A rapid, non-invasive population assessment technique for marine burrowing macrofauna inhabiting soft sediments

Campbell, L., Wood, L., Allen Gerwing, A. M., Allen, S., Sizmur, T. ORCID: https://orcid.org/0000-0001-9835-7195, Rogers, M., Gray, O., Drewes, M., Juanes, F. and Gerwing, T. G. (2019) A rapid, non-invasive population assessment technique for marine burrowing macrofauna inhabiting soft sediments. Estuarine, Coastal and Shelf Science, 227. 106343. ISSN 0272-7714 doi: 10.1016/j.ecss.2019.106343 Available at https://centaur.reading.ac.uk/86128/

It is advisable to refer to the publisher's version if you intend to cite from the work. See Guidance on citing.

the End User Agreement.

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1   A Rapid, Non-invasive Population Assessment Technique for Marine Burrowing Macrofauna

2   Inhabiting Soft Sediments

3

4 Lily Campbell[1]*, Lisa Wood[2], Alyssa M. Allen Gerwing[2], Shaun Allen[2], Tom Sizmur[3], Megan Rogers[4],

5 Olivia Gray[4], Mitch Drewes[4], Francis Juanes[1], Travis G. Gerwing,[1,2]

6

7 [1]Department of Biology, University of Victoria. Victoria, British Columbia, Canada.

8 2 Ecosystem Science and Management Program, University of Northern British Columbia. Prince

9 George, British Columbia, Canada.

10 [3] Department of Geography and Environmental Science, University of Reading. Reading, UK.

11 [4] Hidden River Environmental Management. Terrace, British Columbia, Canada.

12

13

14 *Corresponding Author: Lily Campbell. Email: lilyncampbell@gmail.com

15

Abstract

17   Population assessment techniques for soft-sediment infauna (invertebrates within the substrate)

18 requires excavation of specimens, damaging or killing the specimen and surrounding habitat, while

19 being time-consuming and costly. Rapid population assessments of some marine burrowing decapods

20 have been possible by counting burrow openings to estimate abundance, and while they may be used as

21 indicator species, these decapods are not ubiquitous to environments requiring monitoring. Additionally,

22 the presence of other burrowing macrofauna (invertebrates living in the sediment and retained on 1mm

23 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

36

37

38

39

40

41

42

43

44

45

46

47

Introduction

Understanding the impact of human activity on ecosystem health and biodiversity is a

fundamental aspect of applied scientific research (Gonzalez et al. 2016; Vackar et al. 2012). Ecologists

and conservation biologists often estimate species abundance, or use population dynamics to achieve a

variety of research goals including the assessment of anthropogenic impacts (Cox et al. 2017; Schlacher

et al. 2016b; Simao et al. 2006). Although compiling counts of organismal abundance is easy in theory,

precise and accurate counts are difficult, and may require invasive techniques (Butler and Bird 2007;

Cox et al. 2017; Schlacher et al. 2016b). For example, in marine soft-sediment ecosystems many

invertebrates burrow into the substrate (infauna), requiring excavation of individuals from the sediment

to assess density and presence/absence. Such methods are destructive to the habitat, and risk stressing,

damaging, or killing specimens (Butler and Bird 2007; Schlacher et al. 2016b). In addition to habitat

damage, excavations are time consuming, laborious, and costly, limiting the spatiotemporal scale of

investigation (Dumbauld et al. 1996; Gilkinson 2008). Therefore, a variety of methods have been

proposed for monitoring and estimating infaunal densities, including assessing indicator species or

applying ecological indices that can be used as proxies for ecosystem health (Gerwing et al. 2017;

Gesteira and Dauvin 2000; Hereward et al. 2017; Schlacher et al. 2016b). Ecological proxies are

advantageous as they require less time to assess an area than examining a site holistically, and reduce

costs (Butler and Bird 2007; Gilkinson 2008; Schlacher et al. 2016b), although they require pilot studies

to evaluate their efficacy (Gerwing et al. 2017; Gerwing et al. 2015b).

In coastal soft-sediment ecosystems that have been degraded by anthropogenic impacts such as

urbanization and industrial development (Crain et al. 2008; Gerwing and Cox 2017), fossorial

(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 Rosenburg 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

4

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 m^2 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 cm^2 pit was dug to a depth of 20 cm at Kitimat mudflats,

153   whereas at Wolfe Cove a 1 m^2 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

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] $Y = 0.47 + 0.75x$ ($r^2 = 0.740$; 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)

230   (likelihood ratio Chi-square = 72.462, $p < 0.001$). The linear relationship between the predicted values

231   and the observed total population abundance of these species is described by:

232   [2] $Y = 3.8 + 0.45x$ ($r^2 = 0.421$; Figure 4)

233   *A. pacifica* was significantly correlated with ghost shrimp burrows when the independent Spearman's

234   rho values were calculated (Table 1); however, when modeled as total abundance with *A. brandti,* and

235   *M. arenaria*, the total abundance of these species can be modeled more appropriately by lugworm and

236   other burrow opening types than ghost shrimp burrows.

