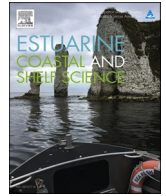




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## Varying intertidal invertebrate taxonomic resolution does not influence ecological findings

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### ABSTRACT

Benthic invertebrate communities are reliable indicators of environmental conditions, and thus often assessed during ecological studies. However, when identifying all specimens to the same taxonomic unit is not possible, the common practice of identifying each specimen to the lowest possible taxonomic level may introduce enough taxonomic noise into datasets to obscure important trends. Using an infaunal-invertebrate community from the Cassiar Cannery mudflat (British Columbia, Canada), we tested if identifying specimens to different taxonomic levels (order, family, genus, species, and lowest possible) produced different statistical interpretations, or observed magnitude of differences in community structure and density between microhabitats. When taxonomic level was varied, statistical interpretations and magnitude of observed differences did not differ. Given the resources required to train taxonomists, and the time required to identify all specimens to species, identifying invertebrates to broader levels may represent an efficient trade-off between taxonomic resolution and resources. This study also showed no difference in conclusions between identifying specimens to species or to the lowest possible taxonomic unit, a mixture of taxonomic resolutions (phylum, class, order, species, etc.). In situations where it is not possible to identify all specimens to species, identifying specimens to the lowest possible unit may offer a similar resolution as would have been achieved with species level investigations.

### 1. Introduction

Within the ecological sciences, invertebrates are powerful study species. Their diversity, large population sizes, replication across a landscape, and wide distribution makes invertebrates perfect candidates for both mensurative and manipulative studies. As such, invertebrates have been used in the development of general theories of ecology (Andersen et al., 2004; Cowie et al., 2000; Pearson and Rosenberg, 1978), and to elucidate human impacts on the natural world (Doyle et al., 2005; Gerwing et al., 2017b; Pearson and Rosenberg, 1978). Although invertebrates are a useful experimental tool, they have several limitations. For instance, identification of invertebrate taxa requires specialized expertise, and resources required to identify invertebrate specimens and train taxonomists is substantial (Terlizzi et al., 2003). Furthermore, taxonomic expertise is becoming increasingly rare in the biological sciences, making it more difficult to find trained taxonomists

(Hopkins and Freckleton, 2002).

Addressing the inherent challenges within invertebrate taxonomy is the concept of “taxonomic sufficiency,” that establishes what taxonomic level organisms must be identified to in order to detect a difference between contrasts of interest (Bevilacqua et al., 2012; Terlizzi et al., 2003; Timms et al., 2013). Often, identifying invertebrates to the family level is enough to detect meaningful differences, especially between reference areas and those impacted by human activities (Chainho et al., 2007; Terlizzi et al., 2003; Thompson et al., 2003). Additionally, identifying invertebrates to family and not species saves not only processing effort, but also reduces the required taxonomic expertise.

While the concept of taxonomic sufficiency is well supported, it is still an open question with regards to how investigators should deal with datasets in which all specimens cannot be identified to the same taxonomic unit, forcing investigators to identify specimens to the lowest possible (or practical) taxonomic unit (Eertman et al., 2002; Gerwing

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Fig. 1. Location of the Cassiar Cannery (CC) mudflat on the north coast of British Columbia, Canada. Map made using QGIS (QGIS, 2019).

et al., 2015a; Thrush et al., 2003a). It is often impossible to identify all specimens to species, as a subset of any given taxon in a sample may be damaged or lack identifying characteristics due to degree of maturity and/or sex. Further, some taxa may be impossible for most researchers to identify to species. For instance, without the aid of a scanning electron microscope, some members of the phylum Nematoda can only be identified to phylum.

Identifying specimens to the lowest possible taxonomic unit involves analyzing datasets with specimens identified to different taxonomic levels. Researchers assume that identifying specimens to the lowest possible taxonomic unit offers a holistic view of the community (Bevilacqua et al., 2013; Groc et al., 2010). However, it is possible that including specimens identified to different taxonomic units introduces enough taxonomic noise into a dataset that biologically meaningful patterns are obscured. Therefore, we evaluated if analyzing a dataset to various taxonomic resolutions (different taxonomic levels: order, family, genus, and species), including to the lowest possible taxonomic resolution, influenced the observed results. Specifically, we examined if the statistical interpretation or the observed magnitude of differences in community composition and density varied when considering an intertidal-invertebrate dataset, at different taxonomic levels, to elucidate differences between microhabitats.

