

Characterization of diversity and community structure of small planktonic copepods in the Kuroshio region off Japan using a metabarcoding approach

Junya Hirai, Keiichi Yamazaki, Kiyotaka Hidaka, Satoshi Nagai,
Yugo Shimizu, Tadafumi Ichikawa

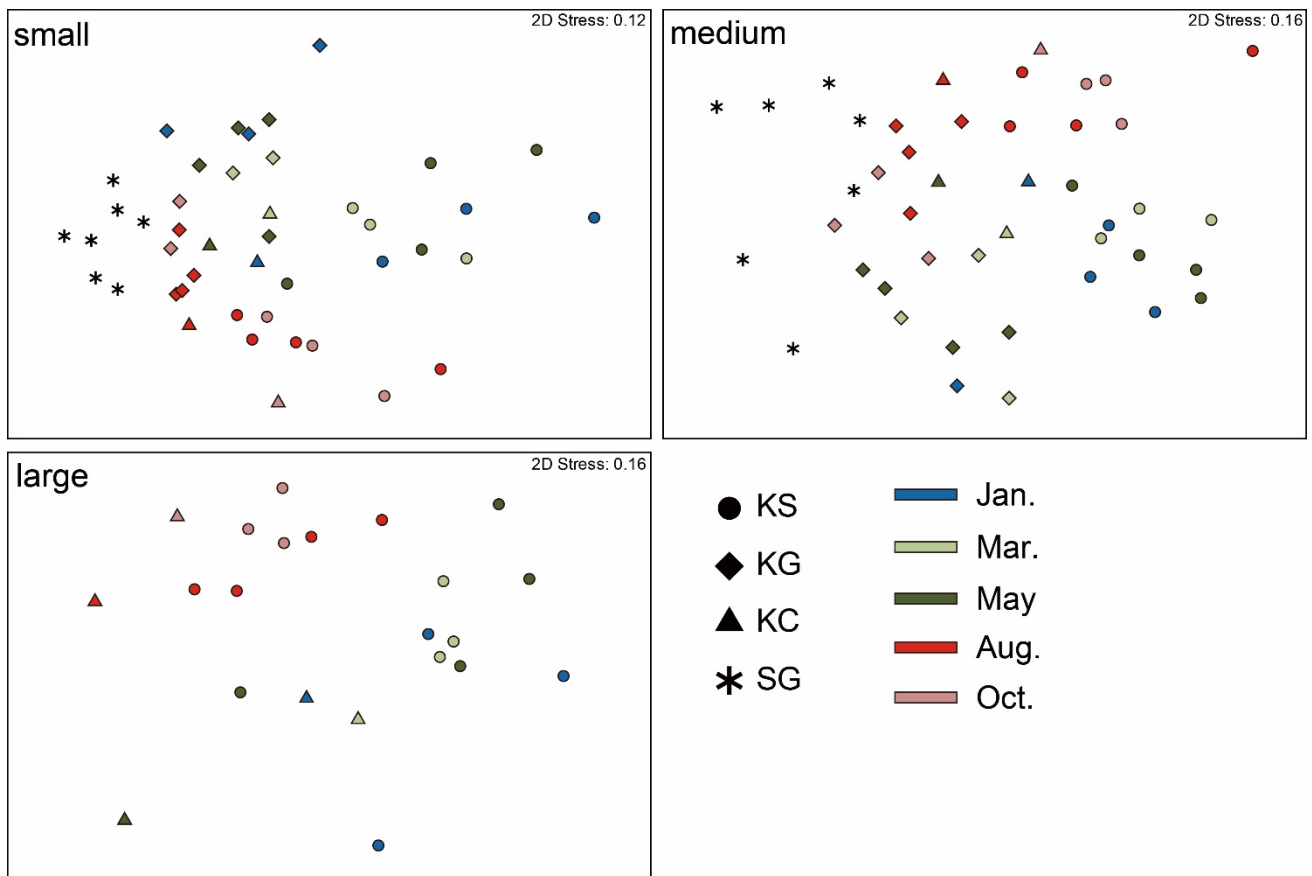


Fig. S1. Multidimensional scaling (MDS) analysis for presence/absence of copepod operational taxonomic units (OTUs) in each size category. KS: Kuroshio Slope, KG: Kuroshio Gyre, KC: Kuroshio Core, and SG: Subtropical Gyre.