237   Lastly, *N. caeca* was modeled by lugworm burrow and other burrow opening counts (covariates), and

238   date of sampling (fixed factor) (likelihood ratio Chi-square = 26.523, $p < 0.001$). A significant

239   interaction effect was noted between lugworm burrows and other burrow openings in the model of *N.*

240   *caeca* ($p = 0.029$). The linear relationship between the predicted values and the observed counts of *N.*

241   *caeca* population abundance is described by the following equation:

242   [3] $Y = 4.42 + 0.28x$ ($r^2 = 0.277$; Figure 5)

243   Although the Spearman's rho value shows a relationship between *N. caeca* abundance and combined

244   ghost shrimp burrows and lugworm burrows, when modeled with other variables of consideration (other

245   burrow openings, sampling date, transect) ghost shrimp burrows became insignificant to the model.

246

247   **Discussion**

248   *Kitimat*

249   The objective of this study was to determine if relationships between the number of burrow

250   openings and the abundance of macrofauna could be modelled at both high and low diversity mudflats

251   on the north coast of British Columbia. At the Kitimat mudflats with only one macrofaunal burrowing

252   species, the positive correlation between burrow openings and the number of *Mya arenaria* was

253 statistically significant; however model residuals were unreliable as they were biased with

254 heteroscedasticity resulting in no significant and meaningful model created with the Kitimat data.

255 Therefore, burrow openings were not a good proxy for *M. arenaria* densities.

256   To the best of our knowledge, the only other study attempting to use burrow opening counts to

257 quantify bivalve abundance used the deep-sea propeller clam *Cyrtodaria siliqua* and examined the effect

258 of dredging on the relationship between burrow openings and *C. siliqua* abundance (Gilkinson 2008).

259 Although not all experimental treatments in their study revealed statistically significant relationships, the

260 ones that did showed moderate to strong relationships with clam densities (r = 0.50-0.72) (Gilkinson

261 2008). However, their study found a temporal change in the ratio of burrows to bivalve abundance, with

262 a decreasing number of burrows but consistent abundance of *C. siliqua* over multiple years (Gilkinson

263 2008). As temporal variation may be a factor in relationships between burrow openings and macrofauna

264 abundance, more data would be required to see if the temporal scale of this research was too short to

265 detect a temporal trend, and perhaps a stronger relationship and more reliable model could be generated

266 by collecting more data during each sampling period, or sampling all year (Bringloe et al. 2013).

267

268 *Wolfe Cove*

269   At Wolfe Cove, high macrofaunal biodiversity made it more difficult to create a single,

270 meaningful statistically significant relationship between burrow openings and species abundance. Of the

271 eight species encountered, only *Clinocardium nuttallii* abundance was not significantly correlated with

272 any of the observed burrow types. This was likely due to the low number of *C. nuttallii* encountered, as

273 only a total of seven individuals were found throughout the sampling period. Therefore, more data

274 would be required to properly assess the relationship between *C. nuttallii* abundance and the number of

275 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 m^2

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   Thalissinidean 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

References

Beninger PG, Boldina I, Katsanevakis S. 2012. Strengthening statistical usage in marine ecology. Journal of Experimental Marine Biology and Ecology. 426:97-108.

Bringloe TT, Drolet D, Barbeau MA, Forbes MR, Gerwing TG. 2013. Spatial variation in population structure and its relation to movement and the potential for dispersal in a model intertidal invertebrate. Plos One. 8(7).

Butler S, Bird FL. 2007. Estimating density of intertidal ghost shrimps using counts of burrow openings. Is the method reliable? Hydrobiologia. 589:303-314.

Carr-Harris C, Gottesfeld AS, Moore JW. 2015. Juvenile salmon usage of the Skeena River estuary. Plos One. 10(3).

Carty SE. 2003. *Zostera marina* and *Neotrypaea californiensis* as indicators of ecosystem integrity in Grice Bay, British Columbia [thesis]. University of British Columbia. 172p. Accessed from Open Collections UBC Theses and Dissertations.