## 2. Materials and methods

### 2.1. Study location

This study examined the infaunal (animals living within the sediment) intertidal-invertebrate community at the Cassiar Cannery (CC) mudflat (Fig. 1). This mudflat's sediment is dominated by fine silts (<63  $\mu\text{m}$ ), with small amounts of fine-grained sand (125–250  $\mu\text{m}$ ) also present (Campbell et al., 2019; Gerwing et al., 2018c; McLaren, 2016). At the CC

mudflat, 40 infauna taxa have been identified, and the number of taxa observed within a 1  $\text{m}^2$  plot ranges from 4 to 10, with a mode of 6 (Gerwing et al., 2017a, 2018b; Sizmur et al., 2019).

### 2.2. Infaunal sampling

The CC mudflat was separated into four sampling locations with microhabitats distinguished by potential disturbance regimes (Fig. 2). The Beach location was in an area where logs drifting through Inverness Passage become stranded on shore, causing sediment scour that in other systems has been observed to have negative impacts upon infaunal invertebrates (Gerwing et al., 2015b; Herbert et al., 2009). Located approximately 25m north of the Beach location, is the Dock location. This dock is part of a historic Pacific salmon cannery that was established in 1889. Sampling in the Dock location occurred under the historical dock, in a habitat that has not seen direct sunlight in ~130 years, and whose sedimentation and hydrological regimes are likely influenced by the dock. Finally, located ~100m north and south of the Dock and Beach locations, we established two reference areas. These are areas outside of the historical footprint of the cannery, and are not currently impacted by log scour or the dock.

Within each location three transects were established, stretching from the start of the mudflat to the low waterline (Cox et al., 2017; Gerwing et al., 2015c). Transects were 60m long, separated by 10m, and stratified into three equal zones based upon distance from shore. Within each zone, one infaunal core was collected, with its distance from shore randomly selected ( $n = 3$  per transect, 9 per location). All four locations were sampled in a day, three times in summer 2017 (May 30, June 21, and July 20) at the lowest low tides, resulting in a total sample size of 27 per location ( $n = 108$  overall). Sampling trips are hereafter referred to as "rounds." Each infauna core had a depth of 10 cm, and a 7 cm diameter. Following collection, sediment was passed through a 250  $\mu\text{m}$  sieve,



Fig. 2. Location of sampling locations at the Cassiar Cannery (CC) mudflat on the north coast of British Columbia, Canada. Map made using QGIS (QGIS, 2019).

Table 1

Details of all taxonomic levels of the infaunal invertebrate community present at the Cassiar Cannery mudflat. \* indicates three species of polychaetes for whom the order designation was not possible, therefore in these cases the infra-class designation was used. Lowest possible is the common lowest possible resolution in mudflat studies when identification to species is impossible.

Specimen	Phylum	Class	Order	Family	Genus	Species	Lowest Possible
1	Mollusca	Bivalvia	Cardiida	Tellinidae	Macoma	<i>Macoma balthica</i>	Species
2	Nematoda	Chromadorea	Monhysterida	Linhomoeidae	Linhomoeus	<i>Linhomoeus undulatus</i>	Phylum
3	Nematoda	Chromadorea	Monhysterida	Linhomoeidae	Paralinhomoeus	<i>Paralinhomoeus buculentus</i>	Phylum
4	Nematoda	Chromadorea	Monhysterida	Linhomoeidae	Terschellingia	<i>Terschellingia longicaudata</i>	Phylum
5	Annelida	Clitellata	Haplotaxida	Naididae	Paranais	<i>Paranais litoralis</i>	Class
6	Arthropoda	Hexanauplia	Calanoida	Pseudodiaptomidae	Pseudodiaptomus	<i>Pseudodiaptomus fobesi</i>	Order
7	Arthropoda	Hexanauplia	Canuelloida	Canuelliidae	Coullana	<i>Coullana canadensis</i>	Order
8	Nemertea	Hoploneurtea	Monostilifera	Emplectonematidae	Paranemertes	<i>Paranemertes peregrina</i>	Species
9	Arthropoda	Malacostraca	Amphipoda	Corophiidae	Americorophium	<i>Americorophium salmonis</i>	Species
10	Arthropoda	Malacostraca	Cumacea	Nannastacidae	Cumella	<i>Cumella vulgaris</i>	Species
11	Arthropoda	Malacostraca	Amphipoda	Anisogammaridae	Eogammarus	<i>Eogammarus confervicolus</i>	Species
12	Arthropoda	Malacostraca	Cumacea	Leuconidae	Nippoleucon	<i>Nippoleucon hinumensis</i>	Species
13	Arthropoda	Ostracoda	Myodocopida	Sarsiellidae	Eusarsiella	<i>Eusarsiella zostericola</i>	Class
14	Annelida	Polychaeta	Phyllodocida	Phyllodocidae	Eteone	<i>Eteone californica</i>	Species
15	Annelida	Polychaeta	Sabellida	Fabriciidae	Fabricia	<i>Fabricia stellaris</i>	Species
16	Annelida	Polychaeta	Scolecida*	Arenicolidae	Abarenicola	<i>Abarenicola pacifica</i>	Species
17	Annelida	Polychaeta	Scolecida*	Capitellidae	Capitella	<i>Capitella Species Complex</i>	Species
18	Annelida	Polychaeta	Scolecida*	Paraonidae	Aricidea	<i>Aricidea hartleyi</i>	Species
19	Annelida	Polychaeta	Spionida	Spionidae	Pygospio	<i>Pygospio elegans</i>	Species

sieved material and invertebrates were stored in vials of 95% ethanol (Gerwing et al., 2017a; Hamilton et al., 2003), and invertebrates were identified to species under a dissecting microscope.

