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A Rapid, Non-invasive Population Assessment Technique for Marine Burrowing Macrofauna
Inhabiting Soft Sediments

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Abstract

Population assessment techniques for soft-sediment infauna (invertebrates within the substrate) requires excavation of specimens, damaging or killing the specimen and surrounding habitat, while being time-consuming and costly. Rapid population assessments of some marine burrowing decapods have been possible by counting burrow openings to estimate abundance, and while they may be used as indicator species, these decapods are not ubiquitous to environments requiring monitoring. Additionally, the presence of other burrowing macrofauna (invertebrates living in the sediment and retained on 1mm mesh such as clams or large worms) may reduce the efficacy of burrow openings in estimating

24 macrofauna abundance. As such, we assessed mudflats along the north coast of British Columbia,
25 Canada, during summer 2017 to determine if macrofauna abundances could be estimated from burrow
26 openings on the sediment surface in regions of low ($n = 1$ species) and high ($n = 8$ species) biodiversity.
27 Abundance could not be estimated at the low diversity sites where only one macrofaunal species created
28 burrows. At the high diversity site, species-specific models estimating abundance from burrow openings
29 could not be constructed; however, the total number of burrow openings observed was useful in
30 estimating total infaunal community abundance. As such, burrow openings may not be an effective tool
31 in assessing species-specific abundances, but may be appropriate to estimate overall community
32 changes.

33

34 **Keywords:** Burrow Openings, Burrowing Organisms Ecological Proxy, Environmental Monitoring,
35 Intertidal Environment, North Coast

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48 **Introduction**

49 Understanding the impact of human activity on ecosystem health and biodiversity is a
50 fundamental aspect of applied scientific research (Gonzalez et al. 2016; Vackar et al. 2012). Ecologists
51 and conservation biologists often estimate species abundance, or use population dynamics to achieve a
52 variety of research goals including the assessment of anthropogenic impacts (Cox et al. 2017; Schlacher
53 et al. 2016b; Simao et al. 2006). Although compiling counts of organismal abundance is easy in theory,
54 precise and accurate counts are difficult, and may require invasive techniques (Butler and Bird 2007;
55 Cox et al. 2017; Schlacher et al. 2016b). For example, in marine soft-sediment ecosystems many
56 invertebrates burrow into the substrate (infauna), requiring excavation of individuals from the sediment
57 to assess density and presence/absence. Such methods are destructive to the habitat, and risk stressing,
58 damaging, or killing specimens (Butler and Bird 2007; Schlacher et al. 2016b). In addition to habitat
59 damage, excavations are time consuming, laborious, and costly, limiting the spatiotemporal scale of
60 investigation (Dumbauld et al. 1996; Gilkinson 2008). Therefore, a variety of methods have been
61 proposed for monitoring and estimating infaunal densities, including assessing indicator species or
62 applying ecological indices that can be used as proxies for ecosystem health (Gerwing et al. 2017;
63 Gesteira and Dauvin 2000; Hereward et al. 2017; Schlacher et al. 2016b). Ecological proxies are
64 advantageous as they require less time to assess an area than examining a site holistically, and reduce
65 costs (Butler and Bird 2007; Gilkinson 2008; Schlacher et al. 2016b), although they require pilot studies
66 to evaluate their efficacy (Gerwing et al. 2017; Gerwing et al. 2015b).

67 In coastal soft-sediment ecosystems that have been degraded by anthropogenic impacts such as
68 urbanization and industrial development (Crain et al. 2008; Gerwing and Cox 2017), fossorial
69 (burrowing) marine decapods have been used extensively as indicator species to detect disturbances

70 across gradients of human impact. The decapods selected as indicator species have traditionally been
71 ghost crabs (*Ocypode* sp.) and shrimp from suborder Pleocyemata (*Upogebia* sp. and *Neotrypaea* sp.), as
72 they are sensitive to anthropogenic impacts and play key ecological roles (Butler and Bird 2007; Carty
73 2003; D'Andrea and DeWitt 2009; Dumbauld et al. 1996; Hereward et al. 2017; Pillay and Branch 2011;
74 Schlacher et al. 2016a; Stelling-Wood et al. 2016). As both ghost crabs and Pleocyemata shrimp have
75 fossorial habits, researchers have estimated species abundances from statistical relationships between the
76 number of burrow openings and population abundance (Carty 2003; Hereward et al. 2017; Schlacher et
77 al. 2016b). Once the relationship has been determined in a given location, monitoring requires only
78 counting the number of burrows as a proxy for abundance, eliminating the need to excavate pits or count
79 individual specimens (Halpern et al. 2015; Hereward et al. 2017; Schlacher et al. 2016b). However,
80 bivalves and polychaetes also create burrow openings, hence this technique of rapid population
81 assessment may not be limited to fossorial decapods. Although both bivalves and polychaetes have been
82 used as indicator species (Guerra-Garcia and Garcia-Gomez 2004; Hutchins et al. 2009; Pearson and
83 Rosenberg 1978; Talmage and Gobler 2010; Waldbusser et al. 2010; Yunker et al. 2011), relationships
84 between bivalve or polychaete abundance and burrow openings have not been examined as extensively
85 as with decapods. For example, only one study examined relationships between burrow openings and
86 bivalve (*Cyrtodaria siliqua*) abundances (Gilkinson 2008), while research that quantifies the relationship
87 between polychaete abundance and the abundance of burrow openings is lacking.