Cox KD, Black MJ, Filip N, Miller MR, Mohns K, Mortimor J, Freitas TR, Greiter Loerzer R, Gerwing TG, Juanes F et al. 2017. Community assessment techniques and the implications for rarefaction and extrapolation with hill numbers. Ecology and Evolution. 7(24):11213-11226.

Crain CM, Kroeker K, Halpern BS. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters. 11(12):1304-1315.

D'Andrea AF, DeWitt TH. 2009. Geochemical ecosystem engineering by the mud shrimp *Upogebia pugettensis* (Crustacea: Thalassinidae) in Yaquina Bay, Oregon: Density-dependent effects on organic matter remineralization and nutrient cycling. Limnology and Oceanography. 54(6):1911-1932.

Dumbauld BR, Armstrong DA, Feldman KL. 1996. Life-history characteristics of two sympatric Thalassinidean shrimps, *Neotrypaea californiensis* and *Upogebia pugettensis*, with implications for oyster culture. Journal of Crustacean Biology. 16(4):689-708.

Gerwing TG, Campbell L, Allen Gerwing AM, Allen S, Cox K, Rogers M, Gray O, Drewes M, Juanes F. 2018a. Potential impacts of logging on intertidal infaunal communities within the Kitimat River Estuary. Journal of Natural History. 52: 2833-2855.

Gerwing TG, Cox K. 2017. Erosion of trust in government consultation will impede the creation of environmental policy. Marine Policy. 83:126-127.

Gerwing TG, Cox K, Allen Gerwing AM, Carr-Harris CN, Dudas SE, Juanes F. 2017. Depth to the apparent redox potential discontinuity (aRPD) as a parameter of interest in marine benthic habitat models. International Journal of Sediment Research. 32:472-480.

Gerwing TG, Gerwing AMA, Drolet D, Barbeau MA, Hamilton DJ. 2015a. Spatiotemporal variation in biotic and abiotic features of eight intert1idal mudflats in the Upper Bay of Fundy, Canada. Northeastern Naturalist. 22:1-44.

Gerwing TG, Gerwing AMA, Hamilton DJ, Barbeau MA. 2015b. Apparent redox potential discontinuity (aRPD) depth as a relative measure of sediment oxygen content and habitat quality. International Journal of Sediment Research. 30(1):74-80.

Gerwing TG, Gerwing AMA, Macdonald T, Cox K, Juanes F, Dudas SE. 2018b. Assessing the relationship between community dispersion and disturbance in a soft-sediment ecosystem. Marine Ecology-an Evolutionary Perspective. 39(4):9.

Gesteira JLG, Dauvin JC. 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Marine Pollution Bulletin. 40(11):1017-1027.

Gilkinson K. 2008. Reliability of abundance estimates of the propellerclam (*Cyrtodaria siliqua*) derived from their burrow openings: A case study from Banquereau, Eastern Canada. Journal of Shellfish Research. 27(2):289-295.

Gonzalez A, Cardinale BJ, Allington GRH, Byrnes J, Endsley KA, Brown DG, Hooper DU, Isbell F, O'Connor MI, Loreau M. 2016. Estimating local biodiversity change: A critique of papers claiming no net loss of local diversity. Ecology. 97(8):1949-1960.

Guerra-Garcia J, Garcia-Gomez J. 2004. Polychaete assemblages and sediment pollution in a harbour with two opposing entrances. Helgoland Marine Research. 58(3):183-191.

Halpern BS, Frazier M, Potapenko J, Casey KS, Koenig K, Longo C, Lowndes JS, Rockwood RC, Selig ER, Selkoe KA et al. 2015. Spatial and temporal changes in cumulative human impacts on the world's ocean. Nature Communications. 6:7.

Harbo RM. 2003. Pacific reef and shore: A photo guide to northwest marine life. Madeira Park, BC: Harbour Publishing.

Harbo RM. 2007. Shells and shellfish of the Pacific Northwest. Madeira Park, BC: Harbour Publishing.

Harbo RM. 2011. Whelks to whales: Coastal marine life of the Pacific Northwest. 2 ed. Madeira Park, BC: Harbour Publishing.

Hereward HFR, Gentle LK, Ray ND, Sluka RD. 2017. Ghost crab burrow density at Watamu Marine National Park: An indicator of the impact of urbanization and associated disturbance? African Journal of Marine Science. 39(1):129-133.

Hewitt JE, Thrush SF, Ellingsen KE. 2016. The role of time and species identities in spatial patterns of species richness and conservation. Conservation Biology. 30(5):1080-1088.

Hutchins CM, Teasdale PR, Lee SY, Simpson SL. 2009. Influence of sediment metal spiking procedures on copper bioavailability and toxicity in the estuarine bivalve *Indoaustriella lamprelli*. Environmental Toxicology and Chemistry. 28(9):1885-1892.

