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Melissa Rohal

David Thistle

Erin E. Easton
The University of Texas Rio Grande Valley, erin.easton@utrgv.edu

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Extraction of metazoan meiofauna from muddy deep-sea samples: operator and taxon effects on efficiency

Melissa Rohal[1], David Thistle*, Erin E. Easton[2]

Department of Earth, Ocean and Atmospheric Sciences, Florida State University, Tallahassee, FL 32306-4520

*Corresponding author. Tel.: +1 850 644 4089, e-mail address dthistle@fsu.edu

[1]Present address: Harte Research Institute, Texas A&M University-Corpus Christi, 6300 Ocean Drive, Unit 5869, Corpus Christi, TX 78412, USA, mrohal@islander.tamucc.edu

[2]Present address: Departamento de Biología Marina, Universidad Católica del Norte, Núcleo Milenio de Ecología y Manejo Sustentable de Islas Oceánicas (EMSIO), Larrondo 1281, Coquimbo, Chile, erineeaston@gmail.com

Abstract

Deep-sea metazoan meiofaunal specimens are usually extracted from muddy samples by centrifugation in a fluid in which meiofauna tend to float and sediment particles tend to sink. Although the procedure is in common use, its efficiency has seldom been examined. The study reported here showed that well-trained operators extracted metazoan meiofauna with efficiencies that were different enough to be a concern in quantitative studies. Therefore, samples should be assigned to operators in a stratified-random manner. In the course of these studies, both operators extracted individuals of the common nematode family Desmoscolecidae significantly less efficiently than other nematode families, a bias that could interfere with studies that compared relative abundances of nematode families.

Keywords: benthos; Desmoscolecidae; Harpacticoida; Ludox®; Nematoda

Highlights

- Most deep-sea meiofaunal samples are extracted by floatation, e.g., in Ludox®.
- Operators differed significantly in extraction efficiency.
- Operators extracted desmoscolecids significantly less well than nondesmoscolecids.

1. Introduction

Ideally, when an investigator takes a sediment sample for the study of an ecological question, no individuals are lost during collection and sample processing. The possibility of loss is of particular concern for students of sediment-dwelling, metazoan meiofauna (hereafter meiofauna), which are difficult to see with the naked eye. The most accurate method of counting meiofauna in a preserved sediment sample is to examine the entire sample, aliquot by aliquot, through a dissection microscope (Nichols, 1979), but this procedure consumes so much time that many investigators (e.g., Jenkins, 1964; Heip et al., 1974) have proposed methods to speed the process. Because the buoyancies of most sediment particles are less than those of most meiofaunal individuals (ostracods are an exception), most of the meiofauna will float in the upper portion of the supernatant in a fluid of appropriate density. Most of the sediment particles will sink and constitute the "sediment fraction" or "pellet." Some workers (e.g., Bowen et al., 1972; de Jonge and Bouwmann, 1977) allowed gravity to do the separation. Others (e.g., Nichols, 1979; Schwinghamer, 1981) used centrifugation to speed the process. Workers have used several fluids of appropriate density, in particular, colloidal silicas with the brand names Ludox® (du Pont) and Levasil® (H. C. Starck, now available from Akzo Nobel Chemicals GmbH as Levasil

CS40-316P). Centrifugation-in-a-fluid-of-appropriate-density (CFAD)methods can extract most shallow-water meiofaunal groups with efficiencies approaching 100% (see, e.g., Burgess, 2001; Du et al., 2009).

Although CFAD methods have been used to extract the meiofauna of muddy deep-sea sediments for more than a decade (see, e.g., Vanreusel et al., 2000; Tselepides and Lampadariou, 2004), the present authors know of only two published studies of extraction efficiency for such samples. Escobar-Briones et al. (2008) removed all the meiofauna from each sample by hand, counted the specimens of each group, returned the specimens to the appropriate sample, and extracted each sample once using a CFAD method based on Ludox-AM®. Their method extracted only 27% of the meiofauna from their continental-slope samples and only 20% from their abyssal-plain samples, extraction rates too low for quantitative ecological studies. Kitahashi et al. (2014) extracted each sample three times using a CFAD method based on Ludox HS40®. They quantified efficiency by searching the pellet by hand for unextracted specimens. Their extraction efficiency for harpacticoids was 100%; they did not report efficiencies for other taxa.