88 Although the majority of research utilizing burrow openings as an ecological proxy has focused
89 on marine fossorial decapods, this group of organisms are not ubiquitous to marine soft-sediment
90 ecosystems. Additionally, it is also possible that the presence of other burrowing macrofauna
91 (invertebrates living in the sediment and retained on a 1mm sieve such as clams or large worms) may
92 decrease the efficacy of using burrow openings as proxies for abundance (Butler and Bird 2007; McPhee

93 and Skilleter 2002). Where only one macrofaunal species is present, monitoring by counting burrow
94 openings may be reliable, but may not be possible when multiple macrofaunal species are present due to
95 the presence of species inhabiting burrows that they didn't create and altering the relationship between
96 the number of burrow openings and abundance (Butler and Bird 2007; McPhee and Skilleter 2002).
97 Conversely, macrofauna often create burrow openings that can be differentiated and identified to species
98 visually, potentially enabling the usage of burrow openings to assess densities outside of monocultures
99 (Harbo 2003; 2007; 2011). For instance, *Neotrypaea californiensis* (ghost shrimp; Suborder Pleocymata)
100 creates distinctive burrows with a vertical shaft and expelled sediment in a volcano shape around the
101 circular burrow opening (Pillay and Branch 2011) while *Abarenicola pacifica* (Pacific lugworm) creates
102 j-shaped burrows with rope-like, coiled fecal castings around the burrow opening (Harbo 2003; 2007;
103 2011; Light 2007). Therefore, it may be possible to estimate abundances of these species from their
104 unique burrow openings even in areas of high macrofaunal diversity, and the applicability of burrow
105 openings counts belonging to macrofauna in estimating organismal abundance should be further
106 examined in biodiverse habitats.

107 We assessed intertidal mudflats in British Columbia, Canada, at both low macrofaunal diverse
108 mudflats near Kitimat, and a high macrofaunal diverse mudflat near Prince Rupert in the Skeena
109 Estuary, to determine the efficacy of burrow openings as proxies for abundance of macrofauna. Both
110 Kitimat and Prince Rupert are cities near estuarine systems in northern BC, Canada, and are important
111 regions for environmental monitoring due to their history of industrial development including an
112 aluminum smelter, logging, and a pulp and paper mill. Future development is also likely in these
113 regions, including potential potash export terminals, and oil and liquefied natural gas pipelines,
114 refineries, and export terminals (Carr-Harris et al. 2015; McLaren 2016; Simpson et al. 1998; Yunker et
115 al. 2011). As such, trends identified in these systems may provide valuable insights applicable to other

116 estuarine systems (Gerwing et al. 2015a; Gerwing et al. 2018b; Hewitt et al. 2016; Little et al. 2017).
117 Therefore, we tested whether a relationship between burrow opening and fossorial organism abundance
118 can be generated in high and low macrofaunal diverse sites, with the goal of creating relationships that
119 could be used to save time and money when assessing macrofaunal populations in the future.

120

121 **Materials and Methods**

122 *Study Sites*

123 Five sheltered intertidal mudflats were sampled for this study: four mudflats with low
124 macrofaunal diversity (i.e. only one macrofaunal species present) in the Kitimat River Estuary and one
125 mudflat with high macrofaunal diversity in the Skeena Estuary (Figure 1). Within the Kitimat Estuary,
126 three mudflats were located within Minette Bay (PL: Pilings; LD: Lodge; LS: Log Sort), while Foxy
127 Beach (FB) was located just outside of Minette Bay. Gerwing et al. (2018a) identified *Mya arenaria* as
128 the sole macrofaunal species in the Kitimat Estuary, therefore, all burrow openings larger than 0.1 cm
129 can be attributed to this bivalve.

130 In the Skeena Estuary near Prince Rupert, Wolfe Cove was the only site surveyed, as it was the
131 only mudflat in the area with a diverse macrofauna community. With ghost shrimp (*Neotrypaea*
132 *californiensis*), bivalves (*Clinocardium nuttallii*, *Macoma nasuta*, *M. arenaria*) and polychaete worms
133 (*Abarenicola pacifica*, *Nephtys caeca*, *Alitta brandti*, and *Glycinde picta*) present (Campbell and
134 Gerwing, Unpublished data), Wolfe Cove is a site of high macrofaunal diversity, with multiple species
135 creating relatively large burrow openings (>0.1 cm) on the substrate surface.

136

137 *Field Methods*

138 At each mudflat, five transects were established, stretching from the start of the mudflat to the
139 low tide waterline (60-200 m long, 25 m apart) (Cox et al. 2017; Gerwing et al. 2015a). Transects were
140 stratified into three equal zones based on distance from shore (near, middle, and far). Within each zone,
141 one sampling location was randomly selected (n = 3 per transect, 15 per site per sampling period) and a
142 1 m² quadrat was established (Gerwing et al. 2015a). Burrow openings greater than 0.1 cm were
143 quantified were differentiated based on physical characteristics and classified into three categories as
144 ghost shrimp burrows, lugworm burrows, or other burrow openings. Ghost shrimp burrows were
145 constructed by *N. californiensis* and identified by the expelled sediment in a volcano shape around the
146 circular burrow opening, characteristic of sheltered mudflats like Wolfe Cove (Pillay and Branch 2011).
147 Lugworm burrows were constructed by *A. pacifica*, identified by circular burrows with rope-like, coiled
148 fecal castings around the burrow opening (Harbo 2003; 2011; Light and Smith 2007). Other burrow
149 openings were the remaining indistinguishable burrows that were small to medium sized non-descript
150 openings created by bivalves and Nephtyidae or Nereididae polychaetes. After burrow openings were
151 classified, a pit was excavated to quantify the abundance of macrofauna (Cox et al. 2017). Due to
152 differences in availability of resources, a 20 cm² pit was dug to a depth of 20 cm at Kitimat mudflats,
153 whereas at Wolfe Cove a 1 m² pit was dug to a depth of 20 cm (Cox et al. 2017; Gerwing et al. 2018a).
154 All mud excavated from each pit was sieved through a No. 35 mesh sieve (0.5 mm) opening. Where
155 possible, macrofauna were identified in the field and immediately released. Specimens that could not be
156 identified in the field were retained and later identified under a dissecting microscope (Light and Smith
157 2007). One mudflat was sampled per day at the lowest low tide during three sampling periods over the
158 summer of 2017 (May 25-31, June 22-28, July 17-24). The LS mudflat was not sampled during the first
159 sampling period, (May 25-31), and PL was not sampled in the last sampling period (July 17-24). This
160 sampling scheme resulted in a total of 30-45 sampling events conducted per mudflat.

