1985), and Index Chemicus (from 1993). Databases were searched using the terms (cope\*) AND (NOT bacter\*), plus all combinations of the terms (element\* ratio), (ecolog\* stoich\*), (C N and P composition), and (chemical composition).

Initial searches returned 4293 papers. Paper selection followed the criteria overviewed in our preferred reporting items for systematic reviews and meta-analyses (PRISMA) diagram (Figure 1, after Moher et al., 2009). We refined the search to include articles written in English, covering relevant research areas (e.g., marine



**FIGURE 1** A PRISMA flow diagram showing the systematic narrowing search results for the systematic review. Diagram after Moher et al. (2009).

ecology, limnology, cell biology), leaving 3398 papers (following methods in Kinlock et al., 2018). We read through paper titles and abstracts, eliminating papers not relevant to copepod elemental content measurements (e.g., soil litter dynamics, insect herbivory on aquatic plants, or on

respiration and nutrient excretion). For the remaining 337 papers, we determined whether copepods were (1) field-collected and not used in laboratory experiments manipulating copepod elemental content; (2) identified to at least genus; and (3) where the elemental content of

copepods was directly measured, not estimated. We excluded papers missing information on replicate numbers, or that only reported stoichiometry for “bulk plankton,” “zooplankton” or “copepods,” or mixed multiple developmental stages or sexes together. Reviews and meta-analyses were not included, but their literature cited was carefully scrutinized to find additional references missed by our keyword search (following methods in Padilla & Savedo, 2013). In total, we found 37 primary studies on a variety of copepod species that met our qualifications for inclusion (Figure 1; see Appendix S1 for included papers and a histogram of publication data).

For each study, we extracted data on elemental contents or elemental ratios measured for each copepod species for every available life history stage. Most of the data we obtained were from tables within the study. Data from figures were obtained using ImageJ (Schneider et al., 2012). If two or more elemental contents were reported within a study, we converted these to molar ratios. Data reported as percent dry weight were converted to molar data by multiplying by the atomic mass of the element. We recorded information on copepod stoichiometry (molar ratios of C:N, C:P, and N:P) in relation to habitat, latitude, taxonomic classification (order, genus, species), life stage, and sex. Current taxonomic designations were checked using WoRMS (Horton et al., 2022) and the World of Copepods (Walter & Boxshall, 2022). If a study included more than one species (average = 1.89 per study; range = 1–6 species) or more than one life history stage (average = 3.89 per study; range = 1–18 life history stages across the 1–3 species in the study), we arbitrarily considered data for observations of each species and/or life history stage from that study-independent observations as long as measurements were separated by 100+ m of depth, or by at least one month. This resulted in multiple observations of copepod stoichiometry being obtained from each of the 37 primary studies. The data in these papers were gathered during the growing season for nondiapausing copepods.

The total body length for marine copepods was obtained from the database created by Brun et al. (2016) and was averaged across all entries for the appropriate stage where total body length was available, regardless of sampling location. For any copepods not within this database, we searched the copepod's genus and species names with the term “total body length” on Google Scholar. These data are available on Figshare for size (<https://doi.org/10.6084/m9.figshare.21841863.v5>) and for references used in this study (<https://doi.org/10.6084/m9.figshare.21841869.v3>).

Based on study location and descriptions by the paper's authors, we categorized collection sites as coastal and open ocean (henceforth “marine”), tidal estuarine rivers and streams (henceforth “estuarine”), or freshwaters (henceforth “inland waters”). Ideally, collection site

was recorded using the latitude and longitude provided by the authors. If no coordinates were provided, we estimated location from the city and country listed in *Materials and methods*. Data were categorized into one of three latitudinal zones: low latitude (0–30° latitude), mid-latitude (30–60°), or high latitude (>60°).

We chose to perform ANCOVA and ANOVA analyses because the nature of the data prevented the use of a meta-analysis or a principal components analysis (PCA). First, we determined the simple mean and SD for elemental ratios for each reported copepod species and/or life history stage within a study. These values were treated as replicate estimates. We then calculated the weighted means of the elemental ratios, weighting the simple means by the number of independent observations for each species and life history stage. We used R (The R Core Team, 2013) to analyze our data. We performed ANOVAs and ANCOVAs using Anova in the car package (Fox & Weisberg, 2019). This was followed by pairwise post hoc Tukey–Kramer tests using pairs in the lsmeans package (Lenth, 2016), where we inferred statistically significant differences between levels if  $p(H_0) \leq 0.05$ .

We performed two sets of analyses. In both, we homogenized the variance and normalized residuals by taking the base 10 logarithm ( $\log_{10}$ ) to transform the data on elemental content, such as C:N, C:P, and N:P, and average total body size (henceforth, “elemental content” and “size”). Size was only available for adult females, and not copepodites. Therefore, we performed ANOVAs for copepodites and ANCOVAs for adult females with  $\log_{10}(\text{body size})$  as a continuous predictor. In the first set of analyses, we used habitat type (inland waters, estuarine, or marine), latitude (low, mid, or high), and  $\log_{10}(\text{size})$  (adult females only) as main effect predictors of elemental content separately for each life history stage (adult females or copepodites). In the second set of analyses, we used taxonomic group (family) and size (adult females only) as main effect predictors for elemental content for each life history stage (adult females or copepodites). Because we were performing multiple comparisons within these analyses, we used Bonferroni corrections to determine the significance of individual  $p$  values by setting the family-wise error rate at  $\alpha = 0.05$  for the full and reduced datasets, and separately for the individual factors and the whole models under consideration. Except in analyses of taxonomic effects for female copepods, there were no significant interactions between elemental content with the predictor variables. The variation explained by each main effect is described by Type II sums of squares, which are insensitive to the order of predictors. These statistical outputs are available in Tables 3 and 4. Lastly, we ran both sets of analyses using the full

dataset, which included all information gathered for this study, and with a reduced dataset, which excluded the families Diaptomidae and Rhincalanidae because they had particularly anomalous elemental content (please see [Results](#) below).

## RESULTS

### Overarching patterns

We found applicable papers for three orders: Calanoida (94.1% of observations), Cyclopoida (5.7% of observations),

and Harpactacoida (0.3% of observations). These data represented 55 genera and 99 species of Calanoida, six families and eight species of Cyclopoida, and one genus and one species of Harpactacoida (Tables 1 and 2).

In total, 85.4% of the observations were collected from marine habitats and 12.4% of the observations were from inland water habitats. Very few observations were for animals from estuarine habitats (Tables 1 and 2, Figure 2). Most copepod data were collected in mid-latitude regions (83.9% of observations) (Tables 1 and 2, Figure 2) and were dominated by data from Europe and North America (Figure 2). We could not find data for copepods in large inland water lakes