Light SF, Smith RI. 2007. The Light and Smith manual: Intertidal invertebrates from Central California to Oregon. Carlton JT, editor. Berkeley, Los Angeles, CA, USA: University of California Press.

Little S, Wood PJ, Elliott M. 2017. Quantifying salinity-induced changes on estuarine benthic fauna: The potential implications of climate change. Estuarine Coastal and Shelf Science. 198:610-625.

McLaren P. 2016. The environmental implications of sediment transport in the waters of Prince Rupert, British Columbia, Canada: A comparison between kinematic and dynamic approaches. Journal of Coastal Research. 32(3):465-482.

McPhee DP, Skilleter GA. 2002. Aspects of the biology of the yabby *Trypea australiensis* (Dana) (Decapoda: Thalassinidea) and the potential of burrow counts as an indirect measure of population density. Hydrobiologia. 485(1-3):133-141.

Pearson TH, Rosenburg R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology. 16:229-311.

Pillay D, Branch GM. 2011. Bioengineering effects of burrowing Thalassinidean shrimps on marine soft-bottom ecosystems. Oceanography and Marine Biology: An Annual Review. 49:137-191.

Saiz-Salinas JI, Gonzalez-Oreja JA. 1999. Measuring redundancy in data from pollution impact studies. Environmental Management. 24(3):415-420.

Schlacher TA, Lucrezi S, Connolly RM, Peterson CH, Gilby BL, Maslo B, Olds AD, Walker SJ, Leon JX, Huijbers CM et al. 2016a. Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. Estuarine Coastal and Shelf Science. 169:56-73.

Schlacher TA, Lucrezi S, Peterson CH, Connolly RM, Olds AD, Althaus F, Hyndes GA, Maslo B, Gilby BL, Leon JX et al. 2016b. Estimating animal populations and body sizes from burrows: Marine ecologists have their heads buried in the sand. Journal of Sea Research. 112:55-64.

Simao DS, Ramos MF, Soares-Gomes A. 2006. Population structure of *Callichirus major* (Say 1818) (Crustacea:Thalassinidea) in a sandy beach of Rio de Janeiro State, Southeast Brazil coast. Journal of Coastal Research.1165-1168.

Simpson CD, Harrington CF, Cullen WR. 1998. Polycyclic aromatic hydrocarbon contamination in marine sediments near Kitimat, British Columbia. Environmental Science & Technology. 32(21):3266-3272.

Stelling-Wood TP, Clark GF, Poore AGB. 2016. Responses of ghost crabs to habitat modification of urban sandy beaches. Marine Environmental Research. 116:32-40.

Talmage SC, Gobler CJ. 2010. Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. Proceedings of the National Academy of Sciences of the United States of America. 107(40):17246-17251.

Vackar D, ten Brink B, Loh J, Baillie JEM, Reyers B. 2012. Review of multispecies indices for monitoring human impacts on biodiversity. Ecological Indicators. 17:58-67.

Volkenborn N, Reise K. 2007. Effects of *Arenicola marina* on polychaete functional diversity revealed by large-scale experimental lugworm exclusion. Journal of Sea Research. 57(1):78-88.

Waldbusser GG, Bergschneider H, Green MA. 2010. Size-dependent pH effect on calcification in post-larval hard clam *Mercenaria* spp. Marine Ecology Progress Series. 417:171-182.

Yunker MB, Lachmuth CL, Cretney WJ, Fowler BR, Dangerfield N, White L, Ross PS. 2011. Biota - sediment partitioning of aluminium smelter related PAHs and pulp mill related diterpenes by intertidal clams at Kitimat, British Columbia. Marine Environmental Research. 72(3):105-126.

