Deep-Sea Research II ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Feeding by *Pseudocalanus* copepods in the Bering Sea: Trophic linkages and a potential mechanism of niche partitioning

Alison C. Cleary *, Edward G. Durbin, Tatiana A. Rynearson, Jennifer Bailey [1]

University of Rhode Island Graduate School of Oceanography, South Ferry Road, Narragansett, RI 02882, USA

ARTICLE INFO

Keywords:
Pseudocalanus
Bering Sea
Trophic interactions
Niche partitioning
18S rDNA

ABSTRACT

Pseudocalanus copepods are small, abundant zooplankton in the Bering Sea ecosystem that play an important role in transferring primary production to fish and other higher trophic-level predators. Four morphologically cryptic species, the primarily arctic *Pseudocalanus minutus* and *Pseudocalanus acuspes*, and the more temperate *Pseudocalanus newmani* and *Pseudocalanus mimus*, are found within the Bering Sea. *Pseudocalanus* are generally considered phytoplanktivores. However, their feeding is poorly known, despite their importance to the ecosystem. *In situ* feeding by the three most abundant *Pseudocalanus* congeners, *P. minutus*, *P. newmani*, and *P. acuspes*, was investigated by sequencing partial 18S rDNA (ribosomal Deoxyribonucleic Acid) of gut contents from 225 individuals sampled from 8 stations across the Bering Sea in May and June of 2010. The 28,456 prey 18S rDNA sequences obtained clustered into 138 distinct prey items with a 97% similarity cut-off, and included diatoms, dinoflagellates, microzooplankton, mesozooplankton, and vascular plants. *Pseudocalanus* diets reflected variations in the environment, with phytoplankton sequences relatively more abundant in copepods from stations with higher water-column chlorophyll *a* concentrations. Feeding differences were observed between species. *P. acuspes* diet contained relatively more heterotrophic dinoflagellate sequences, and was significantly different from that of *P. minutus* and *P. newmani,* both of which contained relatively more diatom sequences, and between which no significant difference was observed. Feeding differences between the two primarily arctic species may be a mechanism of niche partitioning between these spatially co-located congeners and may have implications for the effects of climate change on the success of these abundant zooplankters and their many predators in this ecosystem.

1. Introduction

The competitive exclusion principle in ecological theory suggests that no two sympatric species can occupy precisely the same ecological niche, as one will inevitably eventually outcompete the other (Gause, 1934; Hardin, 1960). In the Bering Sea, four morphologically cryptic species of *Pseudocalanus* copepods have been identified (Frost, 1989; Bailey et al., this issue), which at first glance appear to violate this principle. These four species are similar in both size and overall morphology and traditional morphometric and meristic traits are often unsuccessful at differentiating among them, although they are genetically distinct and can be distinguished by DNA sequencing (Frost, 1989; Bailey et al., this issue). *Pseudocalanus minutus* and *Pseudocalanus acuspes* are considered primarily arctic, while *Pseudocalanus newmani* and *P. mimus* are considered primarily temperate, but all four species ranges overlap in the Bering Sea (Frost, 1989; Coyle et al., 2011). In order for these species to persist in their coexistence in the Bering Sea, they must be different from each other in at least one ecologically meaningful way, such as susceptibility to disease or predators, timing of reproduction, or feeding.

As a location for investigating copepod niche partitioning, the Bering Sea is particularly interesting as it is a region with high levels of interannual variation and alternating 4 to 5 year periods of relatively warm and cool temperatures (Stabeno et al., 2012). Changes in physical forcing may have bottom up ecosystem effects, such as by changing phytoplankton bloom dynamics (Stabeno et al., 2012; Winder and Sommer, 2012). Warming has also been associated with a shift from a largely benthic ecosystem to a more pelagic dominated system, giving small zooplankton such as *Pseudocalanus* spp. an increasingly important role in the transfer of carbon and energy from primary producers to pelagic higher predators (Overland and Stabeno, 2004).