**TABLE 1** Elemental ratios (mol:mol) by family for adult females and copepodites.

Family	Adult females			Copepodites		
	C:N	C:P	N:P	C:N	C:P	N:P
Acartiidae	5.3 ± 1.6 (14)	74 ± 19 (8)	12 ± 3.5 (8)			
Aetideidae	4.5 ± 1.2 (39)			5.3 ± 1.1 (11)		
Augaptilidae	5.1 ± 0.8 (7)					
Calanidae	4.4 ± 1.4 (49)	89 ± 51 (9)	20 ± 8.8 (10)	6.8 ± 2.8 (46)	117 ± 128 (11)	22 ± 15 (12)
Centropagidae	6.7 ± 3.8 (19)	188 ± 86 (17)	18 ± 5.9 (6)	7.4 ± 2.2 (11)	105 ± 57 (10)	
Clausocalanidae	5.8 ± 0.9 (6)	148 ± 43 (4)	30 ± 20 (4)	5.5 (1)		
Diaptomidae	10 ± 2.8 (8)	307 ± 110 (8)	30 ± 15 (6)	11 ± 3.1 (6)	180 ± 67 (6)	16 ± 10 (12)
Eucalanidae	5.3 ± 0.3 (6)	110 ± 64 (8)		4.1 (1)	17 ± 3.4 (2)	
Euchaetidae	6.7 ± 0.9 (20)			6.5 ± 0.7 (9)		
Heterorhabdidae	3.95 (1)					
Lucicutiidae	6.27 ± 1.2 (9)			5.3 ± 0.2 (2)		
Megacalanidae	6.1 ± 1.1 (4)					
Metridinidae	5.7 ± 1.0 (16)			5.4 ± 0.4 (3)		
Paracalanidae	3.6 ± 0.6 (5)					
Phaennidae	5.4 ± 0.6 (5)			6.2 ± 1.3 (2)		
Pontellidae	3.0 ± 0.3 (3)					
Pseudodiaptomidae	3.2 ± 0.2 (2)					
Rhincalanidae	9.6 ± 3.0 (5)	299 ± 171 (4)	27 ± 13 (4)	3.77 (1)		
Scolecitrichidae	5.3 ± 1.2 (19)			8.6 ± 1.9 (2)		
Spinocalanidae	5.8 ± 1.4 (3)					
Subeucalanidae	4.8 (1)	112 (1)	24 (1)			
Temoridae	5.1 ± 0.9 (15)	64 ± 14 (8)	11 ± 2.4 (8)			
Tortanidae	2.7 ± 0.2 (2)					
Corycaeidae (Cyclopoida)	4.4 (1)					
Cyclopidae (Cyclopoida)	6.4 ± 2.4 (3)	280 ± 287 (2)	37 ± 28 (2)	5.4 ± 2.6 (4)	278 (1)	32 (1)
Oithonidae (Cyclopoida)	4.1 (1)					
Oncaeidae (Cyclopoida)	5.9 ± 1.6 (14)					
Ectinosomatidae (Harpactacoida)	4.2 (1)		26 ± 5.4 (6)			

Note: Data presented within each category show the weighted means of the molar elemental ratios, ±1 SD, for the number of independent observations included (*n*). Empty rows indicate no data were available. Stoichiometric data by family are for the order Calanoida unless otherwise noted within parentheses.

**TABLE 2** Elemental ratios (mol:mol) across all data and by major environmental characteristic.

Predictor	Full dataset			Reduced dataset		
	C:N	C:P	N:P	C:N	C:P	N:P
Adult females						
Across all data	5.4 ± 1.8 (275)	158 ± 117 (69)	21 ± 12 (55)	5.2 ± 1.8 (264)	127 ± 89 (57)	19 ± 11 (45)
Latitude						
High	5.5 ± 1.3 (31)	78 ± 29 (22)	15 ± 6.1 (23)	5.5 ± 1.3 (30)	78 ± 29 (22)	15 ± 6.1 (23)
Mid	5.3 ± 2.1 (235)	218 ± 113 (32)	25 ± 16 (21)	5.2 ± 1.8 (227)	188 ± 99 (24)	22 ± 16 (15)
Low	7.3 ± 3.0 (11)	148 ± 135 (15)	26 ± 8.3 (11)	5.2 ± 0.3 (7)	94 ± 66 (11)	26 ± 5.0 (7)
Habitat						
Estuarine	2.7 ± 0.2 (2)			2.7 ± 0.2 (2)		
Inland	8.9 ± 4.0 (16)	267 ± 108 (21)	32 ± 17 (8)	8.9 ± 4.3 (10)	243 ± 102 (13)	37 ± 28 (2)
Marine	5.0 ± 1.4 (257)	110 ± 84 (48)	19 ± 10 (47)	5.1 ± 1.4 (252)	93 ± 46 (44)	18 ± 9.9 (43)
Copepodites						
Across all data	6.7 ± 2.6 (99)	124 ± 99 (30)	19 ± 13 (25)	6.5 ± 2.3 (92)	110 ± 102 (24)	23 ± 15 (13)
Latitude						
High	4.3 ± 1.9 (11)	52 ± 11 (5)	20 ± 4.2 (6)	4.3 ± 2.0 (10)	52 ± 11 (5)	20 ± 4.2 (6)
Mid	7.0 ± 2.6 (88)	162 ± 95 (21)	19 ± 15 (19)	6.7 ± 2.3 (82)	155 ± 106 (15)	25 ± 20 (7)
Low		16 ± 3.4 (4)			16 ± 3.4 (4)	
Habitat						
Estuarine	9.3 ± 1.3 (7)			8.9 ± 1.4 (7)		
Inland	8.4 ± 3.6 (18)	142 ± 77 (17)	17 ± 11 (13)	7.1 ± 2.4 (14)	120 ± 76 (11)	32 (1)
Marine	6.3 ± 2.0 (72)	102 ± 123 (13)	22 ± 15 (12)	6.1 ± 2.3 (71)	102 ± 123 (13)	22 ± 15 (12)

Note: Data presented within each category show the weighted means of the molar elemental ratios, ±1 SD, for the number of independent observations included (*n*). Empty rows indicate no data were available. The full dataset includes all information gathered for this study; the reduced dataset excluded data for Diaptomidae and Rhincalanidae as their C:N contents were greater than many other families.

(e.g., the Great Lakes in North America, Lake Baikal in Russia) and no data for copepods in Africa or Australia (Figure 2).

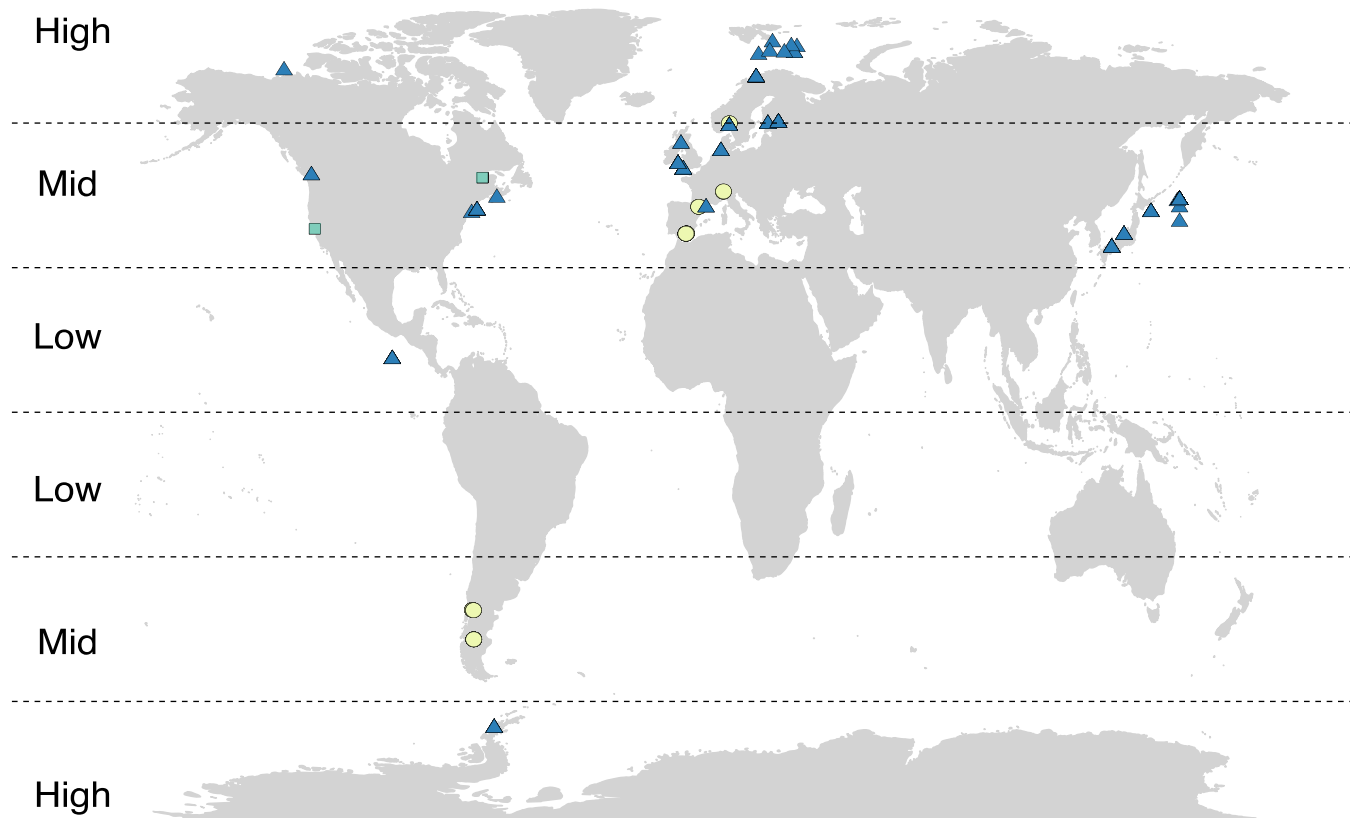
## Taxonomic patterns

Body size of species did not significantly affect among-family differences in elemental composition of adult female copepods. Body size was only significant as a predictor of C:N in adult female copepods in interaction with family (Table 3). As its own factor, body size was not significantly related to any of the stoichiometric ratios considered for adult female copepods (Table 3, Figure 3B,C). Therefore, the correction for body size does not substantially affect the comparisons of elemental ratios among families discussed below.