Zuur A, Leno E, Walker N, Saveliev A, Smith G. 2009. GLM and GAM for count data. Mixed effects models and extensions in ecology with R. Springer. p. 209-243.
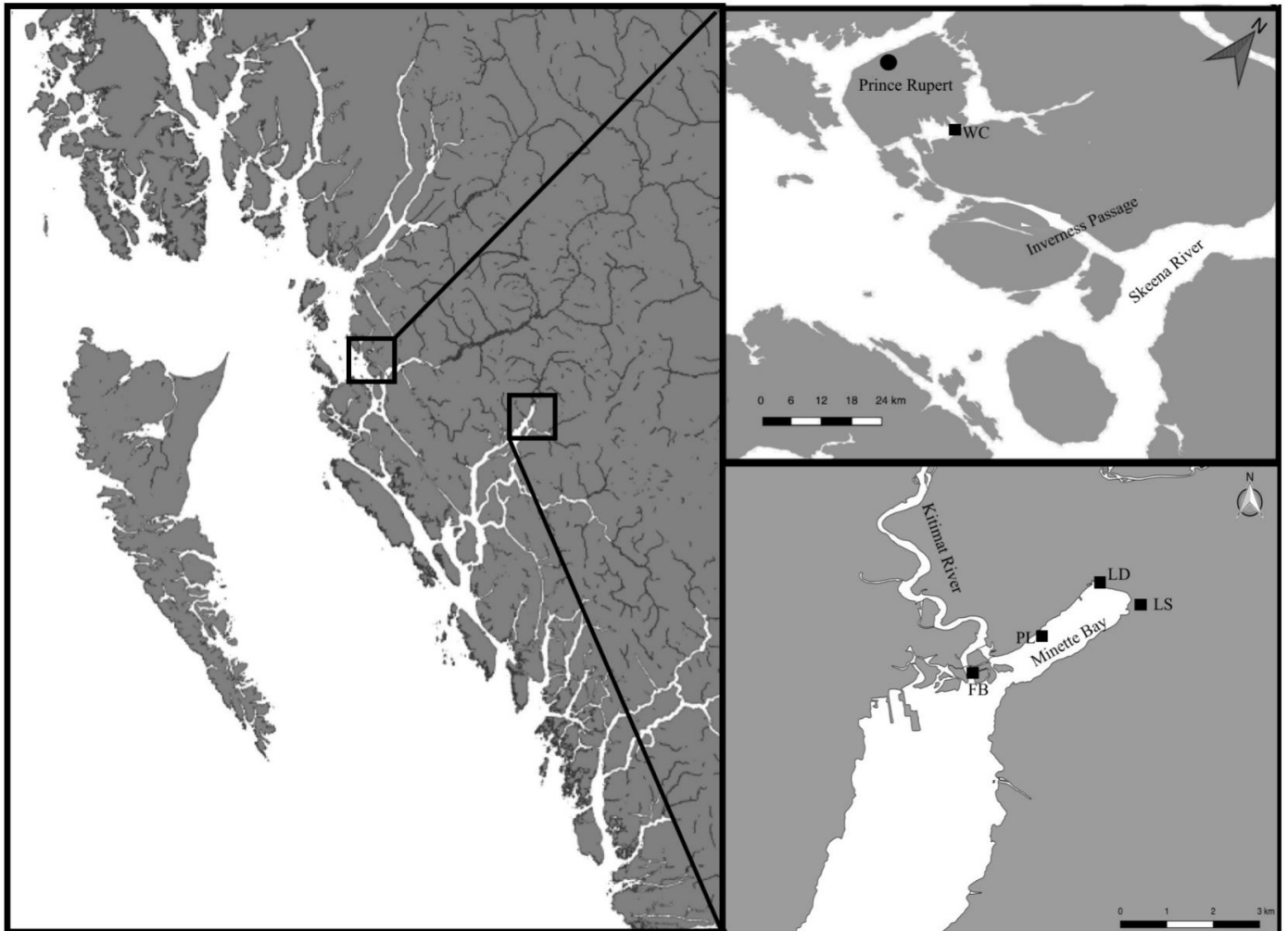
Tables and Figures