Table S1. Location, date, and time information for samples. Observations using both CTD and plankton nets are listed. Location of the Kuroshio axis at 138°E is listed for each research cruise. The samples collected during the day are indicated by an asterisk in local time. Local time is the time of the deployment of plankton nets and is only presented for stations at which zooplankton samples were collected. KS: Kuroshio Slope, KG: Kuroshio Gyre, KC: Kuroshio Core, and SG: Subtropical Gyre.

| Cruise | Region | Station | lat. | lon. | Date | Local time | Kuroshio axis (lat.) |
|-----------------|--------|---------|---------|----------|------------|------------|----------------------|
| Kuroshio (2013) | | | | | | | |
| SY-13-1 | KS | C3400 | 34°00'N | 138°00'E | 17/1/2013 | 10:50* | 33°30'N |
| | KG | C3000 | 30°00'N | 138°00'E | 23/1/2013 | | |
| SY-13-3 | KS | C3400 | 34°00'N | 138°00'E | 7/3/2013 | | 33°35'N |
| | KG | C3000 | 30°00'N | 138°00'E | 3/3/2013 | | |
| SY-13-5 | KS | C3400 | 34°00'N | 138°00'E | 10/5/2013 | 14:50* | No data |
| | KG | C3100 | 31°00'N | 138°00'E | 13/5/2013 | 14:15* | |
| SY-13-8 | KS | C3430 | 34°30'N | 138°00'E | 22/8/2013 | 1:56 | 32°05'N |
| | KG | C3000 | 30°00'N | 138°00'E | 28/8/2013 | 22:13 | |
| SY-13-10 | KS | C3400 | 34°00'N | 138°00'E | 28/10/2013 | 20:11 | 32°30'N |
| | KG | C3130 | 31°30'N | 138°00'E | 31/10/2013 | | |
| Kuroshio (2014) | | | | | | | |
| SY-14-1 | KS | C3400 | 34°00'N | 138°00'E | 17/1/2014 | | 32°45'N |
| | KG | C3000 | 30°00'N | 138°00'E | 23/1/2014 | | |
| SY-14-3 | KS | C3400 | 34°00'N | 138°00'E | 28/2/2014 | 20:40 | 33°00'N |
| | KG | C3000 | 30°00'N | 138°00'E | 7/3/2014 | 20:29 | |
| SY-14-5 | KS | C3400 | 34°00'N | 138°00'E | 10/5/2014 | 0:21 | 33°10'N |
| | KG | C3000 | 30°00'N | 138°00'E | 16/5/2014 | 22:40 | |
| SY-14-8 | KS | C3400 | 34°00'N | 138°00'E | 26/8/2014 | 5:10 | 33°15'N |
| | KG | C3100 | 31°00'N | 138°00'E | 28/8/2014 | 21:07 | |
| SY-14-10 | KS | C3400 | 34°00'N | 138°00'E | 26/10/2014 | 19:06 | 33°00'N |
| | KG | C3000 | 30°00'N | 138°00'E | 29/10/2014 | 5:29 | |
| Kuroshio (2015) | | | | | | | |
| SY-15-1 | KS | C3400 | 34°00'N | 138°00'E | 27/1/2015 | 20:53 | 33°00'N |
| | KC | C3300 | 33°00'N | 138°00'E | 26/1/2015 | 0:38 | |
| | KG | C3000 | 30°00'N | 138°00'E | 21/1/2015 | 22:42 | |
| SY-15-3 | KS | C3400 | 34°00'N | 138°00'E | 26/2/2015 | 19:29 | 32°20'N |
| | KC | C3220 | 32°20'N | 138°00'E | 28/2/2015 | 22:27 | |
| | KG | C3030 | 30°30'N | 138°00'E | 3/3/2015 | 19:13 | |
| SY-15-5 | KS | C3400 | 34°00'N | 138°00'E | 14/5/2015 | 0:14 | 33°00'N |
| | KC | C3300 | 33°00'N | 138°00'E | 15/5/2015 | 23:50 | |
| | KG | C3000 | 30°00'N | 138°00'E | 9/5/2015 | 22:53 | |
| SY-15-8 | KS | C3400 | 34°00'N | 138°00'E | 22/8/2015 | 21:15 | 32°00'N |
| | KC | C3200 | 32°00'N | 138°00'E | 25/8/2015 | 19:10 | |
| | KG | C3000 | 30°00'N | 138°00'E | 25/8/2015 | 21:45 | |
| SY-15-10 | KS | C3400 | 34°00'N | 138°00'E | 24/10/2015 | 21:25 | 33°15'N |
| | KC | C3315 | 33°15'N | 138°00'E | 26/10/2015 | 23:04 | |
| | KG | C3030 | 30°30'N | 138°00'E | 29/10/2015 | 0:24 | |

