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

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

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

Although the majority of research utilizing burrow openings as an ecological proxy has focused on marine fossorial decapods, this group of organisms are not ubiquitous to marine soft-sediment ecosystems. Additionally, it is also possible that the presence of other burrowing macrofauna (invertebrates living in the sediment and retained on a 1mm sieve such as clams or large worms) may decrease the efficacy of using burrow openings as proxies for abundance (Butler and Bird 2007; McPhee and Skilleter 2002). Where only one macrofaunal species is present, monitoring by counting burrow openings may be reliable, but may not be possible when multiple macrofaunal species are present due to the presence of species inhabiting burrows that they didn't create and altering the relationship between the number of burrow openings and abundance (Butler and Bird 2007; McPhee and Skilleter 2002). Conversely, macrofauna often create burrow openings that can be differentiated and identified to species visually, potentially enabling the usage of burrow openings to assess densities outside of monocultures (Harbo 2003; 2007; 2011). For instance, *Neotrypaea californiensis* (ghost shrimp; Suborder Pleocymata) creates distinctive burrows with a vertical shaft and expelled sediment in a volcano shape around the circular burrow opening (Pillay and Branch 2011) while *Abarenicola pacifica* (Pacific lugworm) creates j-shaped burrows with rope-like, coiled fecal castings around the burrow opening (Harbo 2003; 2007; 2011; Light 2007). Therefore, it may be possible to estimate abundances of these species from their unique burrow openings even in areas of high macrofaunal diversity, and the applicability of burrow openings counts belonging to macrofauna in estimating organismal abundance should be further examined in biodiverse habitats.

We assessed intertidal mudflats in British Columbia, Canada, at both low macrofaunal diverse mudflats near Kitimat, and a high macrofaunal diverse mudflat near Prince Rupert in the Skeena Estuary, to determine the efficacy of burrow openings as proxies for abundance of macrofauna. Both Kitimat and Prince Rupert are cities near estuarine systems in northern BC, Canada, and are important regions for environmental monitoring due to their history of industrial development including an aluminum smelter, logging, and a pulp and paper mill. Future development is also likely in these regions, including potential potash export terminals, and oil and liquefied natural gas pipelines, refineries, and export terminals (Carr-Harris et al. 2015; McLaren 2016; Simpson et al. 1998; Yunker et al. 2011). As such, trends identified in these systems may provide valuable insights applicable to other estuarine systems (Gerwing et al. 2015a; Gerwing et al. 2018b; Hewitt et al. 2016; Little et al. 2017). Therefore, we tested whether a relationship between burrow opening and fossorial organism abundance can be generated in high and low macrofaunal diverse sites, with the goal of creating relationships that could be used to save time and money when assessing macrofaunal populations in the future.

Materials and Methods

Study Sites

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

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

Field Methods

At each mudflat, five transects were established, stretching from the start of the mudflat to the low tide waterline (60-200 m long, 25 m apart) (Cox et al. 2017; Gerwing et al. 2015a). Transects were stratified into three equal zones based on distance from shore (near, middle, and far). Within each zone, one sampling location was randomly selected (n = 3 per transect, 15 per site per sampling period) and a 1 m^2 quadrat was established (Gerwing et al. 2015a). Burrow openings greater than 0.1 cm were quantified were differentiated based on physical characteristics and classified into three categories as ghost shrimp burrows, lugworm burrows, or other burrow openings. Ghost shrimp burrows were constructed by *N. californiensis* and identified by the expelled sediment in a volcano shape around the circular burrow opening, characteristic of sheltered mudflats like Wolfe Cove (Pillay and Branch 2011). Lugworm burrows were constructed by *A. pacifica*, identified by circular burrows with rope-like, coiled fecal castings around the burrow opening (Harbo 2003; 2011; Light and Smith 2007). Other burrow openings were the remaining indistinguishable burrows that were small to medium sized non-descript openings created by bivalves and Nephtyidae or Nereididae polychaetes. After burrow openings were classified, a pit was excavated to quantify the abundance of macrofauna (Cox et al. 2017). Due to differences in availability of resources, a 20 cm^2 pit was dug to a depth of 20 cm at Kitimat mudflats, 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). All mud excavated from each pit was sieved through a No. 35 mesh sieve (0.5 mm) opening. Where possible, macrofauna were identified in the field and immediately released. Specimens that could not be identified in the field were retained and later identified under a dissecting microscope (Light and Smith 2007). One mudflat was sampled per day at the lowest low tide during three sampling periods over the summer of 2017 (May 25-31, June 22-28, July 17-24). The LS mudflat was not sampled during the first sampling period, (May 25-31), and PL was not sampled in the last sampling period (July 17-24). This sampling scheme resulted in a total of 30-45 sampling events conducted per mudflat.