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).
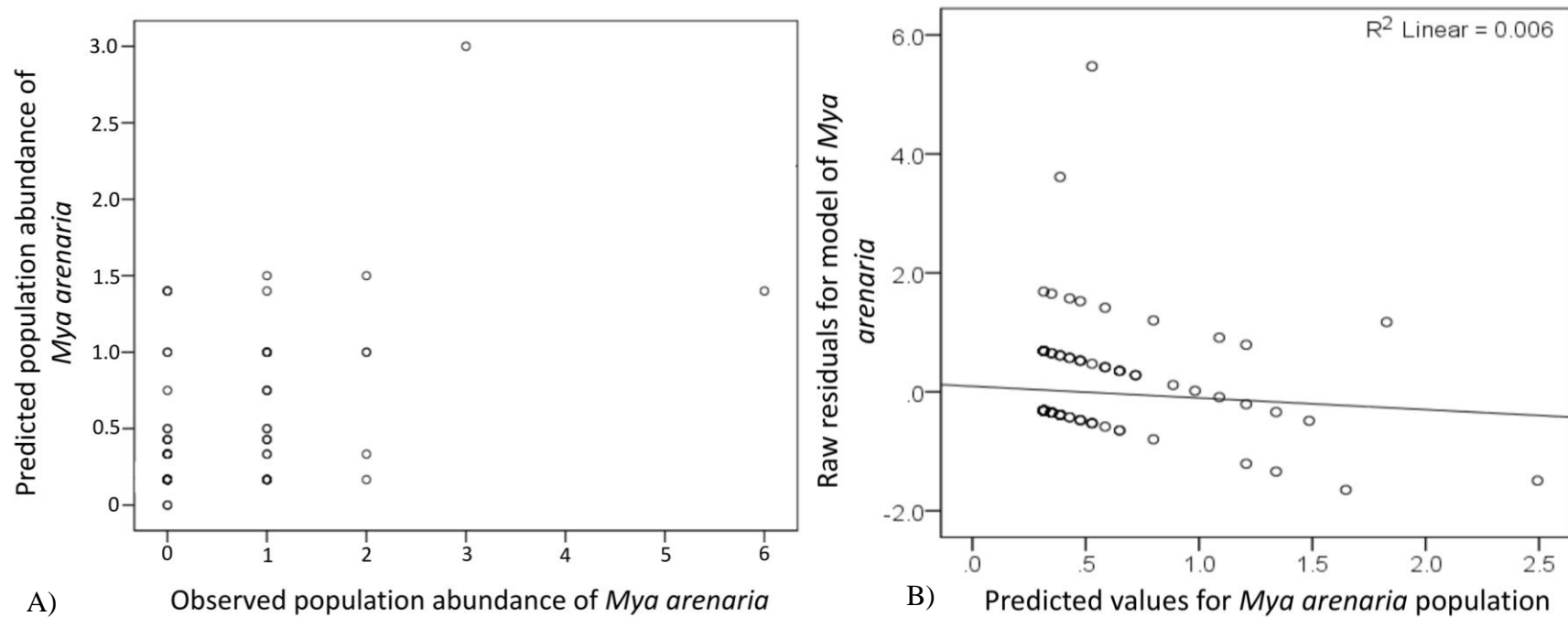
Figure 2: Model output for general linearized model of *Mya arenaria*. A) The relationship between predicted and observed population counts of *M. arenaria* at Kitimat, BC. Predicted values are based on burrow counts. B) The relationship between model residuals and model predicted values for the linear model created for *M. arenaria* populations based on burrow counts.
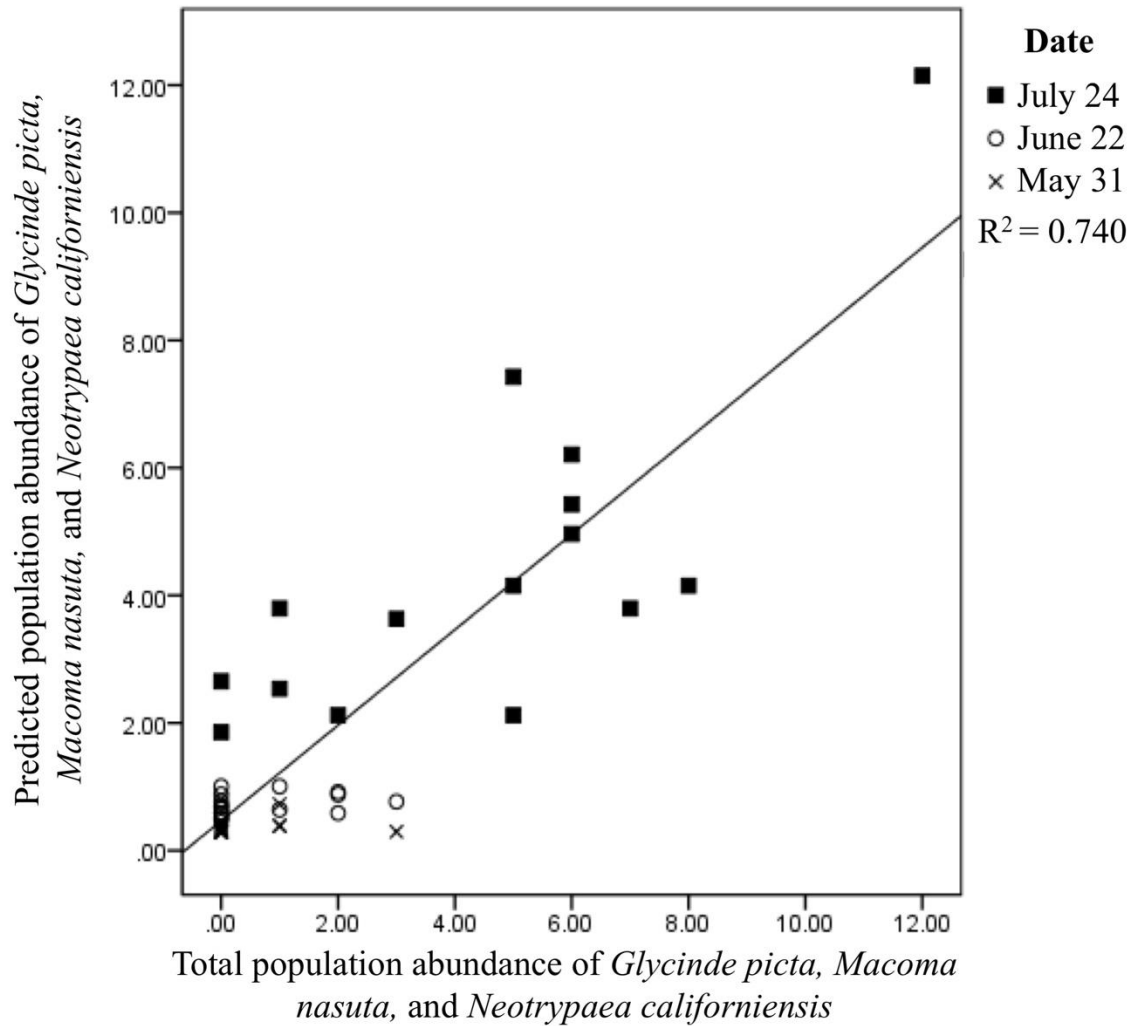
Figure 3. Observed values of *Glycinde picta, Macoma nasuta* and *Neotrypaea californiensis* versus predicted values from other burrow openings