### 2.3. Data analysis

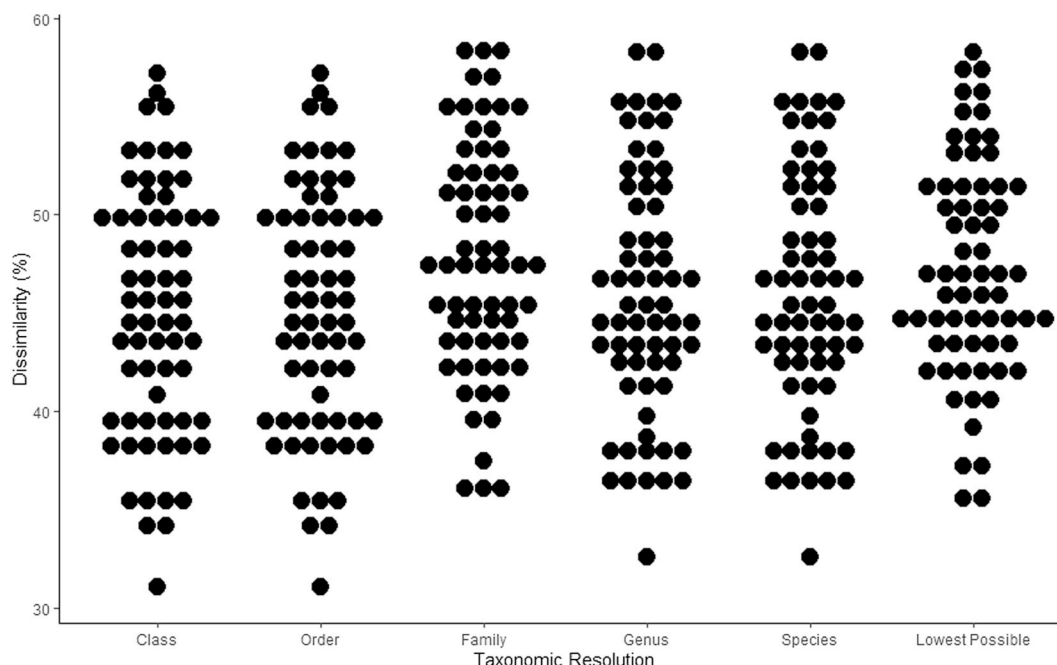
All analyses were conducted in the multivariate statistics program PRIMER with the PERMANOVA add-on (Anderson et al., 2008; Clarke

and Gorley, 2015). To assess if different taxonomic levels influenced the pattern of observed statistical significance between microhabitats ( $\alpha = 0.05$ ), permutational multivariate analyses of variance (PERMANOVA; 9999 permutations) was conducted on specimens identified to different taxonomic levels: order, family, genus, species, and lowest possible (one PERMANOVA per taxonomic resolution). Lowest possible is the only category composed of different taxonomic levels, and this configuration is based upon a common lowest possible resolution in mudflat studies

**Table 2**

Permutational multivariate analysis of variance (PERMANOVA) tables of an infaunal invertebrate community along the north coast of British Columbia, identified to various taxonomic resolutions. Significant  $p$  values ( $\alpha = 0.05$ ) are indicated in bold.

Source	df	Class	Order	Family	Genus	Species	Lowest Possible
Location	3	<b>0.0007</b>	<b>0.001</b>	<b>0.001</b>	<b>0.003</b>	<b>0.003</b>	<b>0.002</b>
Reference Versus Beach	1	0.14	0.14	0.10	0.13	0.14	0.13
Reference Versus Dock	1	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.02</b>	<b>0.02</b>	<b>0.01</b>
Dock Versus Beach	1	0.08	0.08	0.08	0.11	0.12	0.11
Round	2	<b>0.0001</b>	<b>0.0002</b>	<b>0.0002</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>
Transect(Location)	8	<b>0.03</b>	<b>0.02</b>	<b>0.009</b>	<b>0.006</b>	<b>0.008</b>	<b>0.008</b>
Location X Round	6	0.27	0.27	0.24	0.08	0.08	0.18
Round X Transect(Location)	16	0.28	0.28	0.24	0.40	0.39	0.21
Residual	72						
Total	107						