Although *Pseudocalanus* spp. are small (1–2 mm in length), their abundance makes them important components of food webs and carbon cycling in the Bering Sea ecosystem (Frost, 1989; Napp et al., 2002). *Pseudocalanus* are particularly important as prey for walleye pollock, *Theragra chalcogramma,* one of the most commercially

* Corresponding author. Tel.: +14153006556.
E-mail address: alison_cleary@my.uri.edu (A.C. Cleary).
[1] Present address: United States Peace Corps, Pindoyu, Paraguay.

http://dx.doi.org/10.1016/j.dsr2.2015.04.001
0967-0645/

important fish stocks in the US, since larval pollock diet consists of up to 60% small copepods (Coyle et al., 2011). Understanding the range of prey consumed by *Pseudocalanus in situ* and the relative importance of different prey types is essential to understanding the role this abundant consumer plays in transferring primary production to higher trophic levels.

Little is known about the specific prey types consumed by *Pseudocalanus in situ*; results published to date have lumped all *Pseudocalanus* species together (Lebour, 1922; Marshall, 1949; Poulet, 1973). Incubation experiments in natural seawater and water from mesocosms have suggested that *Pseudocalanus* spp. copepods are able to feed effectively on particles from 4 to 102 μm diameter, with a potential preference for particles 25 to 57 μm (Poulet, 1973; Harris, 1982). Microscopic analysis of *Pseudocalanus* gut contents has shown that they consume diatoms, including *Coscinodiscus*, *Paralia*, *Navicula*, and *Thalassiosira* spp., and to a lesser extent, crustaceans, radiolarians, and flagellates (Lebour, 1922; Marshall, 1949).

The relative paucity of data on *Pseudocalanus* feeding is not surprising, since measuring feeding by small zooplankton is methodologically challenging. Previous work used incubation experiments and particle counters (Poulet, 1973), but these may not be representative of feeding *in situ* and give fairly coarse resolution of prey type. Microscopic examination of gut contents provides *in situ* data, but is strongly biased towards prey with distinctive exoskeletons and typically only limited samples can be analyzed by this labor intensive approach (Lebour, 1922; Marshall, 1949). Recent advances in DNA analysis and sequencing have allowed for new molecular approaches to understanding zooplankton feeding. DNA barcodes from gut contents can be sequenced, and by comparing these sequences to reference databases of known organisms, the identity of every known eukaryote the predator, in this case *Pseudocalanus*, had consumed in the preceding minutes can be determined (Cleary et al., 2012; Durbin et al., 2012; O'Rorke et al., 2012; Hu et al., 2014; Craig et al., 2014). Unlike microscopy-based diet analysis, 18S rDNA sequencing allows for identification of the full range of eukaryotic prey items consumed, including soft bodied and morphologically indistinct prey. Feeding differences have been inferred as mechanisms of niche partitioning in other copepods (*e.g.* Von Vaupel Klein, 1997); however, in their study feeding was not directly measured, but rather assumed to be a function of body size and morphology.

This study examined 18S rDNA in *Pseudocalanus* gut contents to elucidate feeding by *P. minutus*, *P. newmani*, and *P. acuspes* in the eastern Bering Sea, to address questions of niche partitioning by these cryptic congeners and improve understanding of food webs and carbon flows through this ecosystem. The gut contents of 225 *Pseudocalanus* individuals from across the shelf and shelf break region were analyzed using Peptide Nucleic Acid (PNA) probes and a high throughput sequencing approach to determine what they had consumed, how environmental factors affected feeding, and whether there were differences in consumption between species which might serve as a mechanism of ecological niche separation.