161

162 *Statistical analysis*

163 Data were analyzed using IBM SPSS software version 24.0. Data were in the form of counts and
164 a large number of zeros were present in the dataset, skewing the dataset significantly to the left. The
165 dataset was deemed non-normal, and therefore a Spearman's rho correlation was used to determine the
166 relationships between each of the species population counts and each burrow type counted. In order to
167 determine if there were significant differences in the relationship between *M. arenaria* and burrow
168 abundance among the four mudflats surveyed at the Kitimat location, a Kruskal-Wallis test was
169 performed.

170 Following the Spearman's correlation analysis, a Poisson log probability distribution was
171 employed to create general linear models (GLMs) based on significant correlations. This distribution is
172 ideal when analyzing non-normal data in the form of counts (Zuur et al. 2009). Sampled population
173 counts were summed for calculating model statistics based on similarities in statistically significant
174 correlations calculated at Wolfe Cove. Abundance for *A. brandti*, *A. pacifica*, and *M. arenaria* were
175 summed, and *G. picta*, *M. nasuta*, and *N. californiensis* were summed because of their common
176 statistically significant correlation in the same direction (negative and positively respectively) to non-
177 descript "other burrow openings." The abundance of lugworm burrows and other burrow openings were
178 used as covariates, while sampling date was a fixed factor, to predict the summed population numbers
179 for *A. brandti*, *A. pacifica*, and *M. arenaria*. The abundance of other burrow openings was modelled as a
180 covariate with sampling date a fixed factor to predict the summed population numbers for *G. picta*, *M.*
181 *nasuta*, and *N. californiensis*. Other dependent variables were modeled, including abundance of *N.*
182 *caeca*, while other covariates and fixed variables were explored in GLMs including transect number and
183 ghost shrimp burrow abundance in order to assess their impact on model significance. Only covariates

184 and fixed factors with an α less than 0.05 were deemed acceptable for use in the models. Where multiple
185 burrow types were entered as covariates in a model, the interaction effect of these openings was also
186 entered as a model variable; for example, lugworm burrows X other burrow openings. Model residuals
187 were graphed to assess model reliability.

188

189 **Results**

190 *Kitimat*

191 At Kitimat, the low macrofaunal region where only one macrofaunal species (*Mya arenaria*) was
192 observed, significant relationships were found between the burrow openings and population abundance
193 of *M. arenaria* at three of the four mudflats ($\rho = 0.458$, $p < 0.001$). No significant relationship was
194 found at the LS site, and therefore this site was excluded from further analyses. No significant
195 differences in the distribution or median *M. arenaria* abundance existed between the three mudflats
196 analyzed, so data were grouped for further analyses.

197 Burrow openings were entered as a covariate in a GLM to predict population abundance of *M.*
198 *arenaria* and were shown to have a significant effect on the model outcome (omnibus test was
199 significant; likelihood ratio Chi-square = 22.48, $p < 0.001$). Given the significance in the GLM, burrow
200 openings were used to assess abundance in a model with a Poisson log distribution; however, when raw
201 model residuals were plotted as a function of predicted values the model showed significant bias and
202 slight heteroscedasticity yielding the model results unreliable (Figure 2). Furthermore, removing one
203 data point made the model insignificant. Therefore, no meaningful model could be derived from the
204 Kitimat data collected.

205

206 *Wolfe Cove*

207 At Wolfe Cove, the high diversity mudflat, partial correlations were determined to calculate the
208 similarity in the variation between population and burrow type, conducted while maintaining a constant
209 distance from shore ($\alpha < 0.1$ to identify patterns (Beninger et al. 2012)). Although eight macrofaunal
210 species were identified at Wolfe Cove, the abundance of *Clinocardium nuttallii* did not show a
211 significant relationship to any type of burrow opening (Table 1). The abundance of some species
212 encountered had statistically significant relationships with the number of burrows, but these
213 relationships were not all positive (Table 1). For example, *Abarenicola pacifica* abundance was
214 positively correlated, while *Nephtys caeca* abundance was negatively correlated to lugworm burrows.
215 The number of *Glycinde picta*, *Macoma nasuta*, and *Neotrypaea californiensis* individuals were all
216 positively correlated with the abundance of other burrow openings, while *Alitta brandti*, *A. pacifica* and
217 *M. arenaria* population numbers were negatively correlated to other burrow openings and positively
218 correlated with lugworm burrow openings (Table 1). Population counts for species that shared common
219 variability were summed to form the dependent variables of the subsequent general linear models,
220 therefore individual correlations shown in Table 1 are not related to the significance of covariates used
221 in these models.

222 The following models revealed significant predictive relationships:

223 Total population abundance of *G. picta*, *M. nasuta*, and *N. californiensis* was predicted by other burrow
224 openings (covariate) and the date of sampling (fixed factor) (likelihood ratio Chi-square = 97.892, $p <$
225 0.001). The linear relationship between the predicted values and the observed population abundance of
226 *G. picta*, *M. nasuta*, and *N. californiensis* is described by the following equation:

227 [1]
$$Y = 0.47 + 0.75x \text{ (} r^2 = 0.740; \text{ Figure 3)}$$

228 The total population abundance of *A. brandti*, *A. pacifica*, and *M. arenaria* was predicted by the number
229 of lugworm burrows and other burrow openings (covariates) and the date of sampling (fixed factor)

(likelihood ratio Chi-square = 72.462, $p < 0.001$). The linear relationship between the predicted values and the observed total population abundance of these species is described by:

$$[2] \quad Y = 3.8 + 0.45x \text{ (} r^2 = 0.421; \text{ Figure 4)}$$

A. pacifica was significantly correlated with ghost shrimp burrows when the independent Spearman's rho values were calculated (Table 1); however, when modeled as total abundance with *A. brandti*, and *M. arenaria*, the total abundance of these species can be modeled more appropriately by lugworm and other burrow opening types than ghost shrimp burrows.