Where statistically significant differences existed among families, adult female Rhincalanidae and Diaptomidae were richer in C, with the marine Rhincalanidae being poorer in N and the freshwater

Diaptomidae poorer in P. In contrast, the largely marine Calanidae tended to be more enriched in N and P, relative to C (Table 1, Figure 4). The C:N of the Rhincalanidae was 1.7- to 2.2-fold greater than that of the Aetideidae, Calanidae, Centropagidae, Metridinidae, Phaennidae, and Scolecitrichidae, and the C:P of Rhincalanidae was 3.4-fold greater than that of the Calanidae (Table 1, Figure 4A). The C:P of the Diaptomidae was between 2.8- to 4.8-fold greater than that of the Acartiidae, Calanidae, Eucalanidae, and Temoridae, all largely marine families (Table 1, Figure 4C). The N:P of Diaptomidae was 2.8-fold greater than that of the Temoridae (Table 1, Figure 4E). In addition to having lower C:N than Rhincalanidae, the C:N of Calanidae was 0.7- to 0.8-fold than that of Metridinidae, Aetideidae and the Oncaidae (a cyclopid) (Table 1, Figure 4A). In addition to having lower C:P than the Diaptomidae, Calanidae C:P was only 0.5-fold than that of the Centropagidae (Table 1, Figure 4C).

For copepodites, stoichiometric ratios were not significantly different among families (Tables 1 and 3, Figure 4B,D,F).



**FIGURE 2** Global distribution of copepod stoichiometric data. Dashed horizontal lines indicate low (0–30°), mid- (30–60°), and high (>60°) latitudes. Inland water data are shown by yellow circles, estuarine water data are shown by green squares, and marine data are shown by blue triangles.

## Taxonomy and explained variance

Differences among families accounted for statistically significant and substantial amounts of variance for all three elemental ratios for adult female copepods (Tables 1 and 3). For adult females, family explained 45%, 47%, and 38% of the variance in C:N, C:P, and N:P, respectively. Removing the Rhincalanidae and Diaptomidae from the data reduced the explained variances to 35% and 33% in C:N and C:P, respectively, but the models were still significant. For copepodites, family was not significantly related to stoichiometric ratio in either the full or reduced data (Tables 1 and 3).

## Patterns by latitude and habitat

For adult female copepods, the factors of body size, latitude, and habitat were all statistically significant for C:N in the full dataset, and the factors of body size and habitat were statistically significant in the reduced dataset, with total  $R^2$  values of 0.26 and 0.16, respectively (Tables 2 and 4). Body size

explained at most 2.2% and 3.2% of the variance in the full and reduced datasets, respectively. Habitat was the best predictor of adult female elemental ratios, accounting for 23%, 12%, and 15% of total variance in C:N, C:P, and N:P, respectively, within the full dataset. When Rhincalanidae and Diaptomidae were excluded from the data, habitat was only significantly related to C:N in adult females, with 15% of the variance explained, which suggests that these two families contributed to the differences among habitats. Latitude was related to a small (4.3%) but statistically significant amount of variance in adult female C:N for the full dataset.

Copepodite results differed from those for adult females in that latitude, rather than habitat, was the best predictor of elemental ratios (Tables 2 and 4). For C:N models using the full dataset, latitude explained a slightly greater amount of variance (13%) compared with habitat (11%). This difference was larger (13% vs. 7.7%) when Rhincalanidae and Diaptomidae were excluded using the reduced dataset, again suggesting that these families influenced the effect of habitat on C:N ratios. More impressively, latitude was related to 64% and 67% of variance in C:P ratios in the full and reduced datasets,



**TABLE 3** Comparisons of elemental ratios (mol:mol) for copepods of different families, with respect to body size and developmental stage.

Ratio	Stage	Body size			Family			Interaction: Body size and family			Res.	Adj. $R^2$	df	$p$
		SS	% var.	$p$	SS	% var.	$p$	SS	% var.	$p$				
Full dataset														
C:N	AF	0.2	0.5	0.090	14	45	<b>&lt;0.001</b>	4	13	<b>&lt;0.001</b>	13	0.50	227	<b>&lt;0.001</b>
	C				3.3	23	0.025				11	0.12	86	0.025
C:P	AF	0.3	0.7	0.37	21	47	<b>&lt;0.001</b>				23	0.38	58	<b>&lt;0.001</b>
	C				10	39	0.013				16	0.29	25	0.013
N:P	AF	0.1	0.3	0.65	6.2	38	<i>0.0073</i>				10	0.30	44	<b>0.003</b>
	C				0.7	7.4	0.43				9.1	-0.01	22	0.43
Reduced dataset														
C:N	AF	0.3	1.3	0.020	8.4	35	<b>&lt;0.001</b>	3	12	<b>&lt;0.001</b>	12	0.38	218	<b>&lt;0.001</b>
	C				1.2	10	0.53				11	-0.01	81	0.53
C:P	AF	0.4	1.1	0.38	11	33	<i>0.0045</i>				21	0.23	48	<b>0.0073</b>
	C				6.6	30	0.061				15	0.20	20	0.061
N:P	AF	0	0.2	0.78	4	32	0.038				8.5	0.26	36	0.013
	C				0.4	5.1	0.46				6.6	-0.04	11	0.46

Note: An ANCOVA and ANOVA were performed for adult females (AF) and copepodites (C), respectively. For C:N in adult female copepods only, there was a significant interaction between body size and family; no other adult interactions were significant. No body size data were available for copepodites. Values for molar elemental ratios and body size were log-transformed to ensure normality. Bonferroni corrections were used to determine the significance of individual  $p$  values by setting the family-wise error rate at  $\alpha = 0.05$  separately for the full and reduced datasets, and separately for the 12  $p$  values estimated for factors, and 6  $p$  values for the whole models. The  $p$  values in boldface are significant ( $p \leq 0.0042$  and  $0.0083$  for the factors and whole models, respectively). The  $p$  values in italics are marginally significant ( $0.0042$  and  $0.0083 \leq p \leq 0.0083$  and  $0.017$  for the factors and whole models, respectively). Full and reduced datasets as indicated in Figure 1 legend.

Abbreviations: Adj.  $R^2$ , adjusted  $R^2$ ; Res., residuals; % var., percent variance, calculated by  $(100 \times (\text{predictor's SS}/\text{total SS}))$ .

respectively. By contrast, models predicting copepodite N:P were statistically insignificant, possibly because of the lack of studies measuring both N and P for this life stage.

