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Required sampling intensity for community analyses of intertidal infauna to detect a mechanical disturbance

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Abstract In coastal ecosystems, infaunal (animals living within the sediment) invertebrates are used to study and monitor disturbances. However, it is an open question as to the minimal required sampling intensity to detect that a disturbance has influenced such communities. As such, we implemented a manipulative experiment using an infaunal community with a known response (community composition and population abundances) to a mechanical disturbance (sediment scour), to determine the minimum sampling intensity required to detect differences in the infaunal community. Statistically significant differences ($\alpha = 0.05$) between the infaunal community of the disturbed and reference replicates were observed in case studies consisting of 99 (4 samples per m^2) to 5 (0.2 samples per m^2) samples per treatment. Below 5 samples, the known statistical and biological difference was undetectable. However, at least 10 samples per treatment (0.4 samples per m^2) were required for the observed infaunal community to be within 93% similarity of our most accurate assessments of the infaunal community. These findings suggest that studies attempting to identify disturbances may require a minimal sampling intensity equivalent to 0.2 samples per m^2, while studies attempting to determine how the infaunal community varies with disturbances may require 0.4 samples per m^2. These potential minimal required sampling intensities will be of use in the theoretical exploration of disturbances, as well as in applied conservation, restoration, and monitoring projects.

Keywords British Columbia · Sample size · Skeena · North Coast

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Introduction

In coastal ecosystems, infaunal (animals living in the sediment) invertebrate communities are often used to study or identify disturbances (Drylie et al., 2020; Fukuyama et al., 2014). Disturbances in these coastal systems can take many forms; for instance, physical disturbances of soft-sediment intertidal flats may occur via scour by loose logs (Gerwing et al., 2018a), dredging, or ice (Norkko et al., 2006), as well as via smothering by released sediments (Thrush et al., 2003). Chemical disturbances, such as those associated with hydrocarbons (Fukuyama et al., 2014), heavy metals, or other toxic substances (Sizmur et al., 2019), can also substantially impact community structure. Likewise, the influence of nutrient additions and organic enrichment upon infaunal

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invertebrate community structure is well-documented (Drylie et al., 2020; Pearson & Rosenberg, 1978).

While infaunal invertebrates can be used to study or identify disturbances, considerable information is required before they can be used effectively. For instance, it is critical to understand how infaunal communities recover in the aftermath of a disturbance. Increasing evidence suggests that the local species pool plays a predominant role, and following disturbance, infaunal communities become more similar to neighboring communities as time progresses (Campbell et al., 2019a; Thrush et al., 2003; Norris et al., 2022). Based upon this general information, biodiversity and total abundance of infaunal individuals (Campbell et al., 2019b; Sherman & Coull, 1980) can be used to identify and study disturbances; however, such a broad approach often lacks nuance. Infaunal invertebrate species can respond to a given disturbance in a variety of ways. For instance, intertidal amphipods and cumacea, as well as small bivalves, are often sensitive to disturbances, decreasing in abundances with disturbances (Gerwing et al., 2022; Sánchez-Moyano & García-Gómez, 1998). Conversely, other taxa such as Oligochaeta (Cowie et al., 2000), Nematoda (Mazzola et al., 2000), and *Capitella* species complex (Pearson & Rosenberg, 1978) are known to be tolerant of disturbances, and can survive, or even thrive in disturbed systems. Therefore, investigators must, in essence, calibrate the infaunal community under observation to a given disturbance before they can be used to study or identify a disturbance. Yet once the responses of individual species to a disturbance are known, investigators can use infaunal community composition and changes in density to holistically identify and study disturbances (Campbell et al., 2019a; Gerwing et al., 2018a; Thrush et al., 2003).

Unfortunately, infaunal invertebrates reside within sediment; therefore, they are predominantly studied by extracting them via invasive methods (but see Campbell et al. (2019b)). As such, designing sampling schemes can be problematic, as it is difficult to a priori assess the spatiotemporal extent and variation of the infaunal community. Often, considerable in situ knowledge of a study area is required before infaunal communities can be effectively used to study or identify disturbances. The consequences of lacking this information can be dramatic. Gerwing et al. (2022) showed that as sampling schemes become increasingly divergent from the spatial scale of a disturbance, the frequency and magnitude of type I and II errors in analyses elucidating how infaunal communities respond to a disturbance increase. However, even in situations where detailed knowledge of the infaunal community exists, it is still unclear as to what sampling intensity is required to use infaunal communities to study or identify disturbances.