Because the extraction of meiofauna from a sample by hand takes an inordinate amount of time, students of the muddy deep sea will certainly continue to take advantage of the greater

efficiencies of CFAD methods. At the same time, more information is needed about the problems of the CFAD approach and their potential effects on quantitative ecological studies. The study reported here showed that two carefully trained operators extracted some groups with significantly different efficiencies. Given the likelihood that extraction efficiency is affected by sediment properties, we looked for effects of differences in sediment grain-size distributions and for effects of differences in the concentrations of chloroplastic pigment equivalents (hereafter CPE).

2. Materials and methods

2.1. Core collection

We planned to sampled each of four latitudes once at ~2700 m and once at ~3700 m, but bad weather made this impossible for stations 1 and 2 (see Table 1 and Fig. 1). Samples were collected with a MC 800 Multi Core (Ocean Instruments, San Diego) that had eight tubes of 10-cm inner diameter. Three of the deployments from each station were chosen at random. From each, one of the high-quality cores was selected at random for the analysis of meiofauna. One of the remaining high-quality cores was selected at random from each deployment for determination of the grain-size distribution of the sediment and the concentration of CPE.

2.2. On-board core processing

For each core used for meiofauna, the water overlying the sediment was siphoned off and poured onto a sieve with 30-µm apertures. The top 1 cm of sediment was sliced off and placed in a sample jar, the sieve contents were added, and the jar was filled with 95% ethanol.

For sediment grade and CPE, cut-off syringes were used as subcores. Samples for sediment grade were preserved in a 10% solution of formalin and artificial salt water (salinity = 35) and buffered to neutrality with sodium bicarbonate. Those for CPE were stored in the dark at -20°C.

2.3. Laboratory processing

2.3.1. Extraction of meiofauna

Operator 1 extracted 14 samples; operator 2 extracted 10 samples. Each sample was washed with deionized water on a 300-µm-aperture sieve, and the material that passed through was caught on a 30-µm-aperture sieve. The meiofauna were removed from the 30-µm fraction by a method inspired by that of Burgess (2001); see also Lebreton et al. (2012). Thirty ml of Ludox® HS-40 (specific gravity = 1.30) and 10-15 ml of the 30-µm fraction were added to a 50-ml centrifuge tube, and the process was repeated until the entire sample was divided among centrifuge tubes. The contents of each tube were homogenized on a vortex mixer for 5 min and centrifuged at 900 × **g** for 5 min. The

supernatant was washed onto a 30-µm sieve, and the pellet was retained in the centrifuge tube. The homogenization and centrifugation steps were repeated on the pellet. The supernatants from the two extractions were combined, and the combined supernatant and the final pellet were stored in separate jars of 95% ethanol.

2.3.2. Hand sorting

The supernatant and the pellet samples were stained with rose bengal, and all were searched for meiofauna under a dissection microscope at 25× by a third operator. Six groups were counted and removed: copepods, nematodes of the family Desmoscolecidae (hereafter desmoscolecids), kinorhynchs, nauplii, nematodes other than desmoscolecids (hereafter nondesmoscolecids), and ostracods.