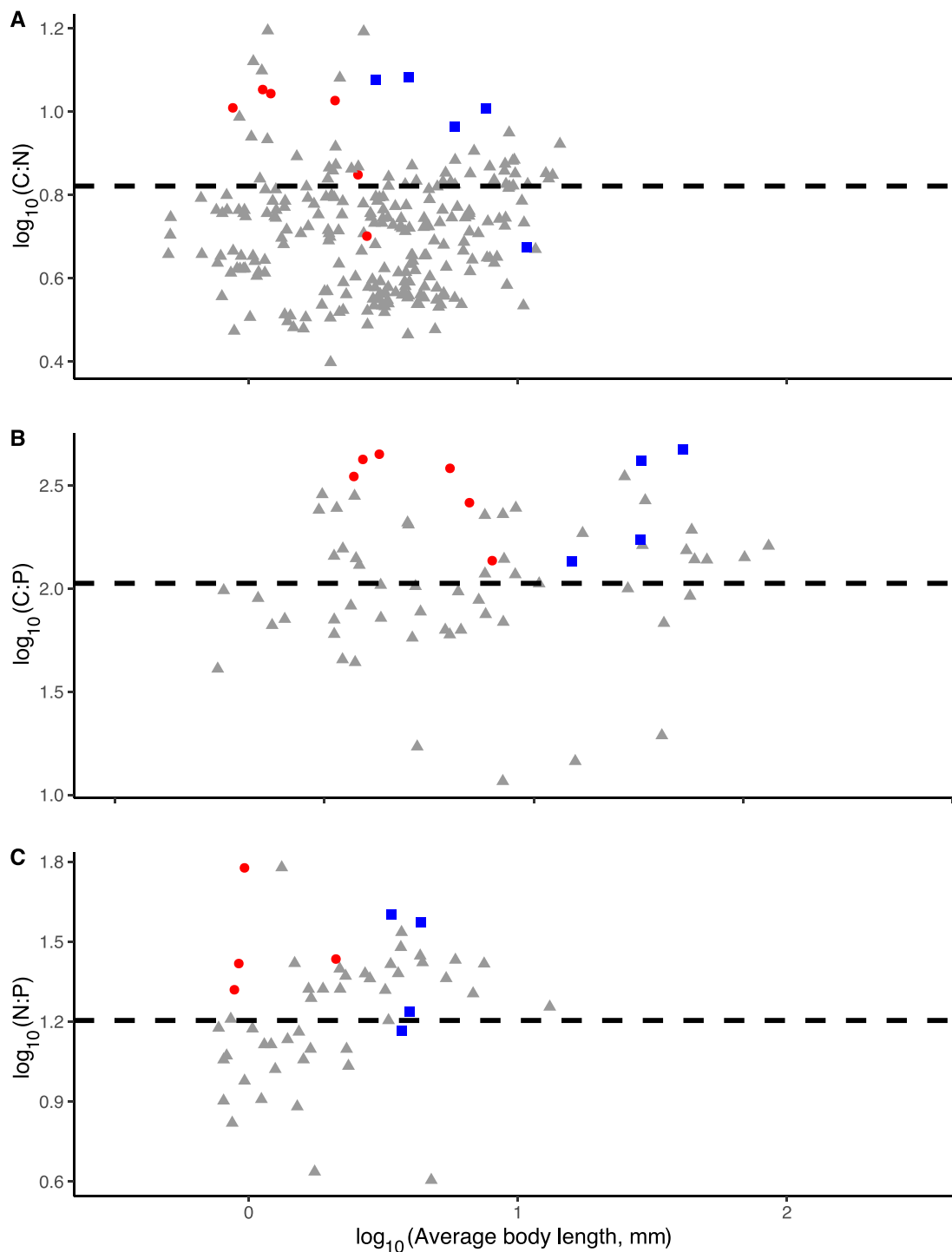
Differences in elemental ratios between inland freshwater and marine habitats followed the same pattern for adult females and copepodites (Table 2). For both life stages, C:N and C:P ratios from inland waters were more C-rich (relative to N and P) than marine environments, with adult females exhibiting larger differences. The C:N of adult female copepods and copepodites from inland freshwater habitats was 1.8- and 1.3-fold greater, respectively, than that of copepods from marine habitats (Figures 5B and 6B). Estuarine copepodites had similar C:N to inland water copepodites (Figure 6B). Differences in C:P among habitats were even more pronounced, being 2.4- and 1.4-fold greater for inland water adult females and copepodites, respectively (Figures 5D and 6D). N:P did not differ significantly among habitats for copepodites, but for adult females, N:P was 1.7-fold greater in inland waters compared with copepods from marine environments (Figure 5F). These patterns in C:P and N:P both suggest relatively low P content in

copepods from inland freshwater habitats compared with animals from marine habitats.

In cases where latitude explained >10% of variance in elemental ratios, mid-latitude copepods tended to be more C-rich than both high- and low-latitude copepods (Table 2). The C:N of copepodites from mid-latitudes was 1.6-fold greater than that of high-latitude animals (Table 2, Figure 6A). Differences in C:P were even more substantial, with mid-latitude copepodites having 10.1-fold greater C:P than low-latitude copepodites, and 3.1-fold greater C:P than high-latitude copepodites (Table 2, Figure 6C). For adult females, latitude only had a substantial effect on C:P when the Rhincaliniidae and Diaptomidae were removed from the data, whereupon mid-latitude animals had twofold greater C:P compared with low-latitude copepods (Table 2, Figure 5C).

## Ontogenetic patterns

There were eight families where there were more than three observations for both adult females and copepodites: Aetideidae, Calanidae, Centropagidae,

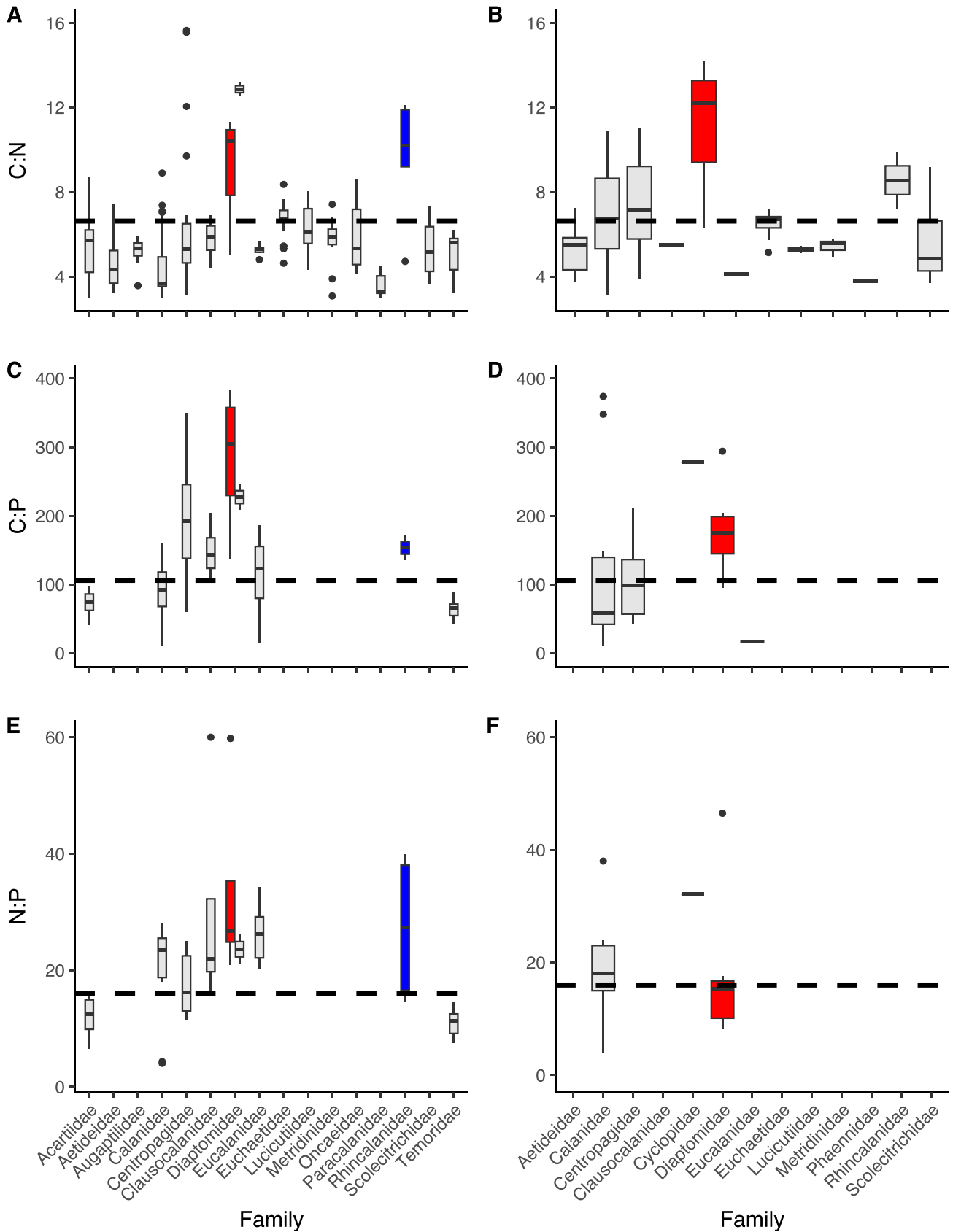


**FIGURE 3** Somatic molar elemental ratios for adult female copepods by size. Scatterplots show the base 10 logarithm ( $\log_{10}$ ) of the stoichiometric content plotted against the  $\log_{10}$  of the average body size of copepods. Red circles represent Diaptomidae, blue squares represent Rhincalanidae, and gray triangles show any other family. Dashed horizontal lines represent the  $\log_{10}$  of the Redfield ratios.