Therefore, we implemented a manipulative experiment using a well-studied infaunal community found on the northern coast of British Columbia (BC), Canada. Here, a mechanical disturbance (sediment scour) was applied to an intertidal mudflat to determine the minimum sampling intensity required to detect differences in the infaunal invertebrate community following a disturbance. A greater understanding of the sampling intensity required to detect disturbances in coastal ecosystems using infaunal invertebrates will be of use not only to practitioners studying theoretical concepts related to disturbance and recovery, but also to applied ecologists conducting environmental assessments, as well as monitoring for disturbances.

Materials and methods

Study site

This experiment was conducted at an intertidal mudflat in Inverness Passage adjacent to the historic former Pacific salmon cannery turned ecotourism lodge, Cassiar Cannery (CC; Fig. S1). This area is strongly estuarine, 4–10 PSU, and mudflat sediment is composed primarily of fine silts, with average ($n=60$, mean ± standard error) volume weighted particle sizes approximately 60.60 μm ± 6.31 μm (Campbell et al., 2020). The infaunal community is dominated by meiofauna, or invertebrates that are retained on a 45-μm sieve but pass through a 1-mm sieve. The infaunal community is dominated by Cumacea (primarily *Nippoleucon hinumensis* with *Cumella vulgaris* observed less frequently), Polychaetes (families Phyllodocidae [*Eteone californica*], Capitellidae [*Capitella* species complex], and Spionidae [*Pygospio elegans*]), Oligochaetes (*Paranais litoralis*), Nematodes, Copepods (order Harpacticoida), Amphipods (*Americorophium salmonis*), and the bivalve *Macoma balthica* (Campbell et al., 2020; Gerwing et al., 2017).

Experimental design

In the mid-beach intertidal zone of an extensive mudflat, 50 plots of 1 m^2 were established. Each plot was further subdivided into four 0.50 m by 0.50 m quadrats (Fig. S2; $n=200$ quadrats). These quadrats are the base unit of replication for this experiment. Sediment was uniformly mechanically disturbed within selected quadrats to a semi-liquefied state on June 23, 2016, to a depth of ~25 cm (Gerwing et al., 2017), simulating physical disturbance. Plots had their constituent quadrats disturbed in different configurations (Fig. S2): Reference (no disturbance), C1 (plots 25% disturbed: 1/4 quadrats per plot), C2 (plots 50% disturbed: 2/4 quadrats), C3 (plots 75% disturbed: 3/4 quadrats), and C4 (plots 100% disturbed: 4/4 quadrats). Configurations were randomly assigned to individual 1 m^2 plots ($n=10$ plots per configuration), and the appropriate proportion of the plot (number of quadrats) was disturbed. This resulted in 99 reference quadrats and 101 disturbed quadrats.

Sixteen days following disturbance, sediment samples were collected from the center of each quadrat. Two weeks is enough time for the recovery of this community to have begun during this time of year, but not to have been completed (Gerwing et al., 2017, 2018b; Norris et al., 2022). Samples were collected using a 10 cm sediment corer, with a 7 cm diameter. To retain infauna, sediment was passed through a 250-μm sieve and preserved in 95% ethanol. All sediment samples were processed under a dissecting microscope, and specimens were identified to the lowest possible taxonomic unit (Gerwing et al., 2020).

Statistical analysis

This infaunal community is known to statistically and biologically (community composition and population abundances) differ between disturbed and reference quadrats (Campbell et al., 2019a; Gerwing et al., 2018b, 2022). Specifically, consistently higher densities of Oligochaeta, Nematoda, and *Capitella* species complex are observed in disturbed versus reference quadrats, while consistently higher densities of *N. hinumensis* and *M. balthica* are observed in reference quadrats (Gerwing et al., 2022). As such, we can use this nested experimental design to determine the sampling intensity required to detect a disturbance. More specifically, a series of case studies using a decreasing number of randomly selected samples (Table 1) were used to elucidate the minimum sampling intensity required to detect a statistical difference between disturbed and reference quadrats. The maximum number of samples used per treatment was 99 to ensure equal sample sizes. An analysis of similarities (ANOSIM; 9999 permutations) was performed to test for significant differences between the infaunal community in each case study (Anderson et al., 2008; Clarke & Gorley, 2015). The response variable for the ANOSIM was a resemblance matrix, constructed using Bray–Curtis distances that were