2.3.3. Particle-size distribution

The sample was first poured onto a 30-µm sieve, and the sieve was gently moved up and down for 5 min in a container filled with enough deionized water to cover the sediment in the sieve. The sediment was rinsed on the 30-µm sieve through 500-, 350-, 250-, 177-, 125-, 88-, 62.5-, 45-, and 30-µm–aperture sieves. The content of each sieve was vacuum filtered onto a dried and tared Whatman GF/D filter. The loaded filters were dried overnight at 60°C, allowed to cool in a desiccator for one hour, and weighed. The less-than-30-µm fraction of a sample was

filtered onto a stack consisting of a dried and tared Whatman GF/D filter on top of a dried and tared 0.1-µm Whatman polycarbonate membrane filter. The stack was dried overnight at 60°C, allowed to cool for one hour in a desiccator, and weighed. Note that in the text the term "mud" is used in the sense of the Wentworth scale, i.e., to mean the fraction that passes through a 62.5-µm sieve.

2.3.4. Chloroplastic-pigment equivalents

Each sample for CPE was extracted in the dark and transferred to a tared microcentrifuge tube. It was weighed, freeze-dried, and reweighed. Six hundred µl of 90% acetone was then added, and the contents were vortexed for 15–30 s and frozen overnight. The following day, the sample was vortexed long enough to resuspend the sediment and centrifuged at 1500 × **g** for 2 min, long enough to remove particles from the supernatant. The fluorescence of chlorophyll *a* and phaeopigments was measured with a Varian Cary Eclipse Fluorescence Spectrophotometer set at 5 nm excitation and emission slits, excitation at 425 nm, and emission from 600 to 880 nm. Peak emission (659–674 nm) and emission at 750 nm were recorded for each sample. After addition of two drops of 10% HCl, the fluorescence was remeasured. The methods of Parsons et al. (1984) were used for calculation of CPE concentrations.

2.4. Statistical analysis

The individual-test significance level was 0.05 and was not corrected for multiple testing. A resampling procedure (Simon, 1999) written in the Statistics101 (www.statistics101.net) programming language was used to test for a difference in means (two-tailed). Ninety-five percent confidence limits were calculated of means by resampling; the code can be found in Simon (1999). The Spearman rank-difference method (Tate and Clelland, 1957) as implemented in Statistix 10© (Analytical Software) was used to test for correlations.

Desmoscolecids and nondesmoscolecids were analyzed separately because preliminary data suggested that the extraction efficiencies of these groups differed.

2.5. *Extraction efficiency*

2.5.1. Approach

Extraction efficiencies are reported in the usual way, i.e., the number in the supernatant divided by the number in the pellet plus the number in the supernatant. Significance tests followed Atchley et al. (1976), Prothero (1986), and Berges (1997) and operated on the ratio of the number of individuals found in the pellet to the number found in the supernatant, so that the numerator and denominator would be independent.

2.5.2. Operator extraction efficiency per group

For each of the six groups, we asked whether the average extraction efficiency differed between operators 1 and 2.

2.5.3. Operator extraction efficiency: correlations with percentage mud and amount of CPE

For each of the six groups, Spearman rank correlation was used to determine whether operator efficiency for a given group was correlated with the percentage of mud in the sample and with the amount of CPE in the sample.

3. Results and discussion

3.1. Differences in efficiency between operators

One would expect that operators trained in exactly the same way using the same equipment would differ very little in their ability to extract meiofauna. Dr. Jeffery Baguley (pers. comm.) made us aware that this assumption can be false. Because no test of this assumption appears in the literature, the performances of our operators were compared. The proportion of the total individuals originally present in the sample that remained in the pellet was less for operator 1 than for operator 2 for all six groups (Tables 3 and 4 and Figs. 2 to 4). The differences were significant for desmoscolecids (64.0% versus 84.6%), nauplii (95.4% versus 99.5%), and nondesmoscolecids (83.6% versus 96.9%). These differences in extraction efficiency could make real patterns more difficult to perceive. To minimize this problem, investigators should assign samples to operators in a stratified random manner; i.e., each operator

should process approximately equal numbers of samples from each station.