Diaptomidae, Eucalanidae, Euchaetidae, Metridinidae, and Cyclopidae (a cyclopoid) (Table 1). Within the Calanidae, the C:N of adult females was significantly lower than that of copepodites ( $t$  ratio =  $-6.13$ ,  $df = 235$ ,  $p < 0.0001$ ; Table 1, Figure 7A). None of the other adult-copepodite data were significantly different for C:N, C:P, or N:P (Table 1, Figure 7).

## DISCUSSION

More than three decades after the summary of Båmstedt (1986), we found that there are still substantial gaps in data on copepod elemental composition, and that these gaps limit our ability to detect large-scale patterns for different life history stages. Broadly, we observed that



**FIGURE 4** Somatic molar elemental ratios by family: (A, C, E) adult females; (B, D, F) copepodites. Data are shown for families with a minimum of five stoichiometric observations. For box-and-whisker plots, boxes show medians with first and third quartiles, whiskers represent the minimum and maximum values, and outliers are shown as points. Red bars represent Diaptomidae, blue bars represent Rhinocalanidae, and gray bars show any other family. Dashed horizontal lines represent the Redfield ratio (C:N = 6.63, C:P = 106, N:P = 16).

**TABLE 4** Comparisons of elemental ratios (mol:mol) for copepods from different habitats and latitudes, with respect to body size and developmental stage.

Ratio	Stage	Body size			Latitude			Habitat			Res. SS	Adj. R <sup>2</sup>	df	p
		SS	% var.	p	SS	% var.	p	SS	% var.	p				
Full dataset														
C:N	AF	0.7	2.2	<b>0.0035</b>	1.4	4.3	<b>0.0003</b>	7.3	23	<b>&lt;0.0001</b>	22	0.26	267	<b>&lt;0.0001</b>
	C				1.7	13	<b>0.0002</b>	1.4	11	<b>0.0022</b>	11	0.25	95	<b>&lt;0.0001</b>
C:P	AF	0.1	0.4	0.56	1.6	4.8	0.16	3.8	12	0.0039	27	0.35	64	<b>&lt;0.0001</b>
	C				15	64	<b>&lt;0.0001</b>	1.2	5.4	0.044	7.1	0.70	26	<b>&lt;0.0001</b>
N:P	AF	0.8	5.1	0.075	0.4	2.1	0.41	2.3	15	<b>0.0030</b>	12	0.26	50	<b>0.0007</b>
	C				0.2	1.6	0.56	0	0	0.95	9.6	-0.06	22	0.74
Reduced dataset														
C:N	AF	0.8	3.2	<b>0.0013</b>	0.5	1.8	0.046	3.6	15	<b>&lt;0.0001</b>	20	0.16	254	<b>&lt;0.0001</b>
	C				1.5	13	<b>0.0002</b>	0.9	7.7	0.017	9	0.21	88	<b>&lt;0.0001</b>
C:P	AF	0.4	1.9	0.26	3.1	12	0.016	2	8.7	0.018	18	0.39	52	<b>&lt;0.0001</b>
	C				15	67	<b>&lt;0.0001</b>	1.9	8.7	0.015	5.3	0.72	20	<b>&lt;0.0001</b>
N:P	AF	0.8	6.4	0.083	0.3	1.7	0.55	1.3	10	0.032	10	0.18	40	0.016
	C				0.2	2.1	0.64	0.5	6.5	0.42	6.5	-0.11	10	0.68

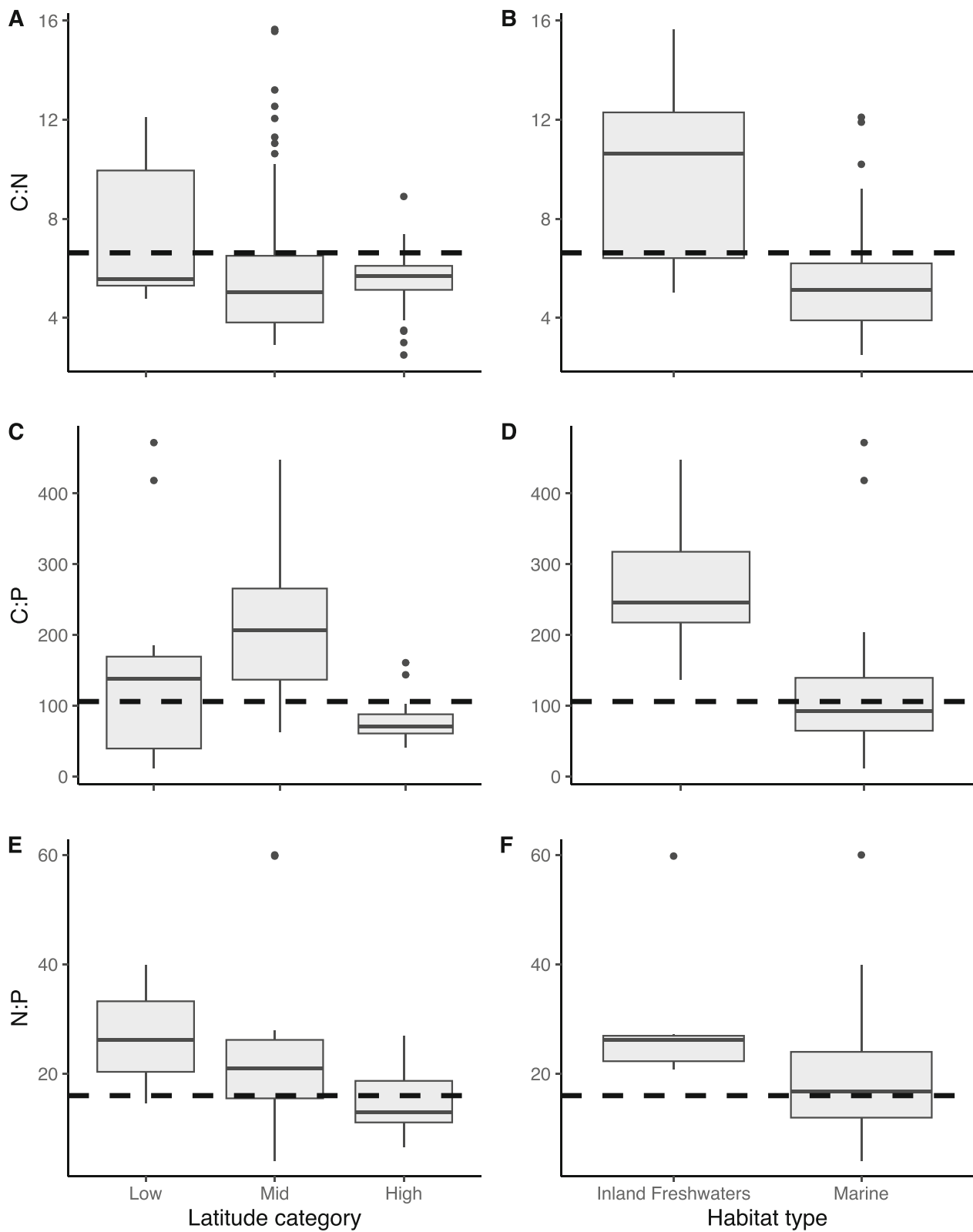
Note: An ANCOVA and ANOVA were performed for adult females (AF) and copepodites (C), respectively. For C:N in adult female copepods only, there was a significant interaction between body size and family. No body size data were available for copepodites. Values for molar elemental ratios and body size were log-transformed to ensure normality. Bonferroni corrections were used to determine the significance of individual *p* values by setting set family-wise error rate at  $\alpha = 0.05$  separately for the full and reduced datasets, and by separately for the 15 *p* values estimated for factors, and 6 *p* values for the whole models. The df and *p* columns are for the full model. The *p* values in boldface are significant ( $p \leq 0.0033$  and  $0.0083$  for the factors and whole models, respectively). The *p* values in italics are marginally significant ( $0.0033$  and  $0.0083 \leq p \leq 0.0067$  and  $0.017$  for the factors and whole models, respectively). Full and reduced datasets as indicated in Figure 1 legend.