Table 1 Results of analysis of similarities (ANOSIM) of infaunal community density and species composition between disturbed and reference treatments. Dashed line indicates where the ANOSIM could no longer detect significant differences in the infaunal community (species composition and population abundances) between disturbed and reference treatments ($\alpha=0.05$)

Samples per treatment	Volume of sediment sampled per treatment (m^3)	Sampling intensity per treatment (samples per m^2)	p
99	0.0381	4	0.0004
75	0.0288	3.03	0.0003
60	0.0231	2.42	0.004
50	0.0192	2.02	0.007
25	0.0096	1.01	0.004
20	0.0077	0.81	0.005
15	0.0058	0.61	0.003
10	0.0038	0.40	0.001
5	0.0019	0.20	0.05
4	0.0015	0.16	0.2
3	0.0012	0.12	0.3
2	0.0008	0.08	0.6
1	0.0004	0.04	0.4

calculated from fourth root transformed densities of the 16 infaunal taxa observed in this experiment. We selected an $\alpha = 0.05$ to denote statistical significance, as this value is commonly used. In each case study, we did not correct for the inflation of the family-wise error rate, as the permutation *p* values already provide an exact test of individual null hypothesis, minimizing the need for *p* value correction (Anderson et al., 2008). Also, each randomly selected subset of the data can be considered independent.

To determine how sampling intensity influenced observed resolution of community composition, and to identify the optimal number of samples required, non-metric multidimensional scaling (nMDS) plots were used to visualize variation in the infaunal community (species assemblage and densities) between disturbed and reference habitats and sample sizes (100 restarts; infaunal community resemblance matrix calculated using Bray–Curtis similarities on fourth root transformed density data). While a biological and statistical difference is known to result due to disturbances within this infaunal community, it is subtle, a product of relative abundances, and some variation in species assemblages (Gerwing et al., 2017, 2018b; Gerwing et al., 2020; Gerwing et al., 2022; Norris et al., 2022). As such, points on the nMDS plot that cluster near the point representing 99 samples (most accurate representation of the infaunal community) produced a relatively accurate representation of the infaunal community. Points further away are less accurate.

Results and discussion

Along the northern coast of BC, Canada, we used a manipulative experiment to determine the minimum sampling intensity required to detect the impact of a mechanical disturbance within an infaunal community. In general, a statistically significant difference in the infaunal community between disturbed and reference quadrats was observed in the case studies consisting of 99 to 5 samples per treatment (Table 1). Below 5 samples, the known statistical and biological differences between disturbed and reference quadrats (Gerwing et al., 2022) was undetectable. These findings suggest that studies attempting to identify or study disturbances may require a minimal sampling intensity equivalent to 0.2 samples per m^2, 5 replicates per treatment, or 0.0019 m^3 of sediment assessed per treatment (Table 1). However, while a statistically significant difference between disturbed and reference quadrats was detectable, the observed infaunal community (species assemblage and densities) as elucidated by five samples per treatment or less did not produce a highly accurate representation of the infaunal community (Fig. 1). Instead, 10 samples per treatment (0.4 samples per m^2 or 0.0038 m^3 of sampled sediment) were required before the observed infaunal community clustered (93% similarity) with the higher sample sizes, indicating a more accurate representation of the infaunal community. It is important to note that increasing sample sizes beyond this point resulted in increased clustering with the 99-sample treatment and thus a more accurate representation of the infaunal community, although this was not a considerable improvement beyond the observed 93% clustering threshold.

While this study suggests a minimal sampling intensity required to use infaunal communities to study or identify disturbances, care must be taken not to over extrapolate these findings. The impact of a disturbance upon a community is known to vary by many factors, including disturbance severity (Cowie et al., 2000), habitat type (Schratzberger & Warwick, 1998), type and predictability of disturbance (Radchuk et al., 2019), population densities (Denslow, 1995), biodiversity (Drylie et al., 2020), life history stages and strategies of affected individuals (Thistle, 1981), time of year (Hobbs & Huenneke, 1992), site history (Nelson et al., 2021), and community successional stage (Cadotte, 2007). As such, the usefulness of this suggested minimal sampling intensity should be confirmed in any system of study. Despite this need for caution, similar, albeit not identical, infaunal community responses to other physical (Gerwing et al., 2015) and chemical (Fukuyama et al., 2014) disturbances, as well as deposition of sediment (Thrush et al., 2003) and organic enrichment (Gerwing et al., 2018a; Pearson & Rosenberg, 1978), have been observed in other intertidal systems. Given these broad similarities, it is possible that the minimum sampling intensity suggested in this study may be applicable to a broad range of soft-sediment intertidal systems, as well as disturbing agents. However, more research is required to evaluate this relationship.