We sought a property of the samples that could explain the differences in operator performance. The efficiency of neither operator was correlated with CPE for any group. The efficiency of operator 2 (the better operator) was not correlated with percentage mud. In contrast, the efficiency of operator 1 was significantly negatively correlated with the percentage mud in the sample for copepods ($p < 0.034$), nondesmoscolecids ($p < 0.007$), and nauplii ($p < 0.001$). The statistic for the test was the number in the pellet divided by the number in the supernatant, so the negative correlation indicates that operator 1's extraction efficiency increases with increasing percentage mud. In other words, operator 1 was less efficient when extracting sandier samples. The reason for this result is not obvious, but during the vortexing step, the operators might have achieved different intensities of mixing. If so, and if the sample becomes harder to mix as the degree of sandiness increases, the observed pattern could arise. A more detailed protocol for vortexing the sample may be necessary to minimize differences in technique among operators. Other aspects of the extraction procedure may be involved. For example, at several steps the samples is transferred with the aid of a jet of water. Water will dilute the CFAD and decreases its density.

Differences in extraction efficiency between operators could arise from differences in the amount of water they used in transfers. Finally, the efficiency of the procedures, in general, may be subject to improvement. For example, given that meiofaunal individuals must be reach the surface of the sediment to be extracted, it might be worth exploring whether extraction efficiency increases as the depth of the sediment in the extraction vessel decreases.

3.2. Absolute extraction efficiency

Because we planned to sort the pellet, our samples were only extracted twice. Because three extractions are commonly used (see, e.g., Rose et al. 2005, Ingles et al. 2011, Miljutin et al. 2011), our study does not assess the extraction efficiency of the de facto standard method. Still, each extraction requires time and creates opportunities for loss, so we report our results for two extractions (Table 4) to help future workers decide whether two extractions might be sufficient.

3.3. Extraction efficiency of desmoscolecids

In the course of our work, we discovered that desmoscolecids were extracted much less efficiently than nondesmoscolecids. Because the extraction efficiency of the two groups has apparently not been compared, we give our results here. For operator 1, average extraction for the former (64.0%)

was significantly less ($p < 0.005$) than that of the latter (83.6%). For operator 2, average extraction for the former (84.6%) was significantly less ($p < 0.005$) than that of the latter (96.9%). Although operator 2 extracted a higher proportion of desmoscolecids than did operator 1, her average efficiency was still only 84.6%.

Substantial proportions of the desmoscolecids in a sample can fail to be extracted. In some cases, more desmoscolecids were found in the pellet than in the supernatant. Such inefficiencies could make certain inquiries difficult, for example, comparing abundances of nematode families.

Why extraction of desmoscolecids was less efficient than that of nondesmoscolecids is unknown. One explanation could be that two extractions were not enough, and desmoscolecids and nondesmoscolecids would have been extracted with comparable efficiencies if three extractions had been done. At least one other explanation is possible. Unlike other nematodes, most desmoscolecids have conspicuous rings around their bodies composed of foreign particles (including sediment grains) and secretions from the worm (Decraemer and Smol, 2006). The composition of the rings of some individuals may decrease their buoyancy such that they sink below the level in the supernatant that is decanted. If so, an increase in the density of the extraction fluid should increase the extraction efficiency of

desmoscolecids. Unfortunately, any increase in the density of the extraction fluid will increase the amount of sediment retained in the supernatant. Preliminary research will be needed to find a compromise appropriate to the questions to be addressed by a study.

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nematode community structure. Deep-Sea Res. I 47, 1855-1879.

Table 1. Station information. The positions and depths are the averages of those of the multiple-corer deployments from a given station.

Station	Position	Depth (m)
1	44.0012°N 130.3946°W	3242
2	42.5594°N 131.9228°W	3591
3	39.9917°N 125.8781°W	3676
4	40.0011°N 125.4447°W	2694
5	36.7975°N 123.6998°W	3676
6	36.6806°N 122.8213°W	2720
7	32.8739°N 120.6151°W	3852
8	32.7977°N 120.3709°W	2704

Table 2. For each sample, the percentage of mud (= mud) and the concentration of chloroplastic pigment equivalents (= CPE) in nanograms per gram sediment dry weight are given. The first digit in the sample number is the station number.