at Wolfe Cove. Invertebrate populations were counted by excavating and collecting all specimens from a 1 m^2 pit to a depth of 20 cm, while burrow openings were counted visually on the surface during low tide in the summer of 2017.
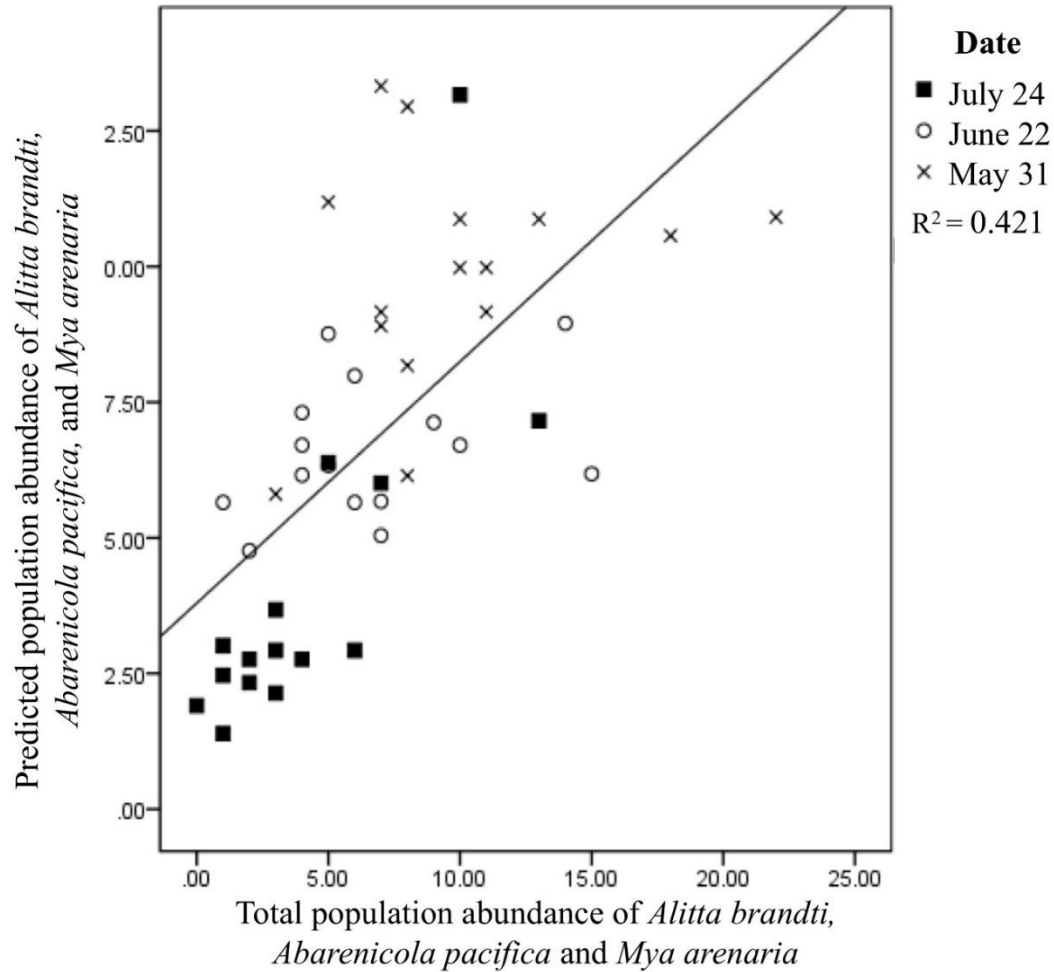
Figure 4. Observed values of *Alitta brandti, Abarenicola pacifica,* and *Mya arenaria* populations versus predicted values using lugworm burrows and other burrow openings as predictors at Wolfe Cove. Invertebrate populations were counted by excavating and collecting all specimens from a 1 m² pit to a depth of 20 cm, while burrow openings were counted visually on the surface during low tide in the summer of 2017.