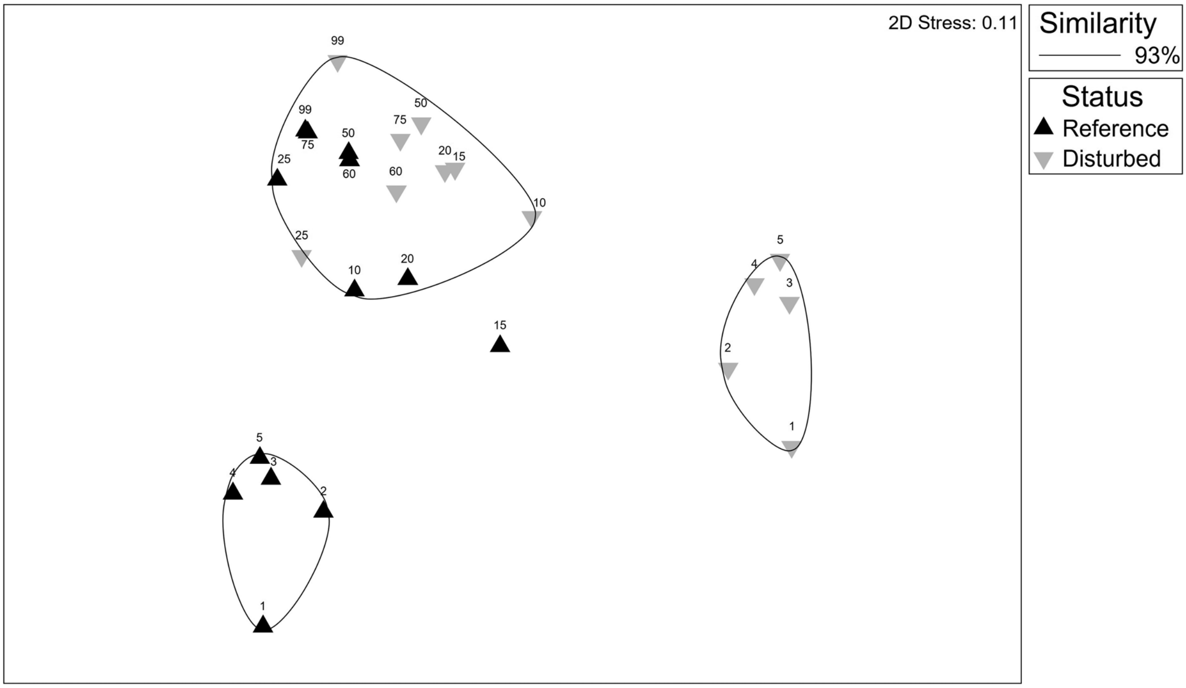

Fig. 1 Non-metric multidimensional scaling (nMDS) plots exploring the variation in the infaunal community between disturbed and reference plots. Numbers above each plot correspond to the sample size per treatment (see Table 1). Points encompassed by polygons represent infaunal communities that were at least 93% similar

Conclusions

Regardless of the need for further research, our findings postulate a potential minimum sampling intensity (0.2 samples per m^2) required to use infaunal invertebrates to study or detect a disturbance. However, at least 0.4 samples per m^2 may be required for the observed infaunal community to be within 93% similarity of our most accurate assessments of the infaunal community. As such, we suggest that investigators only concerned with detecting that a disturbance has occurred and can use the lower threshold of 0.2 samples per m^2. However, if investigators are interested in how the infaunal community varies with disturbance, then a minimal sampling intensity of 0.4 samples per m^2 is more appropriate. These potential minimal required sampling intensities will be of use to researchers engaged in the theoretical exploration of disturbances and their role in influencing and structuring ecosystems. More practically, this potential minimal sampling intensity will also be of use to researchers and land use managers who are engaged in environmental or impact assessments, conserving and restoring ecosystems, or monitoring coastal habitats for disturbances.

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Data availability Data is available upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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