| | | | | | | | |
|------------------|----|-------|-----------|------------|------------|--------|---------|
| Kuroshio (2016) | | | | | | | |
| SY-16-1 | KS | C3400 | 34°00'N | 138°00'E | 21/1/2016 | 19:55 | 33°00'N |
| | KG | C3130 | 31°30'N | 138°00'E | 28/1/2016 | 2:20 | |
| SY-16-3 | KS | C3345 | 33°45'N | 138°00'E | 2/3/2016 | 19:40 | 33°10'N |
| | KG | C3000 | 30°00'N | 138°00'E | 5/3/2016 | 20:20 | |
| SY-16-5 | KS | C3400 | 34°00'N | 138°00'E | 15/5/2016 | 22:31 | 32°45'N |
| | KG | C3000 | 30°00'N | 138°00'E | 11/5/2016 | 0:25 | |
| SY-16-8 | KS | C3400 | 34°00'N | 138°00'E | 31/8/2016 | 22:54 | 33°00'N |
| | KG | C3200 | 32°00'N | 138°00'E | 3/9/2016 | 2:46 | |
| SY-16-10 | KS | C3400 | 34°00'N | 138°00'E | 27/10/2016 | | 33°00'N |
| | KG | C3000 | 30°00'N | 138°00'E | 30/10/2016 | | |
| Subtropical gyre | | | | | | | |
| KH-13-7 | SG | St. 0 | 20°00'N | 160°00'E | 16/12/2013 | 10:51* | |
| KH-14-3 | SG | St. 0 | 20°00'N | 160°00'E | 28/6/2014 | 3:24 | |
| KH-16-7 | SG | N02 | 23°00'N | 141°00'E | 10/12/2016 | 11:01* | |
| | SG | N04 | 23°00'N | 127°00'E | 13/12/2016 | 7:07* | |
| | SG | T15 | 25°59.5'N | 126°26.8'E | 18/12/2016 | 13:38* | |
| | SG | RM1 | 25°49.9'N | 126°57.4'E | 21/12/2016 | 18:58 | |
| KH-17-4 | SG | St.17 | 23°00'N | 137°00'E | 2/10/2017 | 0:26 | |

Table S2. Numbers of sequence reads during bioinformatics analysis.

| | Reads per sample | Total reads |
|--|------------------|-------------|
| Raw reads | 22,071-137,194 | 6,732,598 |
| Initial quality-filtering | 14,131-57,311 | 3,520,653 |
| Selection of copepods | 10,872-55,482 | 3,099,194 |
| Standardization of reads before alignments | 10,872 | 1,195,920 |
| Further quality filtering | 6,621-9,687 | 949,571 |
| Standardization of reads before OTU clustering | 6,621 | 728,310 |
| Final reads for community and diversity analyses | 6,589 | 724,790 |

Table S3. Top five operational taxonomic units (OTUs) contributing to similarity in each cluster group of medium and large sizes using similarity percentage (SIMPER) analysis. The cluster groups are presented in Figure 4A. KS: Kuroshio Slope, KG: Kuroshio Gyre, KC: Kuroshio Core, and SG: Subtropical Gyre.