Sample	Mud	CPE
1181	92.4	52.54
1331	91.8	113.00
1421	91.1	202.03
2341	96.4	154.89
2431	97.5	124.32
2661	97.1	108.91
3271	96.9	360.34
3331	93.8	279.89
3421	94.4	364.25
4111	72.3	1067.80
4271	70.8	1342.91
4561	92.0	1650.62
5231	95.4	2206.25
5351	95.1	3373.32
5521	95.2	2310.17

6111	90.6	3373.06
6261	95.4	4312.13
6311	91.4	2915.08
7161	93.7	867.51
7381	96.2	498.54
7451	94.5	378.87
8151	86.7	1794.04
8481	76.5	341.40
8521	78.8	1051.40

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Table 3. Operator identity and abundances of meiofaunal groups in the supernatant (= super) and pellet fractions of the 0-1 cm layer of 78.5 cm^2 samples. Desmos = nematodes of the Desmoscolecidae. Kinos = kinorhynchs. Nondesmos = all other nematodes.

Station	Operator	Sample	Fraction	Copepods	Desmo	Kinos	Nondesmos	Nauplii	Ostracods
1	2	1181	Super	84	152	16	1664	99	13
			Pellet	2	68	1	102	2	2
1	1	1331	Super	69	104	7	1134	48	8
			Pellet	1	48	3	254	3	7
1	1	1421	Super	120	107	9	638	113	11
			Pellet	5	83	2	157	12	10
2	2	2341	Super	73	134	7	1591	109	5
			Pellet	0	25	1	16	0	5
2	1	2431	Super	123	56	7	1357	102	3
			Pellet	5	67	0	298	1	6
2	2	2661	Super	109	137	21	1105	177	12
			Pellet	3	19	0	14	0	4
3	2	3271	Super	117	247	31	2196	159	11
			Pellet	1	97	2	65	2	6

3	1	3331	Super	147	82	16	924	222	6
			Pellet	1	145	1	253	9	12
3	1	3421	Super	151	210	26	1427	509	17
			Pellet	5	116	0	122	9	6
4	1	4111	Super	194	232	21	714	176	8
			Pellet	46	206	8	588	32	14
4	2	4271	Super	204	594	31	2471	226	11
			Pellet	1	58	6	101	1	14
4	2	4561	Super	230	808	77	4382	265	17
			Pellet	0	83	1	24	0	8
5	1	5231	Super	176	732	91	2369	324	5
			Pellet	2	214	1	201	7	5
5	1	5351	Super	148	932	114	2131	285	4
			Pellet	2	97	1	62	0	4
5	2	5521	Super	118	833	52	1922	129	5
			Pellet	0	41	2	27	0	0
6	1	6111	Super	286	314	68	1522	425	15
			Pellet	16	255	0	356	38	7
6	1	6261	Super	270	546	102	1915	327	7
			Pellet	1	96	3	140	7	5
6	2	6311	Super	274	608	202	3658	353	8

			Pellet	2	97	4	60	2	7
7	1	7161	Super	149	158	22	1490	191	6
			Pellet	0	45	0	170	5	11
7	2	7381	Super	197	217	11	1623	201	5
			Pellet	4	17	0	48	0	12
7	1	7451	Super	143	117	15	1398	189	1
			Pellet	2	77	0	203	3	11
8	1	8151	Super	110	373	21	3023	201	2
			Pellet	10	176	4	1022	19	4
8	1	8481	Super	215	161	31	2128	247	13
			Pellet	4	114	0	348	6	11
8	2	8521	Super	260	231	33	2444	312	9
			Pellet	1	69	2	253	3	17

Table 4. Extraction efficiencies (the ratio of the number in pellet divided by the number in supernatant and pellet combined) as percentages and the results of tests for differences between operators (as assessed by the ratio of the number in the pellet to the number in the supernatant).