Lastly, *N. caeca* was modeled by lugworm burrow and other burrow opening counts (covariates), and date of sampling (fixed factor) (likelihood ratio Chi-square = 26.523, $p < 0.001$). A significant interaction effect was noted between lugworm burrows and other burrow openings in the model of *N. caeca* ($p = 0.029$). The linear relationship between the predicted values and the observed counts of *N. caeca* population abundance is described by the following equation:

$$[3] \quad Y = 4.42 + 0.28x \text{ (} r^2 = 0.277; \text{ Figure 5)}$$

Although the Spearman's rho value shows a relationship between *N. caeca* abundance and combined ghost shrimp burrows and lugworm burrows, when modeled with other variables of consideration (other burrow openings, sampling date, transect) ghost shrimp burrows became insignificant to the model.

Discussion

Kitimat

The objective of this study was to determine if relationships between the number of burrow openings and the abundance of macrofauna could be modelled at both high and low diversity mudflats on the north coast of British Columbia. At the Kitimat mudflats with only one macrofaunal burrowing species, the positive correlation between burrow openings and the number of *Mya arenaria* was

statistically significant; however model residuals were unreliable as they were biased with heteroscedasticity resulting in no significant and meaningful model created with the Kitimat data. Therefore, burrow openings were not a good proxy for *M. arenaria* densities.

To the best of our knowledge, the only other study attempting to use burrow opening counts to quantify bivalve abundance used the deep-sea propeller clam *Cyrtodaria siliqua* and examined the effect of dredging on the relationship between burrow openings and *C. siliqua* abundance (Gilkinson 2008). Although not all experimental treatments in their study revealed statistically significant relationships, the ones that did showed moderate to strong relationships with clam densities ($r = 0.50-0.72$) (Gilkinson 2008). However, their study found a temporal change in the ratio of burrows to bivalve abundance, with a decreasing number of burrows but consistent abundance of *C. siliqua* over multiple years (Gilkinson 2008). As temporal variation may be a factor in relationships between burrow openings and macrofauna abundance, more data would be required to see if the temporal scale of this research was too short to detect a temporal trend, and perhaps a stronger relationship and more reliable model could be generated by collecting more data during each sampling period, or sampling all year (Bringloe et al. 2013).

Wolfe Cove

At Wolfe Cove, high macrofaunal biodiversity made it more difficult to create a single, meaningful statistically significant relationship between burrow openings and species abundance. Of the eight species encountered, only *Clinocardium nuttallii* abundance was not significantly correlated with any of the observed burrow types. This was likely due to the low number of *C. nuttallii* encountered, as only a total of seven individuals were found throughout the sampling period. Therefore, more data would be required to properly assess the relationship between *C. nuttallii* abundance and the number of burrow openings.

276 The number of burrows identified as belonging to ghost shrimp showed weak correlations to
277 three of the eight species investigated, including between these burrows and *Neotrypaea californiensis*
278 abundance. While significant, this correlation was expected to be stronger as numerous *N. californiensis*
279 were observed in the sediment at the time of sampling. Furthermore, previous studies have found
280 significant and stronger relationships between the number of burrow openings and abundance of *N.*
281 *californiensis* (Carty 2003; Dumbauld et al. 1996). While unexpected, both Carty (2003) and Dumbauld
282 et al., (1996) used either a suction or large core rather than digging a pit as was done in this study. The
283 vertical shaft of *N. californiensis*’ burrow can be up to 90 cm deep (Dumbauld et al. 1996), therefore,
284 excavating a pit to 20 cm depth may not have been sufficient to capture all specimens present in the 1 m²
285 quadrat. However, this method was chosen because at this mudflat below 20cm depth the sediment
286 particle size became larger and transitioned into gravel, reducing the likelihood that *N. californiensis*
287 were present below this depth, and eliminating the ability to use suction as an extraction technique. The
288 high number of other burrowing infauna at this site may have also introduced too much variability into
289 the habitat, reducing the ability to create strong relationships between *N. californiensis* abundance and
290 burrow openings (Butler and Bird 2007; McPhee and Skilleter 2002).

291 Previous research has also noted that burrow opening counts cannot distinguish between
292 uninhabited and inhabited burrow openings, which may have influenced our results, and is one of the
293 reasons burrow opening/population abundance relationships may produce highly variable population
294 estimates (Schlacher et al. 2016b). This is especially a problem for mobile, errant taxa like
295 Thalassinidean shrimp and certain polychaetes (e.g. Nephtyidae or Nereididae), as they can vacate their
296 burrows or burrow through the sediment. Additionally, when excavating pits, mobile Nereididae worms
297 were observed moving into burrows belonging to bivalves like *M. arenaria*. Therefore, counting burrow
298 openings as estimators of population abundance may not be appropriate for mobile invertebrates.

299 The abundance of the lugworm *Abarenicola pacifica* was significantly positively correlated to
300 the number of burrows identified as lugworm burrows, although a statistically significant GLM could
301 not be created with just *A. pacifica* and lugworm burrows. Of interest, *Nephtys caeca* was also
302 significantly correlated with burrows identified as lugworm burrows, although the correlation was
303 negative. This may be due to the bioturbating activities of lugworms that can influence polychaete
304 assemblages, and their presence can negatively affect abundances of other polychaetes, especially
305 mobile predatory worms (Volkenborn and Reise 2007).

306 The abundance of *Macoma nasuta*, *N. californiensis* and *Glycinde picta* were all positively
307 correlated to the number of ‘other burrow openings’ (burrow openings identified as not belonging to
308 ghost shrimp or lugworms), while *Alitta brandti*, *A. pacifica* and *M. arenaria* were negatively correlated
309 to these openings. This result provides major challenges for using burrow openings as estimates of
310 individual species densities, as it eliminates our ability to assign burrow openings to a given species.
311 However, it does allow for the ability to create models which express the relationship between
312 population abundance and the type of burrow opening found (Equations 1-3), with applications for
313 monitoring population declines.

