# ARTICLE IN PRESS

Journal of Experimental Marine Biology and Ecology xxx (xxxx) xxx-xxx



Contents lists available at ScienceDirect

# Journal of Experimental Marine Biology and Ecology

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



# Kinorhynch assemblages in the Gulf of Mexico continental shelf collected during a two-year survey

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#### ARTICLE INFO

#### Keywords: Echinoderes Scalidophora Community analysis Granulometry

#### ABSTRACT

Kinorhynch assemblages along the northern continental shelf of the Gulf of Mexico were examined to better understand the distributional trends of this understudied group of meiofauna. Samples were collected with a multicorer from 37 stations at 36-187 m depths in the years 2013 and 2014. Sediment was collected and the animals were isolated from the mud using Ludox® centrifugation. Kinorhynchs were isolated with a stereomicroscope and the adult animals were identified to species level either by light microscopy or scanning electron microscopy. A total of 1328 animals were recovered, including juveniles and adults. After processing, 812 adults were identified and classified to 6 families, 12 genera and 32 species. The identifications were used to explore relationships among the species, and to determine if the animal assemblages at each site revealed relationships to each other. Sediment characteristics were determined at each site, to reveal if abiotic factors correlated with animal densities. Cluster analysis revealed distinct assemblages in Louisiana versus Florida sediment. The highest abundance of kinorhynchs was found in western sediments in Louisiana, which are characterized by higher levels of silt and clay, and aluminum-associated trace metals. These sediment characteristics indicated a strong influence from the outflow of the Mississippi River. The most abundant species were Echinoderes bookhouti, E. skipperae, E. augustae, and E. spinifurca, which comprised 79% of the identified animals. This was the first kinorhynch survey to cover a geographic area as large as the northern Gulf of Mexico, over multiple years, and to statistically analyze the animals in relation to their sediment type.

### 1. Introduction

One of the lesser studied groups of meiofauna are the kinorhynchs, also known as "mud dragons". This group is generally not very abundant in most benthic samples when compared to organisms such as nematodes and copepods (Giere, 2009; Neuhaus, 2013). In the Gulf of Mexico (GOM) few studies have identified these animals to species level, as they are usually reported as undetermined "Kinorhyncha". Until recently the only known kinorhynch species in the GOM were Echinoderes coulli, E. remanei, E. steineri, Centroderes barbanigra, C. cf. drakei, and Campyloderes cf. vanhoeffeni (Blake, 1930; Chitwood, 1951; Higgins, 1964; Higgins, 1977; Neuhaus, 2013; Neuhaus et al., 2014; Zelinka, 1913). Recent meiofauna studies analyzing kinorhynchs from the GOM beginning in 2007 (Landers et al., 2012) have resulted in new species descriptions, namely Echinoderes romanoi, E. joyceae, E. charlotteae, E. skipperae, E. augustae, Paracentrophyes sanchezae, Semnoderes lusca, and Sphenoderes aspidochelone (Landers and Sørensen, 2016;

#### Sørensen and Landers, 2014, 2017a, 2017b; Sørensen et al., 2016).

Since 2013 our sampling strategy has employed a multicorer, which has resulted in a much greater abundance of kinorhynchs being collected. The present study analyzes the kinorhynchs recovered in 2013 and 2014. The study covers multiple stations over multiple years on the northern GOM continental shelf, resulting in the identification to species level of over 800 animals from shelf sediments. With the data collected at these stations, the goal of this study was to use statistical methods to provide a better understanding of the abiotic factors that influence kinorhynch distribution and community structure. Our hypothesis is that kinorhynch densities will correlate to collection locations enriched by the Mississippi River outflow, which can be identified by specific granulometric characteristics (silt + clay percentages) and trace metals (aluminum [Al] and zinc [Zn] concentrations). Regarding species distributions across the various collection locations, our null hypothesis is that kinorhynch species will be distributed randomly.

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http://dx.doi.org/10.1016/j.jembe.2017.05.013

Received 1 December 2016; Received in revised form 26 May 2017; Accepted 30 May 2017 0022-0981/ © 2017 Elsevier B.V. All rights reserved.