| Cluster group | OTUs | Blast-hit species | Contribution (%) |
|--|--------|--------------------------------|------------------|
| Cluster 1 KS large (summer–autumn) | OTU 3 | <i>Subeucalanus subtenuis</i> | 17.33 |
| | OTU 9 | <i>Subeucalanus crassus</i> | 14.94 |
| | OTU 1 | <i>Calanus sinicus</i> | 7.45 |
| | OTU 6 | <i>Pleuromamma xiphias</i> | 6.78 |
| | OTU 30 | <i>Pareucalanus attenuatus</i> | 6.06 |
| Cluster 2 KS large (winter–spring) | OTU 6 | <i>Pleuromamma xiphias</i> | 17.97 |
| | OTU 1 | <i>Calanus sinicus</i> | 15.21 |
| | OTU 11 | <i>Candacia bipinnata</i> | 9.34 |
| | OTU 12 | <i>Paraeuchaeta russelli</i> | 8.34 |
| | OTU 5 | <i>Neocalanus gracilis</i> | 6.41 |
| Cluster 3 KS middle (winter–spring) | OTU 1 | <i>Calanus sinicus</i> | 24.45 |
| | OTU 12 | <i>Paraeuchaeta russelli</i> | 7.72 |
| | OTU 18 | <i>Pleuromamma abdominalis</i> | 7.24 |
| | OTU 21 | <i>Pleuromamma gracilis</i> | 4.98 |
| | OTU 3 | <i>Subeucalanus subtenuis</i> | 4.3 |
| Cluster 5 KS middle (summer–autumn) | OTU 3 | <i>Subeucalanus subtenuis</i> | 6.94 |
| | OTU 1 | <i>Calanus sinicus</i> | 6.23 |
| | OTU 20 | <i>Subeucalanus pileatus</i> | 6.11 |
| | OTU 8 | <i>Acrocalanus longicornis</i> | 5.74 |
| | OTU 9 | <i>Subeucalanus crassus</i> | 5.56 |
| Cluster 6 KG middle (summer–autumn) | OTU 3 | <i>Subeucalanus subtenuis</i> | 10.64 |
| | OTU 2 | <i>Cosmocalanus darwinii</i> | 8.24 |
| | OTU 5 | <i>Neocalanus gracilis</i> | 6.93 |
| | OTU 15 | <i>Pareucalanus</i> sp. | 5.47 |
| | OTU 32 | Euchaetidae OTU | 4.69 |
| Cluster 7 KG middle (winter–spring) | OTU 2 | <i>Cosmocalanus darwinii</i> | 12.18 |
| | OTU 5 | <i>Neocalanus gracilis</i> | 10.82 |
| | OTU 13 | <i>Mesocalanus tenuicornis</i> | 9.44 |
| | OTU 18 | <i>Pleuromamma abdominalis</i> | 6.62 |
| | OTU 33 | <i>Euchaeta media</i> | 5.24 |
| Cluster 8 SG middle | OTU 2 | <i>Cosmocalanus darwinii</i> | 11.97 |
| | OTU 5 | <i>Neocalanus gracilis</i> | 10.78 |
| | OTU 16 | <i>Lucicutia flavicornis</i> | 5.77 |
| | OTU 7 | <i>Delibus nudus</i> | 5.52 |
| | OTU 32 | Euchaetidae OTU | 5.34 |

Table S4. Average proportions of the sequence reads of major copepods in the small fraction of copepod community. KS: Kuroshio Slope, KG: Kuroshio Gyre, KC: Kuroshio Core, and SG: Subtropical Gyre.

| | KS | KG | KC | SG |
|---|-----------|-----------|-----------|-----------|
| Major OTUs of medium-sized/large copepods | 28.8 | 24.7 | 16.3 | 11.9 |
| Major OTUs of small copepods | 54.7 | 37.2 | 37.5 | 44.6 |
| Non-major copepods | 16.5 | 38.2 | 46.2 | 43.5 |