314 Of particular interest is the significant effect of sampling date on these models, suggesting that
315 temporal variation is an important consideration for modelling invertebrate abundances from burrow
316 opening counts. Previous research has found temporal variation to be a component of these models for
317 bivalves as previously mentioned, and for Pleocyemata shrimp species (Dumbauld et al. 1996; Gilkinson
318 2008; Schlacher et al. 2016b). As such, future research should be directed at furthering our knowledge
319 of temporal variation in these relationships, and understanding how to determine the appropriate
320 sampling date or sampling interval.

321

322 *Efficacy of Counting Burrow Openings as Organismal Abundance Proxies*

323 Although using burrow opening counts to estimate individual species abundance may not be
324 effective in low diversity sites, burrow counts in high macrofaunal diverse sites may still be a useful tool
325 for monitoring. For instance, in a heavily polluted estuary, simply counting macrofauna burrows without
326 assigning the burrow to a given taxa was sufficient to detect responses of the infaunal community along
327 the gradient of pollution (Saiz-Salinas and Gonzalez-Oreja 1999). Although burrow openings were
328 unable to predict individual infaunal abundances at our high diversity sites, openings were still able to
329 predict overall infaunal abundances, and therefore may be able to detect changes in habitat condition
330 over time in these systems. Burrow opening counts may therefore be an appropriate monitoring method
331 to identify potential infaunal population changes and relate them to alterations in habitat condition.
332 Counting burrow openings would be quicker, cheaper, and less destructive than excavation and
333 identification of infauna to a given taxonomic unit (Gilkinson 2008; Saiz-Salinas and Gonzalez-Oreja
334 1999; Schlacher et al. 2016b). As such, counting burrows could still be a useful monitoring tool when
335 the goal is to detect overall community changes.

336

337 **Conclusion**

338 In order to evaluate if burrow openings are a good predictor of infaunal abundance, we examined
339 mudflats with either a monoculture or with high macrofaunal biodiversity along the north coast of BC. A
340 model predicting macrofaunal abundance from burrow openings was not possible at low diversity
341 mudflats, while total macrofaunal abundance rather than individual species abundance was predicted at
342 the high diversity mudflat. Based upon our findings we therefore recommend considering these three
343 points for burrow opening counts as a rapid and reliable method for estimating the abundance of
344 macrofaunal organisms:

- 345 1. Timing of sampling appears to be relevant to macrofaunal counts and future research should be
346 directed at elucidating temporal variation in relationships between burrow openings and
347 invertebrate abundance.
- 348 2. At high macrofaunal diverse sites, complex interactions exist and therefore burrow opening
349 counts may be more appropriate for predicting total macrofaunal population abundance.
- 350 3. Regardless of species found, relationships between burrow openings counts and macrofaunal
351 abundance must be empirically tested in the system of interest.
- 352 Although designing a sampling protocol requires the above considerations, burrow opening counts can
353 be powerful tools for ecosystem monitoring. Monitoring population abundance through burrow opening
354 counts has the ability to detect overall changes in abundances, while being less destructive, quicker, and
355 cheaper than traditional excavation methods.