Abbreviations: Adj.  $R^2$ , adjusted  $R^2$ ; Res., residuals; % var., percent variance, calculated by  $(100 \times (\text{predictor's SS}/\text{total SS}))$ .

measurements of C:N for field-collected copepods were ~5–6, in agreement with laboratory-based studies (e.g., Meunier et al., 2016) and within the C:N range reported in previous reviews (Båmstedt, 1986; summarized in Mauchline, 1998). However, there are few measurements of elemental composition other than those for adult female calanoids collected from mid-latitude coastal-shelf marine habitats. We need more such direct measurements of copepod stoichiometry for species from certain regions, like from the southern hemisphere, from large freshwater lakes, and from low- and high-latitude habitats. Additionally, we found that the number of observations on copepodites generally was one third of that on adult females, which reduces both the number and statistical power of comparisons that can be made. Due to high mortality at certain copepodite stages, younger life stages can comprise a large fraction of copepod biomass (Bi et al., 2011; Eiane & Ohman, 2004). To better understand the effect of community composition on biogeochemical processes and the demographic constraints imposed on copepods imposed by nutrient availability, more effort must be made to gather comparable direct, field-based stoichiometric measurements of specific life history stages.

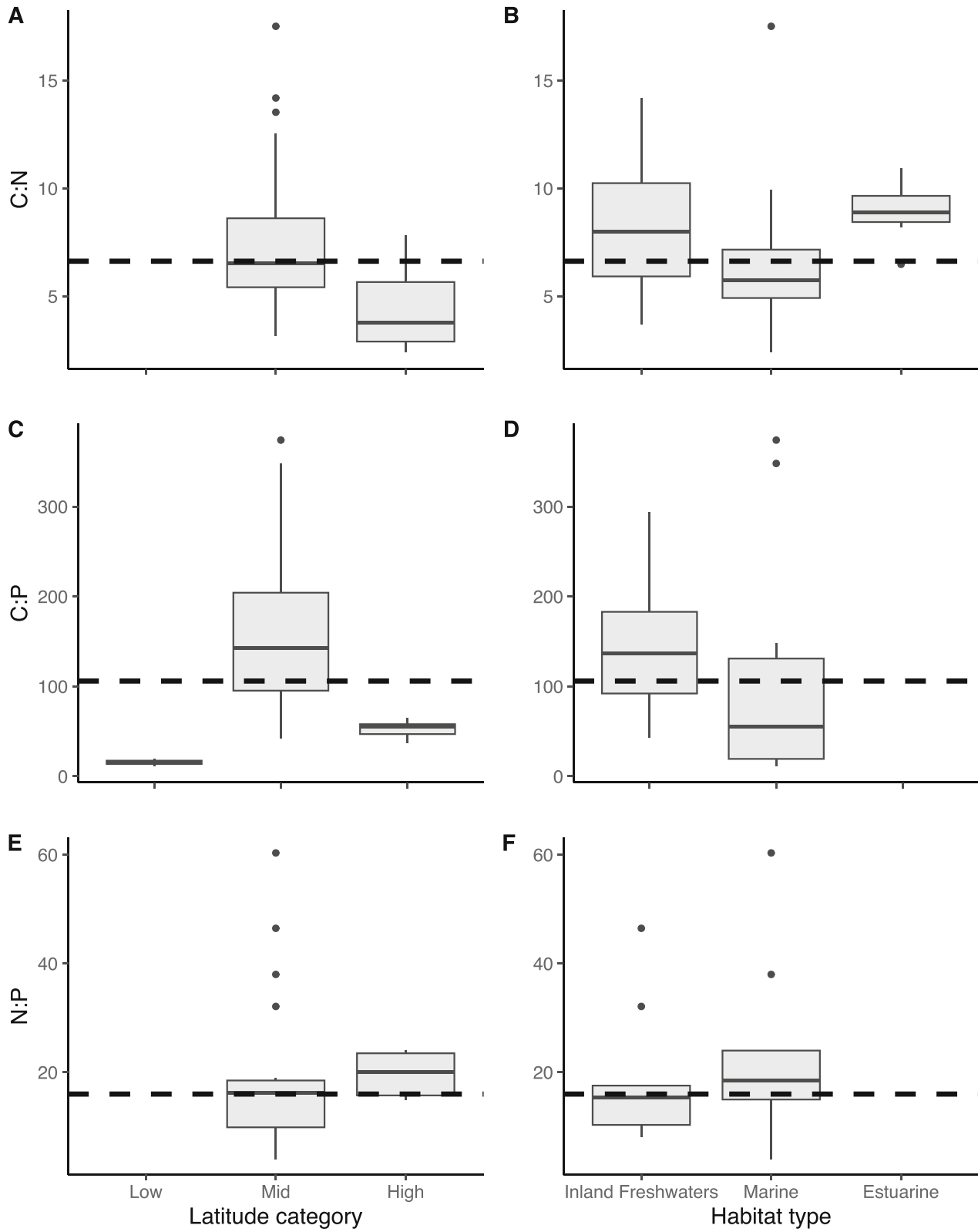
We found large differences in elemental composition among families that pose challenges to interpretation of observed patterns. Such inter-family differences have not been a focus of previous reviews, but here they accounted for more variation in copepod elemental composition than either habitat or latitude. Specifically, within the adult female copepods considered here, the Rhincaliniidae and the Diaptomidae tended to have higher C:N and C:P than other families, whereas the Calanidae tended to have lower values of these ratios (Table 1, Figure 4). Body size cannot account for these differences among families (Figure 3). Consequently, differences in copepod elemental composition among latitudinal and habitat categories may reflect which families are predominately represented by data from these locales. Ideally, one would statistically correct differences in average elemental composition among latitudes for differences in family representation or use phylogenetic correction to be more certain that traits that were correlated with stoichiometry were not responsible for observed patterns (Westoby et al., 1995). However, there is no sufficiently resolved phylogeny for copepods (Khodami et al., 2017) and limited data for most families (Table 1). Additionally, there is greater diversity in marine