	Average extraction efficiency (%)		Test for difference
Group	Operator 1	Operator 2	Probability
Copepods	96.3	99.1	> 0.109
Desmoscolecids	64.0	84.6	< 0.008
Kinorhynchs	92.7	94.7	> 0.465
Nauplii	95.4	99.5	< 0.014
Nondesmoscolecids	83.6	96.9	< 0.013
Ostracods	45.7	60.6	> 0.227

Figure captions

Fig. 1. Chart showing the locations of the stations and the 2700-m and 3700-m isobaths. The insert shows the position of the chart relative to the west coast of the United States.

Fig. 2. Extraction efficiency of Copepoda and Kinorhyncha for operator 1 (circles) and operator 2 (triangles).

Fig. 3. Extraction efficiency of Desmoscolecidae and nondesmoscolecid nematodes for operator 1 (circles) and operator 2 (triangles).

Fig. 4. Extraction efficiency of nauplii and Ostracoda for operator 1 (circles) and operator 2 (triangles).

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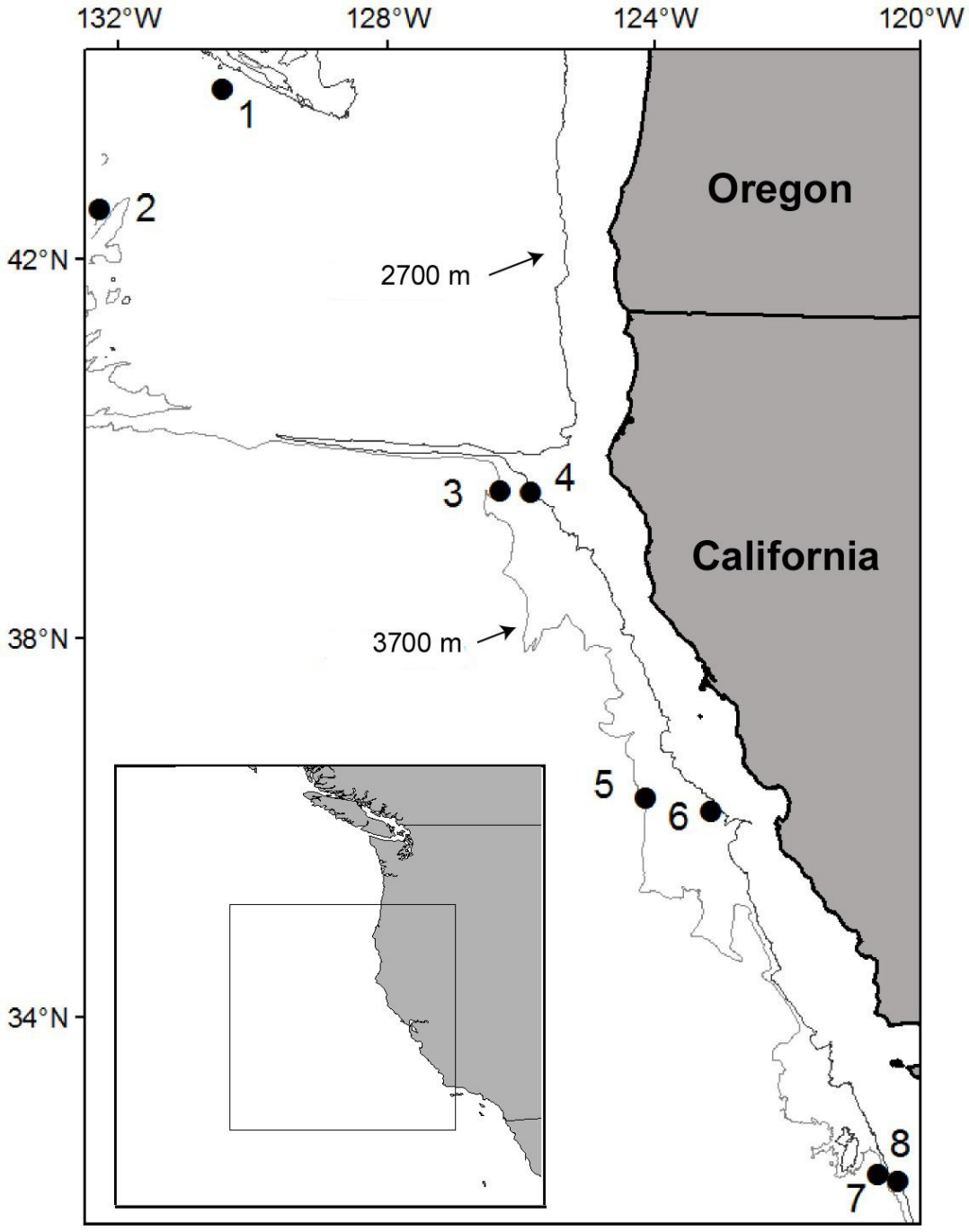
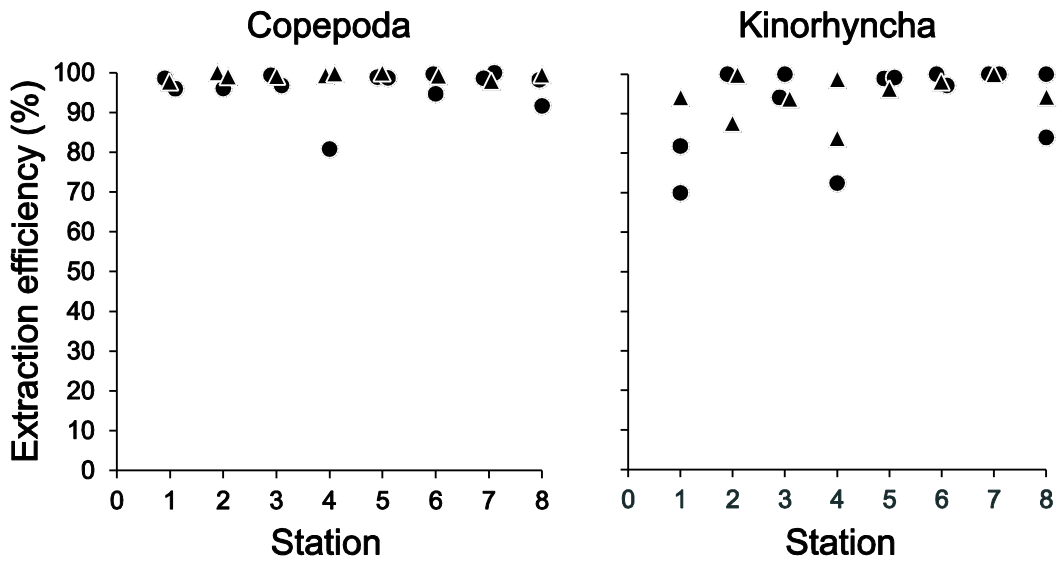