356

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Tables and Figures

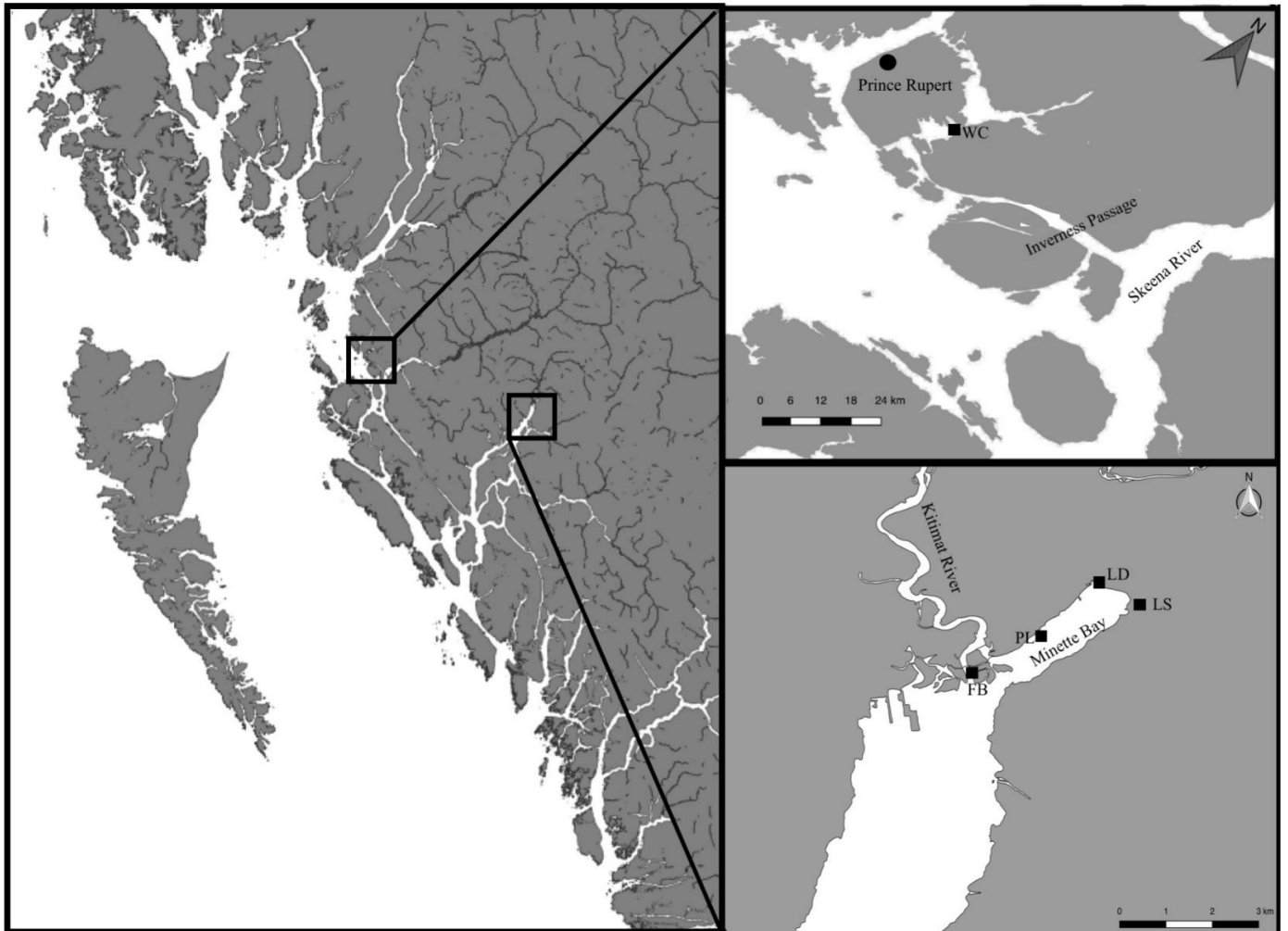


Figure 1. Map of intertidal mudflats sampled during summer 2017 near Kitimat and Prince Rupert, British Columbia, Canada. WC: Wolfe Cove, LS: Log Sort, LD: Log Dump, and FB: Foxy Beach. Mudflat near Prince Rupert in the Skeena River Estuary (WC: Wolfe Cove 54.242433, -130.273033) had high macrofaunal diversity ($n = 8$ species). Mudflats in the Kitimat River Estuary (LS: Log Sort 54.0248815, -128.610411, LD: Log Dump 54.031088, -128.621355, PL: Pilings 54.015791, -128.632238, and FB: Foxy Beach 54.005785, -128.660710) had low macrofaunal diversity ($n = 1$ species).

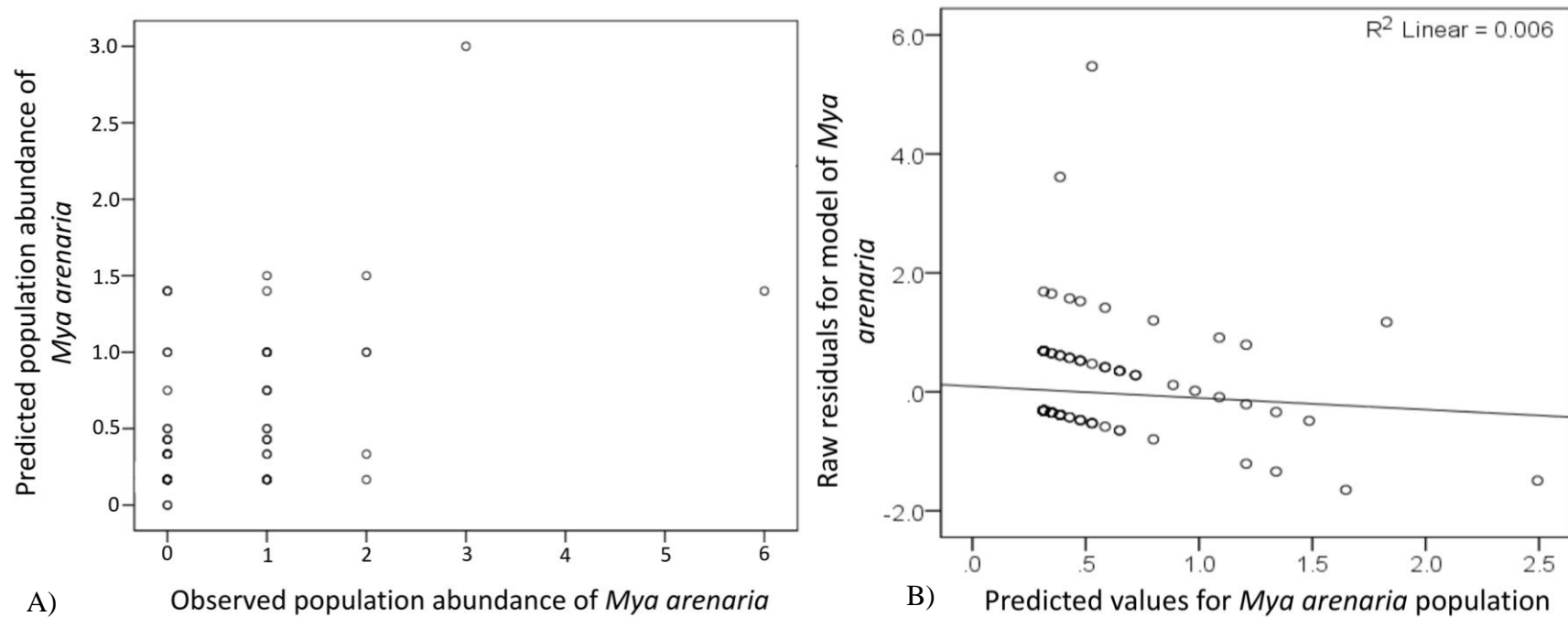


Figure 2: Model output for general linearized model of *Mya arenaria*. A) The relationship between predicted and observed population counts of *M. arenaria* at Kitimat, BC. Predicted values are based on burrow counts. B) The relationship between model residuals and model predicted values for the linear model created for *M. arenaria* populations based on burrow counts.