## Stoichiometric data (mol:mol) for adult female copepods

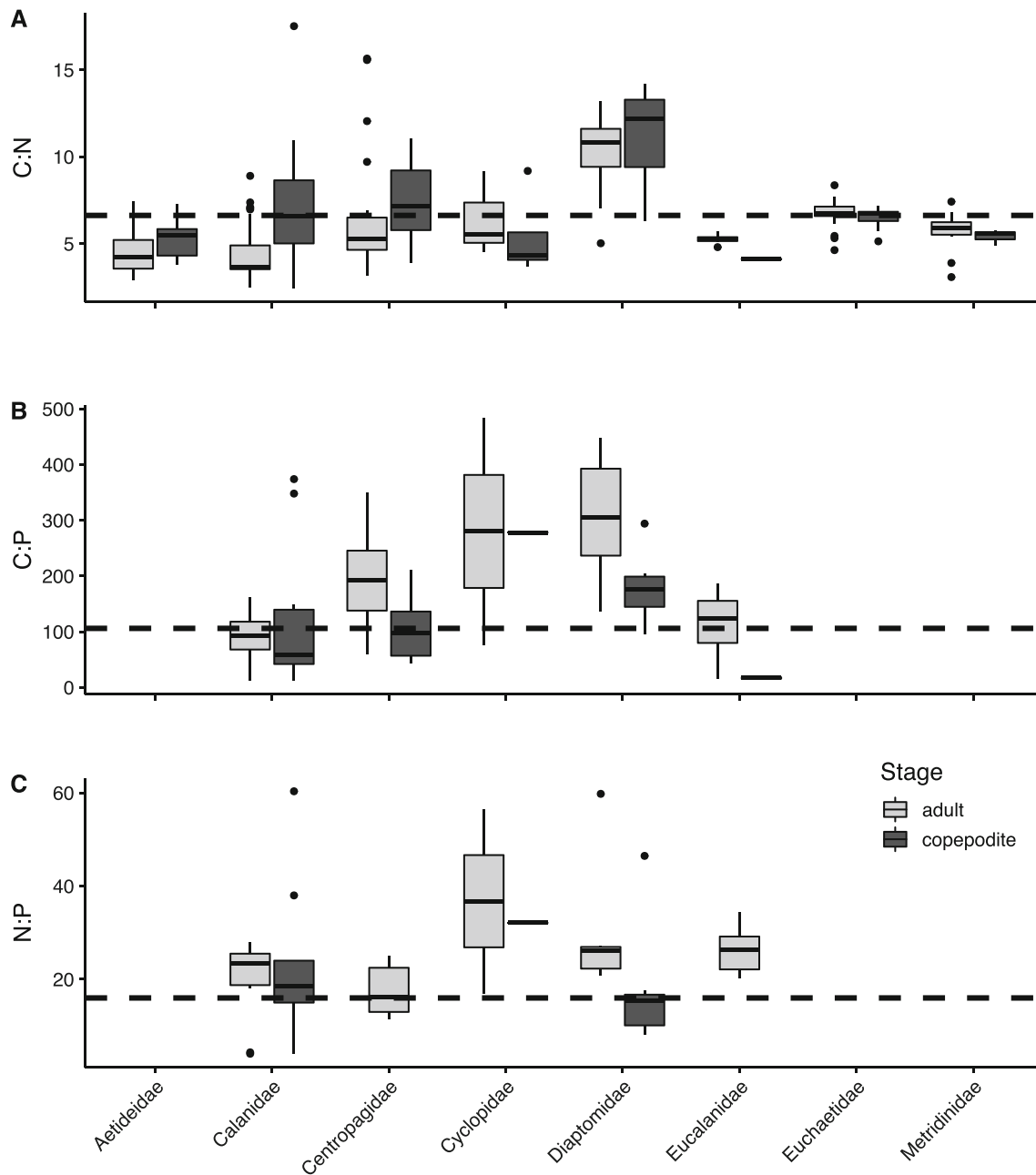


**FIGURE 5** Somatic molar elemental ratios for adult female copepods by environmental characteristics. Data are shown for families with a minimum of five stoichiometric observations. The box-and-whisker plots show stoichiometric data by either latitude (A, C, E) or habitat type (B, D, F); boxes show medians with first and third quartiles, whiskers represent the minimum and maximum values, and outliers are shown as points. Dashed horizontal lines represent the Redfield ratio (C:N = 6.63, C:P = 106, N:P = 16).

## Stoichiometric data (mol:mol) for copepodites



**FIGURE 6** Somatic molar elemental ratios for copepodites by environmental characteristics, as described in Figure 5.



**FIGURE 7** Somatic molar elemental ratios for adult females and copepodites within the same family. Data are shown for families with a minimum of three stoichiometric observations at each stage. The box-and-whisker plots show stoichiometric data; boxes show medians with first and third quartiles, whiskers represent the minimum and maximum values, and outliers are shown as points. Box color indicates ontogeny; light gray boxes show adult female copepods, and dark gray boxes show copepodites. Dashed horizontal lines represent the Redfield ratio (C:N = 6.63, C:P = 106, N:P = 16).

copepods and little overlap between marine and freshwater copepod families (Boxshall & Defaye, 2008), and an uneven distribution of copepods by taxonomy across latitudes (Rombouts et al., 2009). We expect, but cannot prove with extant data, that the different spatial distributions of stoichiometrically distinct families may reflect evolutionary adaptations to prevailing conditions like nutrient availability, temperature, and seasonality.

With this in mind, we note when stoichiometrically distinct families contribute to differences in copepod elemental composition in ways that complicate our conclusions.

Latitude affected elemental composition of the two life history stages, adult females and copepodites, in different ways. Adult female C:P and N:P did not vary much with latitude, whereas adult female C:N declined

significantly, albeit weakly, from low to high latitude (Figure 5). These results conflict with Båmstedt (1986) who found that C:N in marine copepods increased as latitude increased, presumably because of higher lipid content at higher latitudes (see also Mauchline, 1998; Miller et al., 2000). One possible reason why our data did not match this pattern is our inclusion of freshwater copepod species, which tended to be more C-rich than marine species and which were typically sampled from mid-latitudes. By contrast, Båmstedt (1986) was solely concerned with marine copepods. Furthermore, the inclusion of more low-latitude data on Rhincalanidae, which are more C-rich than other marine taxa, may also have contributed to the lack of a pattern. Latitudinal differences in adult female C:N became statistically insignificant when these families were excluded (Tables 2 and 4).

In contrast, latitude had a more substantial effect on the C:N and C:P ratios for copepodites, causing both ratios to decline with latitude (Figure 6). The pattern in copepodite C:P matches a prediction of increasing mRNA content, and thus, somatic P content, to compensate for slower growth by poikilothermic organisms in colder temperatures (Woods et al., 2003). High growth rate also allows for faster development during short growing seasons at high latitudes (Lonsdale & Levinton, 1985). Alternatively, zooplankton grow best on a diet that matches their elemental requirement (Jones et al., 2002; Meunier et al., 2016, 2017), so we expect copepod stoichiometry could have evolved to reflect elemental availability at different latitudes. Therefore, the observed patterns could reflect lower C:N, C:P, and N:P in phytoplankton within cold, high-latitude gyres compared with warm, low-latitude gyres (Martiny, Pham, et al., 2013), which likely reflects variation in nutrient availability at large geographic scales (Galbraith & Martiny, 2015; Martiny, Vrugt, et al., 2013). To determine whether differences in copepod stoichiometry are driven by increased rRNA and thus somatic P in high-latitude copepods, or whether environmental elemental availability influenced the evolution of copepod stoichiometry, future work should gather data on copepod P content and compare stoichiometry of closely related copepods from lower and higher latitudes (Table 5).

Our observed patterns in copepodite stoichiometry with latitude directly contradict the general latitudinal patterns described by Båmstedt (1986), possibly because he did not assess latitudinal variation in stoichiometry separately for copepodites and adults, and adult females dominated his data. Calanoid copepods typically have six copepodite stages, of which the earlier stages (C1–C4) are not as prone to accumulate C-rich wax esters and triglycerides compared with later stages (Lee et al., 2006). About 27% of our data were for copepodites in stages

C1–C4; lower accumulation of C-rich fatty acids by these younger copepodites may have partially driven the observed copepodite's stoichiometric response to latitudinal variations in nutrient availability, temperature, and/or seasonality, making the importance of these environmental characteristics more obvious than for older animals, like adult females. In light of this possibility, the lack of a strong effect of latitude on the stoichiometry of adult females may reflect the counteracting effects of adaptation to nutrient availability, which would increase N and P contents with latitude, and increase the storage of C-rich lipids in response to greater seasonal variation and a shorter growing season at high latitudes (Båmstedt, 1986; Mauchline, 1998; Miller et al., 2000). Because we only used data from adult female copepods gathered during the growing season, the lack of a positive relationship between C:N and latitude may also reflect the lack of data from seasons during which lipid accumulation occurs (reviewed in Båmstedt, 1986; Mauchline, 1998). Future work should determine whether the C:N of high-latitude copepods exhibits more seasonal variation than the same or closely related species inhabiting less seasonal lower latitudes (Table 5).