2. Methods

2.1. Field sampling and species identification

Copepods and environmental data were collected in the eastern Bering Sea between May 19 and June 10, 2010. *Pseudocalanus* spp. copepods were collected in vertically integrated net tows from 60 m (or 1 m above the seafloor where depth <60 m) to the surface at 15–20 m min^{-1} with a 153 μm mesh 1 m ring net at 8 stations (Table 1 and Fig. 1). Mixed plankton samples were immediately preserved in 95% ethanol, to minimize effects of digestion and potential net feeding, and ethanol was changed once after 12 to 24 h to maintain concentration (Passmore et al., 2006). Temperature, salinity and fluorescence profiles were obtained with a SBE 19+ CTD at the same stations as copepods were collected, with temperature and salinity at the depth of the *in situ* fluorescence maximum used in station comparisons. Total chlorophyll and chlorophyll >5 μm were measured flourometrically from extracted pigments of water collected at this same depth of maximum *in situ* fluorescence (Lomas et al. 2012, M. Lomas, unpublished data). In the lab, individual *Pseudocalanus* spp. copepods were picked from mixed plankton samples under a dissecting microscope and rinsed thoroughly in clean 95% ethanol (Bailey et al., this issue). DNA was extracted from each whole individual using the DNeasy Blood and Tissue kit (Qiagen) (Bailey et al., this issue). Species identity of each copepod was determined through sequencing of cytochrome oxidase gene fragments by Bailey et al. (this issue).

2.2. Gut contents 18S rDNA amplification and sequencing

Peptide Nucleic Acid Polymerase Chain Reaction (PNA-PCR) was used to amplify the partial 18S rDNA of all eukaryotes in *Pseudocalanus* gut contents from pools of DNA extracts from 5 conspecific individuals from a single net tow (Cleary et al., 2012; O'Rorke et al., 2012). Triplicate pools were analyzed of each species at each of the stations, for 15 copepods in total from each species in each net tow where sufficient individuals were collected (Table 1). One technical replicate, with all stages of analysis after DNA extraction run separately, was run on one of the pools of 5 copepods from *P. acuspes* at station 99, for an overall total of 46 samples. Each reaction contained 1x GoTaq Green master mix (Promega), 0.5 μmol L^{-1} each 960F and 1200R primers (Gast et al., 2004), 20 μmol L^{-1} PNA (5′-TGCTCAATCTCGTGCGAC-3′), and approximately 0.5 ng μL^{-1} template DNA. An initial denaturation at 95 °C for 30 s, was followed by 30 cycles of 94° for 30 s, 77° for 30 s, 58° for 30 s, 60° for 45 s, and a final extension at 60° for 5 min. To remove any remaining genomic DNA, amplicons were electrophoresed on a 0.8% agarose gel and the

Table 1
Characteristics of sampling locations and species analyzed at each station. Station (Stn) numbers correspond to Bailey (2012), species numbers indicate how many individuals of each species were analyzed, latitude and longitude are in decimal degrees, all dates are in 2010, total bottom depth is in meters and chlorophyll *a* (Chl*a*) represents the >5 μm size fraction in μg L^{-1} at the depth of maximum chlorophyll *a* fluorescence.

Stn	*P. acuspes*	*P. minutus*	*P. newmani*	Latitude	Longitude	Date sampled	Depth	Chl*a*
49	0	30	30	59.8998	−178.8960	May 19	486	20.1
55	0	15	0	58.2043	−174.2357	May 21	381	0.4
87	0	15	15	55.4315	−168.0608	May 29	205	6.2
99	15	0	15	56.8536	−164.5056	May 31	73	3.8
156	0	15	15	62.1890	−175.1521	June 5	79	3.8
163	0	15	0	59.8934	−178.8983	June 7	657	4.1
175	15	0	0	59.9003	−172.2170	June 9	73	0.8
179	15	15	0	58.8301	−168.1589	June 10	46	0.8

Please cite this article as: Cleary, A.C., et al., Feeding by *Pseudocalanus* copepods in the Bering Sea: Trophic linkages and a potential mechanism of niche partitioning. Deep-Sea Res. II (2015), http://dx.doi.org/10.1016/j.dsr2.2015.04.001