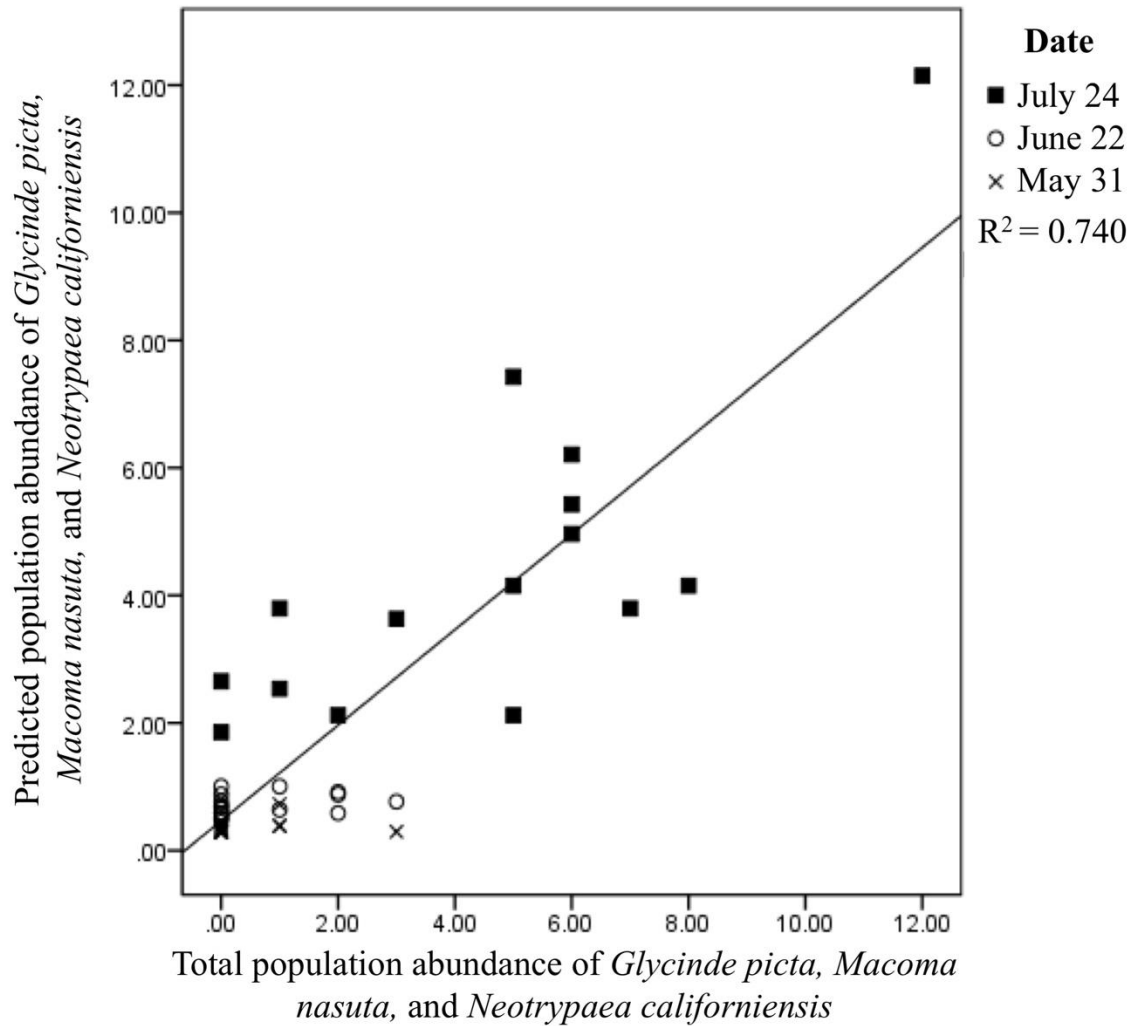


Figure 3. Observed values of *Glycinde picta*, *Macoma nasuta* and *Neotrypaea californiensis* versus predicted values from other burrow openings at Wolfe Cove. Invertebrate populations were counted by excavating and collecting all specimens from a 1 m² pit to a depth of 20 cm, while burrow openings were counted visually on the surface during low tide in the summer of 2017.