Both the C:N and C:P of adult female copepods from marine habitats were lower than in inland freshwater copepods. This pattern held for C:N even when we removed data for Diaptomidae (inland freshwaters) and Rhincalanidae (marine) that had distinct C-rich stoichiometries (Table 2). Although variable, seston in inland water ecosystems like lakes generally has higher C:N and C:P than oceans (Downing, 1997; Hecky et al., 1993), and seston in marine ecosystems tends to have Redfield-like stoichiometric ratios (Hecky et al., 1993; Sommer & Sommer, 2006). If zooplankton can utilize elements within seston, inland water consumers may have evolved higher C:nutrient ratios to avoid the costs of postabsorptive adjustments through increased respiration or DOC excretion (reviewed in Hessen & Anderson, 2008). In contrast, copepods in some marine environments may in fact be energy/C limited, not N or P limited (Sommer & Sommer, 2006; Walve & Larsson, 1999). Furthermore, the availability of other micronutrients, like iron and zinc, can also be low in marine phytoplankton prey, which can subsequently limit copepod production (Baines et al., 2016; Chen et al., 2011; Twining & Baines, 2013). Marine copepods should therefore evolve stoichiometric requirements and elemental compositions that are richer in N and P than in C to ensure maximal growth. Such evolution would strongly tie copepod stoichiometry to habitat, phylogeny, and diet. To explore these patterns, and to untangle the potential confounding of phylogenetic and dietary differences between copepod families, future work should collect C:N data on a single copepod group with a similar



**TABLE 5** Proposed research.

Driver	Predicted pattern	Study type
Environment: Latitude	Higher latitude copepods have greater variation in C:P and N:P seasonally	Gather stoichiometric data for closely related copepods from a range of latitudes, focusing on noncalanoid copepods from locations outside of northern, mid-latitude habitats, and copepods across different life history stages. Copepods from latitudes with stronger seasonal temperature differences are expected to have higher variation in C and P content, associated with seasonal lipid storage and faster growth rates, respectively.
	Mid-latitude latitude copepod species have higher C:N and C:P	Gather stoichiometric data for copepods, seawater, and seston at different latitudes. Increased C in mid-latitude habitats drove evolution of higher somatic C than in low- and high-latitude copepod species collected during the growing season.
Environment: Habitat	C:N of inland water < marine copepods	Disentangle environment characteristics, taxonomy, and diet. Collect stoichiometric data for copepods in a single family with shared trophic mode, from inland water and marine habitats (Centropagidae suggested).
	C:N and C:P of open ocean copepods < coastal species	Gather stoichiometric data for closely related species of open ocean and coastal copepods. Open ocean habitats, which may be more limited by energy (C) and nutrients (like Fe and Zn), will have copepods with lower elemental ratios than coastal animals.
Taxonomy	Proportionally more N-rich somatic material in Calanoida > Cyclopoida > Siphonostomatoida	Motile, active calanoid copepods are more obvious to predators compared with passive-feeding cyclopoid copepods. Calanoids require more N-rich muscle tissue, and will have lower C:N and higher N:P ratios compared with cyclopoids. The Siphonostomatoida are often parasitic and immobile as adults, and will have low N requirements.
Ontogeny	C:P and N:P increase throughout ontogeny; confirm C:N decreases in non-calanoid copepods	Broadly, gather more data that include P measurements, and gather more information on copepodites and nauplii. Track copepod elemental content throughout development, as well as in adult females before, during, and after reproduction. Investigate the potential for stoichiometric bottlenecks throughout ontogeny, and implications for community composition and nutrient cycling.

*Note:* Such work could further illuminate patterns of copepod stoichiometry with respect to drivers considered in this systematic review. Overall, more direct measurements of copepod stoichiometry are needed for animals that are not adult female calanoids collected from coastal marine waters in northern, mid-latitude habitats.

trophic mode inhabiting inland freshwater and marine habitats, like the genus *Limnocalanus* in the family Centropagidae, to determine whether there is evidence of evolutionary divergence related to habitat and food composition (Table 5). Also, because much of the stoichiometric data for marine copepods in our study were gathered relatively close to the coast, future work should also gather information on the elemental composition of mid-ocean copepods, which may be more limited by energy (C) and micronutrients (like iron and zinc) compared with coastal copepod species (Table 5).

There are many avenues for future work, starting with exploring the stoichiometry of other orders, which have different lifestyles compared with Calanoida. We gathered data on elemental ratios for three copepod orders—mostly Calanoida, with few data for Cyclopoida, and one observation for Harpactacoida. This coverage matches previous reviews by Båmstedt (1986) and Ventura (2006), where most data were gathered for Calanoida, with little on Cyclopoida. For example, Cyclopoida are generally sit-and-wait predators or utilize particle feeding, whereas Calanoida tend to be active

feeders (Brun et al., 2017). Differences in trophic mode are associated with differences in predation risk. Active, motile copepods have a greater predation risk than more passive copepods (van Someren Gréve et al., 2017). Future work should gather data on other copepod orders where we did not find data for inclusion in our analysis. For example, it would be interesting to compare the stoichiometry of the parasitic copepod order Siphonostomatoida with the nonparasitic Calanoida and Cyclopoida (Table 5). As many adult parasitic copepods are minimally motile, their N requirements should be quite low compared with more active orders.

Another factor that may help explain some of the unaccounted-for variability in copepod stoichiometry is the animal's reproductive status. While we did not specifically focus on the reproductive status of copepods, future work should measure copepod stoichiometry before, during, and after reproduction to note changes in their elemental content (Table 5). Furthermore, we also observed that size influenced C:N for adult female copepods, but was complicated by taxonomy. For example, while copepods from Diaptomidae and Rhincalanidae fell within the size range of other copepods in our study: these two families consistently differed in their C:N compared with other copepod families considered here. Because size data were difficult to obtain for copepods from inland and estuarine waters, and largely lacking for copepodites, more efforts should be made to examine how size and ontogeny influence copepod stoichiometry throughout development.

Lastly, the importance of ontogeny should be considered by comparing copepodites and adults within the same family. Only the C:N of Calanidae adult females was significantly lower than Calanidae copepodites (Table 1, Figure 7). However, there were also far more data for the Calanidae compared with the other seven families that met the requirements for inclusion in this analysis (107 vs. 7–50 total data points). Gathering more data for these other families may have in fact shown differences in stoichiometry between developmental stages. While we had expected to see ontogenetic shifts in C:P based on previous laboratory work (Meunier et al., 2016), we did not observe significant shifts in either C:P or N:P, which we suspect is due to the low overall availability of P data. Phosphorus content, particularly as percent dry weight, decreases throughout development within copepods, reflecting shifting elemental demands throughout ontogeny (Carrillo et al., 2001; Villar-Argaiz et al., 2002). Copepod development also tracks seasonal seawater N:P trends, likely due to changing stoichiometric demands throughout ontogeny. Thus, the presence of particular copepod stages could result in differential nutrient cycling in aquatic ecosystems (Meunier et al., 2016). Finally, changes in stoichiometric demands throughout

copepod development may result in shifting stoichiometric bottlenecks, wherein elemental limitations hinder organismal development (Villar-Argaiz & Sterner, 2002). Future work should explicitly measure elemental ratios for numerous copepod species, specifically noting the developmental stage of the animals. Such measurements would illuminate the potential for stoichiometric bottlenecks in aquatic communities and help link seasonal changes in community composition with differential elemental cycling by copepods (Table 5).

## CONCLUSIONS

While we were able to expand upon the seminal work of Båmstedt (1986), many of the same gaps in the literature identified >36 years ago still remain. We have several key findings regarding somatic copepod stoichiometry with respect to important environmental characteristics, and copepod taxonomy and ontogeny. First, we observed three families with distinctive stoichiometry—the Rhincalanidae and Diaptomidae were especially C-rich, and the Calanidae were especially N- and P-rich, suggesting that there are different evolutionary and stoichiometric pressures in inland freshwater and marine environments. Much more research should focus on studying the elemental ratios within specific copepod families, particularly those that are not adult female calanoids from coastal marine waters in northern, mid-latitude habitats (Table 5). Next were differences in C:N and C:P ratios between copepodites and adult females from mid- and high latitudes, suggesting nutrient availability or adaptation to cold environments may influence copepod stoichiometry. Due to their selective foraging and differential retention of limiting nutrients, we predict that copepods of different life history stages differentially influence consumer-driven nutrient cycling, and thus may experience stoichiometric bottlenecks, which has implications for community composition (Table 5). Overall, we suggest much more work is needed to address numerous knowledge gaps. Future research should focus on increasing the taxonomic and geographic diversity of sampling and analyze younger life history stages separately from older animals. These data may illuminate whether stoichiometric differences are due to ancient phylogenetic splits in copepod lineages or are evolutionary responses to key habitat characteristics.

## AUTHOR CONTRIBUTIONS

Emily M. Herstoff came up with the idea for this systematic review, gathered the data, and performed the analyses as part of her PhD dissertation research. All authors worked together to write and revise the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data (Herstoff, 2023a, 2023b) are available from Figshare: <https://doi.org/10.6084/m9.figshare.21841860.v5> and <https://doi.org/10.6084/m9.figshare.21841863.v5>.

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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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