**Fig. 3.** Observed magnitude of differences (% dissimilarity) in invertebrate communities, identified to different taxonomic resolutions, in different microhabitats from the Cassiar Cannery mudflat along the north coast of British Columbia, Canada. If dissimilarity values overlap on the dot plot, then observed differences between taxonomic resolutions is minimal.

when identification to species is impossible (Campbell et al., 2019; Cox et al., 2019; Gerwing et al., 2017a; Gray et al., 2002; Thrush et al., 2000): polychaetes, amphipods, bivalves, and nemerteans identified to species, copepods to order, ostracods and oligochaetes to class, and nematodes to phylum. Taxonomic classification is summarised in Table 1. We were able to identify all specimen to species, given the well studied nature of this infaunal community (Gerwing et al., 2017a, 2018b). The response variables for the PERMANOVAs were resemblance matrices calculated using Bray-Curtis coefficients, and densities were fourth-root transformed to improve assessment of rare and common taxa on community structure. Within the PERMANOVAs location was included as a fixed factor (four levels: beach, dock, north and south reference), while round (3 levels), and transect nested within location (3 transects per location) were included as random factors. Within the location term, *a priori* planned contrasts were used to differentiate between locations.

In all analyses we did not correct for the inflation of the family-wise error rate (Benjamini and Hochberg, 1995), as identifying specimens to different taxonomic levels produced independent datasets. Even if this had not been the case, within the PERMANOVAs the permutation  $p$ -values already provide an exact test of each individual null hypothesis of interest, therefore, minimizing the need for  $p$  value correction

(Anderson et al., 2008). Further, most *ad hoc* corrections that could be used, such as Bonferroni, are inexact and overly conservative (Anderson et al., 2008; Day and Quinn, 1989). Therefore, following the advice of Anderson et al. (2008), we have elected to not correct our  $p$  values.

To determine if identifying invertebrates to different taxonomic units impacted the observed magnitude of differences between microhabitats, PRIMER's Similarity Percentage Analysis (SIMPER) routine was used (Clarke, 1993; Clarke and Ainsworth, 1993; Clarke and Gorley, 2015). This routine calculates the percent dissimilarity observed between different microhabitats. For this study we assumed that higher percent dissimilarity values indicate higher observed differences between treatments or sites (Hawkes and Gerwing, 2019). Percent dissimilarity was calculated between microhabitats for each round and then visualized using a dot plot ( $n = 66$  contrasts per round, 396 total). If dissimilarity values overlap on the dot plot, then observed differences between taxonomic resolutions is minimal.

### 3. Results and discussion

When the taxonomic resolution of the infaunal-invertebrate community from the CC mudflat was varied, neither the statistical interpretation (Table 2), nor the magnitude of observed differences (Fig. 3) in

community composition and density varied between microhabitats. Previous investigations into taxonomic sufficiency have reported similar findings, suggesting that identifying invertebrates to broader taxonomic resolutions may not diminish our ability to detect statistical or biological differences between contrasts of interest (Chainho et al., 2007; De Biasi et al., 2003; Thompson et al., 2003). Given the resources required to train taxonomists, as well as the time required to identify all specimens to species, considerable time and resources can be saved by identifying specimens to broader taxonomic levels. Therefore, identifying invertebrates to broader levels may represent a good trade-off between taxonomic resolution and resources (Terlizzi et al., 2003).

This study also observed no difference between identifying specimens to species or to the lowest possible taxonomic unit, as is common in studies using invertebrates (Gerwing et al., 2016; Gray et al., 2002; Thrush et al., 2000). In situations where it is not possible to identify all specimens to species, identifying specimens to the lowest possible unit may offer a similar resolution as would have been achieved with species-level investigations.

We urge caution before investigators apply these findings to other systems. The invertebrate community at the CC mudflat is not very speciose; a fact exemplified as no genus contained more than one species (Table 1). While this pattern of biodiversity is common on mudflats (Gerwing et al., 2016, 2017a, 2018a; Thrush et al., 2003a, 2003b), it is not universal to all mudflat systems (Cox et al., 2019). Other systems that are more speciose (e.g. more species within a genus) may not produce similar results when different taxonomic resolutions are used.

Regardless, our findings contribute to a growing body of literature (Chainho et al., 2007; De Biasi et al., 2003; Thompson et al., 2003) that shows identifying invertebrates to broader taxonomic units may be enough to detect biologically meaningful differences. Further, in situations where it is not possible to identify all specimens to species, identifying specimens to the lowest possible unit may offer a similar resolution as would have been achieved with species-level investigations.

## Author statement

TGG and AMG collected the data. TGG processed invertebrate samples, and analyzed the data. All authors contributed to drafting the manuscript.

## Declaration of competing interest

We have no conflicts of interest to declare.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2019.106516>.

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