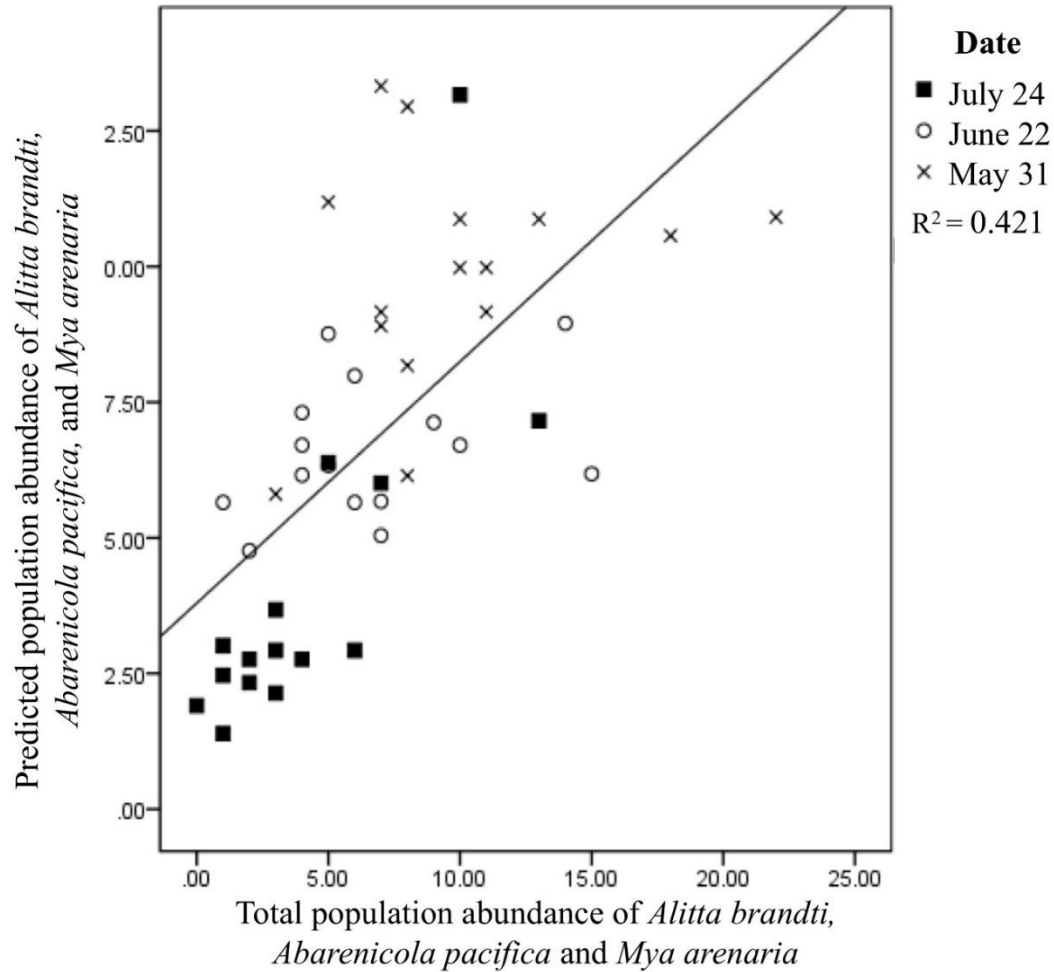


Figure 4. Observed values of *Alitta brandti*, *Abarenicola pacifica*, and *Mya arenaria* populations versus predicted values using lugworm burrows and other burrow openings as predictors at Wolfe Cove. Invertebrate populations were counted by excavating and collecting all specimens from a 1 m² pit to a depth of 20 cm, while burrow openings were counted visually on the surface during low tide in the summer of 2017.

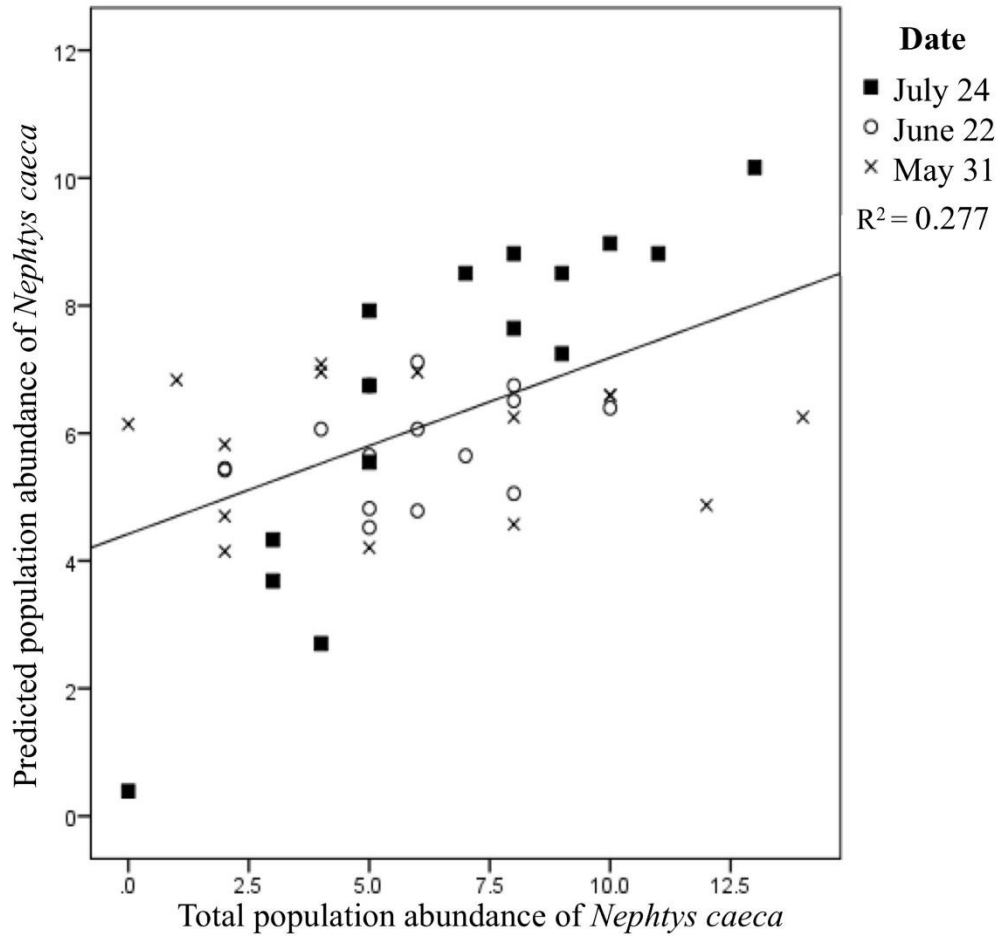


Figure 5. Observed values of the *Nephtys caeca* population versus predicted abundance using lugworm burrows at Wolfe Cove. *N. caeca* individuals were counted by excavating and collecting all specimens from a 1 m² pit to a depth of 20 cm, while burrow openings were counted visually on the surface during low tide in the summer of 2017.

Table 1. Correlation matrix for abundance of macrofauna and type of burrow opening on the substrate surface at Wolfe Cove. Spearman's rho coefficients and associated significance are presented. As we were attempting to identify potential relationships, $\alpha = 0.1$ was used to denote significance and statistically significant correlations are shown in bold (Beninger et al. 2012).

	<i>Alitta brandti</i>	<i>Nephtys caeca</i>	<i>Glycinde picta</i>	<i>Abarenicola pacifica</i>	<i>Clinocardium nuttallii</i>	<i>Mya arenaria</i>	<i>Macoma nasuta</i>	<i>Neotrypaea californiensis</i>
Other Burrow Openings	-0.312, 0.037	0.227, 0.133	0.335, 0.025	-0.293, 0.051	0.092, 0.547	-0.512, 0.001	0.508, 0.001	0.259, 0.086
Ghost Shrimp Burrows	-0.111, 0.469	0.266, 0.077	0.216, 0.153	-0.347, 0.020	-0.156, 0.306	-0.166, 0.277	0.071, 0.642	0.263, 0.081
Lugworm Burrows	0.299, 0.046	-0.376, 0.011	-0.116, 0.448	0.501, 0.001	-0.083, 0.590	0.298, 0.047	-0.121, 0.427	-0.072, 0.641