449

450 Fig. 1.

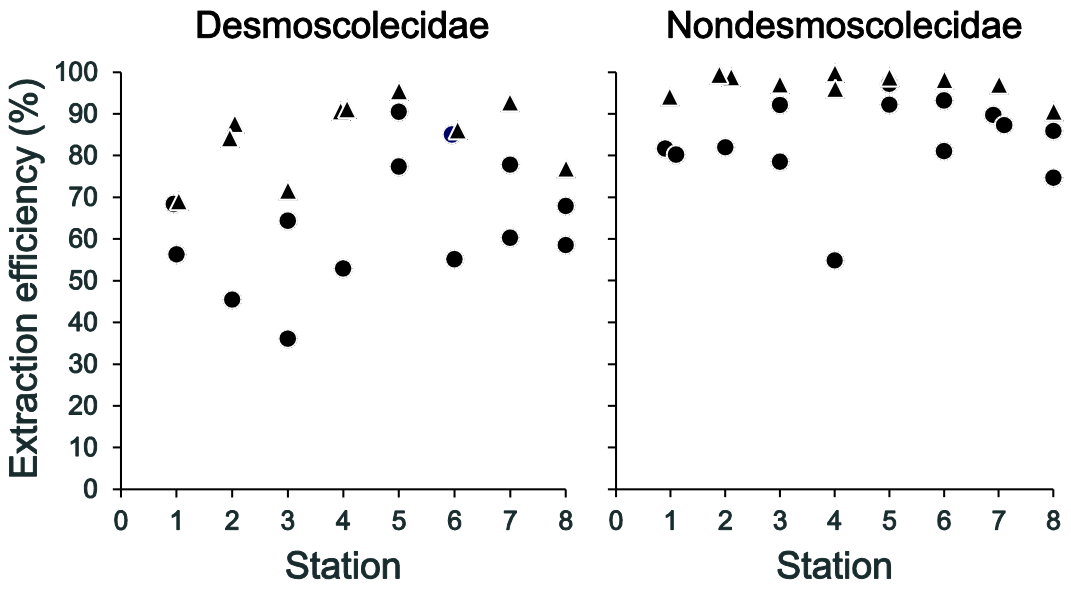
451



452

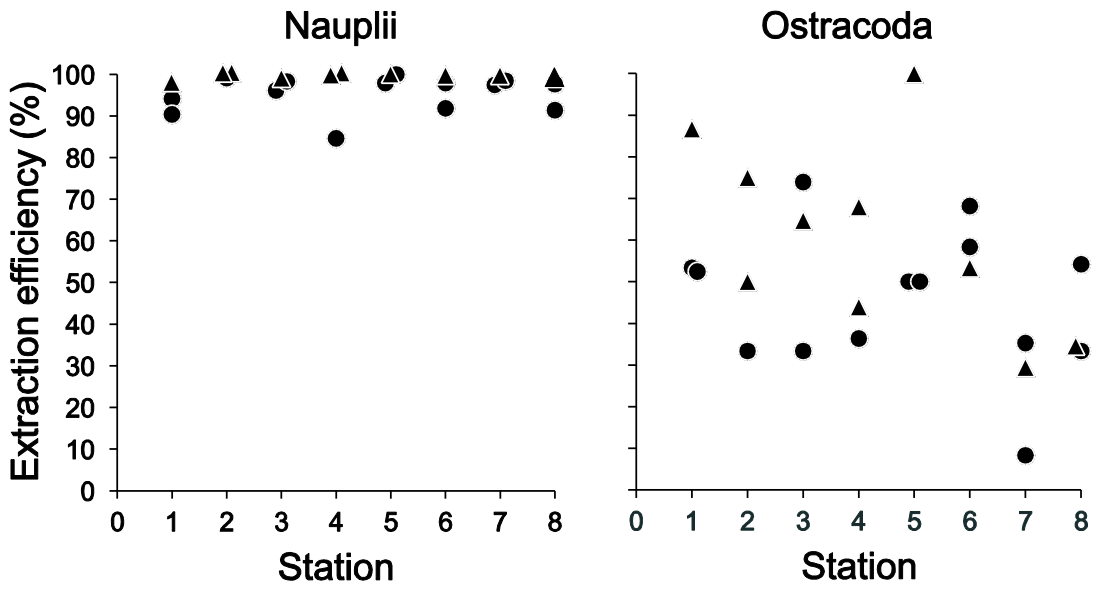
453 Fig. 2.

454



455

456 Fig. 3.


Nauplii
Ostracoda
Extraction efficiency (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8
Station
0 1 2 3 4 5 6 7 8
Station

457

458 Fig. 4.

459