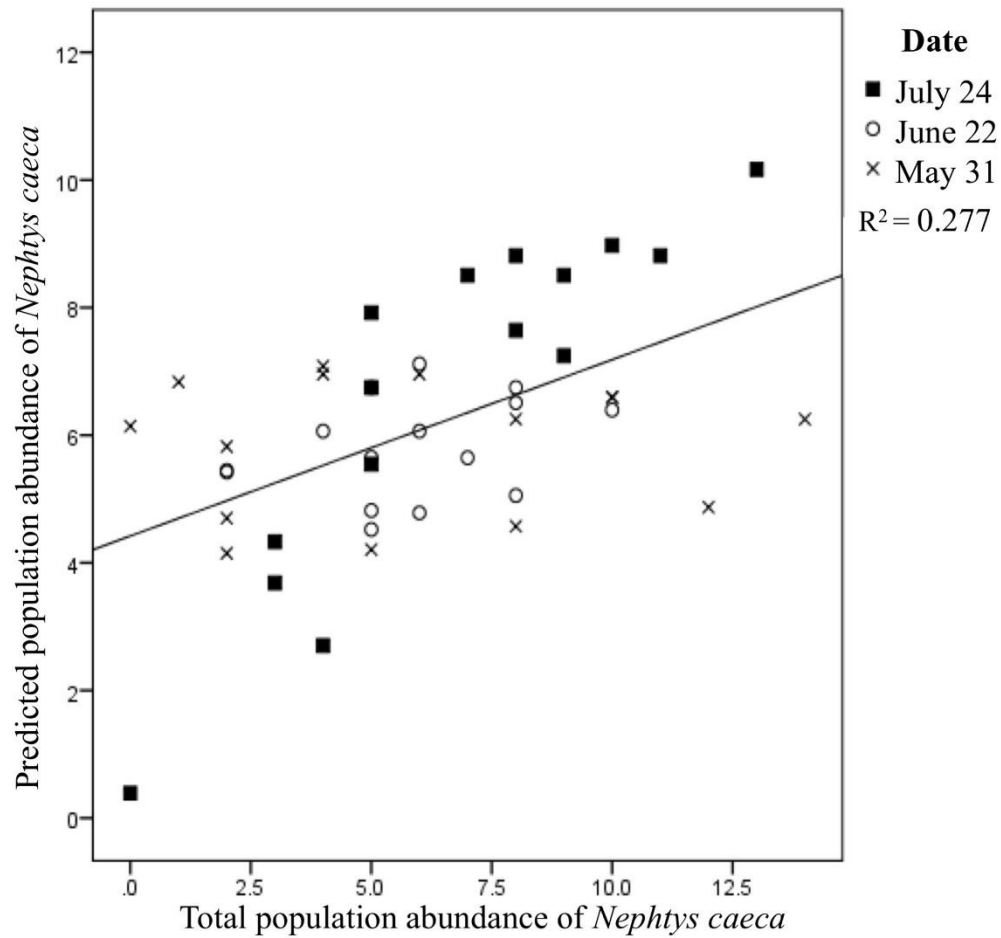
Figure 5. Observed values of the *Nephtys caeca* population versus predicted abundance using lugworm burrows at Wolfe Cove. *N. caeca* individuals were counted by excavating and collecting all specimens from a 1 m^2 pit to a depth of 20 cm, while burrow openings were counted visually on the surface during 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).

	Alitta brandti	*Nephtys caeca*	*Glycinde picta*	*Abarenicola pacifica*	*Clinocardium nuttallii*	*Mya arenaria*	*Macoma nasuta*	*Neotrypaea californiensis*
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