Please cite this article as: Landers, S.C., Journal of Experimental Marine Biology and Ecology (2017), http://dx.doi.org/10.1016/j.jembe.2017.05.013

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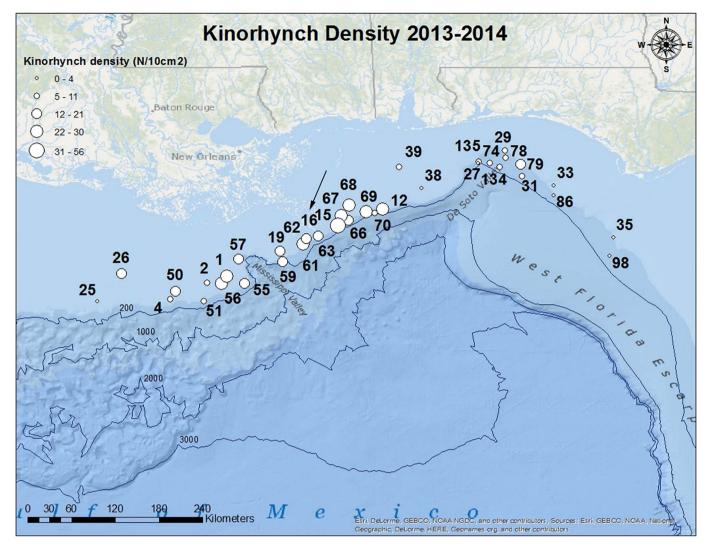


Fig. 1. A map of the 2013–2014 sediment collection sites with kinorhynch densities (animals recovered in the counting wheel). Station numbers are provided. Depth contours are in meters. Latitude and longitude for each site are reported in Table 1. Arrow points to the general area at the outflow of the Mississippi River.

#### 2. Materials and methods

#### 2.1. Sample collection

Samples were collected aboard NOAA ships *Pisces* and *Gordon Gunter* from 37 sites (Fig. 1) on the GOM continental shelf in October and November 2013 and 2014. A mini-multicorer (Ocean Instruments®) was used for sediment collection. Five sediment samples were collected from each site; three were used for kinorhynch isolation and the remaining two for abiotic data (granulometry and trace metal analysis). Sediment samples for kinorhynch isolation were immediately fixed with 5–10% formalin. Sediment for granulometry and trace metals analysis was stored in a refrigerator or frozen. Depth and salinity were recorded at each site. ArcGIS© 10.2.2 was used to create maps of the study area (Fig. 1).

#### 2.2. Kinorhynch isolation

Sediment samples were sieved first through a 500-µm sieve, then through a 45-µm sieve, and centrifuged with Ludox\* HS-40 (Burgess, 2001). Kinorhynchs were picked from the meiofauna using a stereomicroscope with a counting wheel and processed for either light microscopy (LM) or scanning electron microscopy (SEM). For LM, the animals stored in isopropanol were dehydrated to 90% ethanol, and then exposed to increasing concentrations of glycerol and left in 100% glycerol overnight. They were then mounted in Fluoromount G  $^{\circ}$ 

(Sørensen and Pardos, 2008) and photographed using a Nikon E600 light microscope equipped with DIC optics. For SEM, the animals stored in isopropanol were dehydrated to 100% ethanol, critical-point dried, and sputter-coated with either gold or platinum/palladium. Some animals were post-fixed in osmium tetroxide vapor before sputter-coating. The samples were examined at the Natural History Museum of Denmark (NHMD) or at the Auburn University Research Instrumentation Facility (AURIF). At NHMD the specimens were examined with a JEOL JSM-6335F SEM and at AURIF they were examined with a Zeiss EVO 50 SEM.

## 2.3. Trace metals analysis

Trace metals concentrations in the sediment samples were determined at Louisiana State University's Agricultural Center using Inductively Coupled Plasma (ICP) and following EPA method 200.7 (USEPA, 2001).

#### 2.4. Granulometric analysis

Samples from 2013 were analyzed for granulometry at the Louisiana State University Agricultural Center, while 2014 samples were processed at Troy University following U.S. Soil Survey Staff (1996). A mechanical shaker was used to separate the samples into 5 sand size fractions, plus the silt + clay fraction.

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