Statistical analysis

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

Following the Spearman's correlation analysis, a Poisson log probability distribution was employed to create general linear models (GLMs) based on significant correlations. This distribution is ideal when analyzing non-normal data in the form of counts (Zuur et al. 2009). Sampled population counts were summed for calculating model statistics based on similarities in statistically significant correlations calculated at Wolfe Cove. Abundance for *A. brandti, A. pacifica, and M. arenaria* were summed, and *G. picta, M. nasuta,* and *N. californiensis* were summed because of their common statistically significant correlation in the same direction (negative and positively respectively) to non-descript "other burrow openings." The abundance of lugworm burrows and other burrow openings were used as covariates, while sampling date was a fixed factor, to predict the summed population numbers for *A. brandti, A. pacifica, and M. arenaria.* The abundance of other burrow openings was modelled as a covariate with sampling date a fixed factor to predict the summed population numbers for *G. picta, M. nasuta,* and *N. californiensis*. Other dependent variables were modeled, including abundance of *N. caeca,* while other covariates and fixed variables were explored in GLMs including transect number and ghost shrimp burrow abundance in order to assess their impact on model significance. Only covariates and fixed factors with an α less than 0.05 were deemed acceptable for use in the models. Where multiple burrow types were entered as covariates in a model, the interaction effect of these openings was also entered as a model variable; for example, lugworm burrows X other burrow openings. Model residuals were graphed to assess model reliability.

Results

Kitimat

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

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

Wolfe Cove

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

The following models revealed significant predictive relationships:

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

[1] $Y = 0.47 + 0.75x$ ($r^2 = 0.740$; Figure 3)

The total population abundance of *A. brandti, A. pacifica,* and *M. arenaria* was predicted by the number 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] $Y = 3.8 + 0.45x$ ($r^2 = 0.421$; 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] $Y = 4.42 + 0.28x$ ($r^2 = 0.277$; 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.

The number of burrows identified as belonging to ghost shrimp showed weak correlations to three of the eight species investigated, including between these burrows and *Neotrypaea californiensis* abundance. While significant, this correlation was expected to be stronger as numerous *N. californiensis* were observed in the sediment at the time of sampling. Furthermore, previous studies have found significant and stronger relationships between the number of burrow openings and abundance of *N. californiensis* (Carty 2003; Dumbauld et al. 1996). While unexpected, both Carty (2003) and Dumbauld et al., (1996) used either a suction or large core rather than digging a pit as was done in this study. The vertical shaft of *N. californiensis'* burrow can be up to 90 cm deep (Dumbauld et al. 1996), therefore, excavating a pit to 20 cm depth may not have been sufficient to capture all specimens present in the 1 m^2 quadrat. However, this method was chosen because at this mudflat below 20cm depth the sediment particle size became larger and transitioned into gravel, reducing the likelihood that *N. californiensis* were present below this depth, and eliminating the ability to use suction as an extraction technique. The high number of other burrowing infauna at this site may have also introduced too much variability into the habitat, reducing the ability to create strong relationships between *N. californiensis* abundance and burrow openings (Butler and Bird 2007; McPhee and Skilleter 2002).

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

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

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

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

Efficacy of Counting Burrow Openings as Organismal Abundance Proxies

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

Conclusion

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

1. Timing of sampling appears to be relevant to macrofaunal counts and future research should be directed at elucidating temporal variation in relationships between burrow openings and invertebrate abundance.
2. At high macrofaunal diverse sites, complex interactions exist and therefore burrow opening counts may be more appropriate for predicting total macrofaunal population abundance.
3. Regardless of species found, relationships between burrow openings counts and macrofaunal abundance must be empirically tested in the system of interest.

Although designing a sampling protocol requires the above considerations, burrow opening counts can be powerful tools for ecosystem monitoring. Monitoring population abundance through burrow opening counts has the ability to detect overall changes in abundances, while being less destructive, quicker, and cheaper than traditional excavation methods.

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Tables and Figures

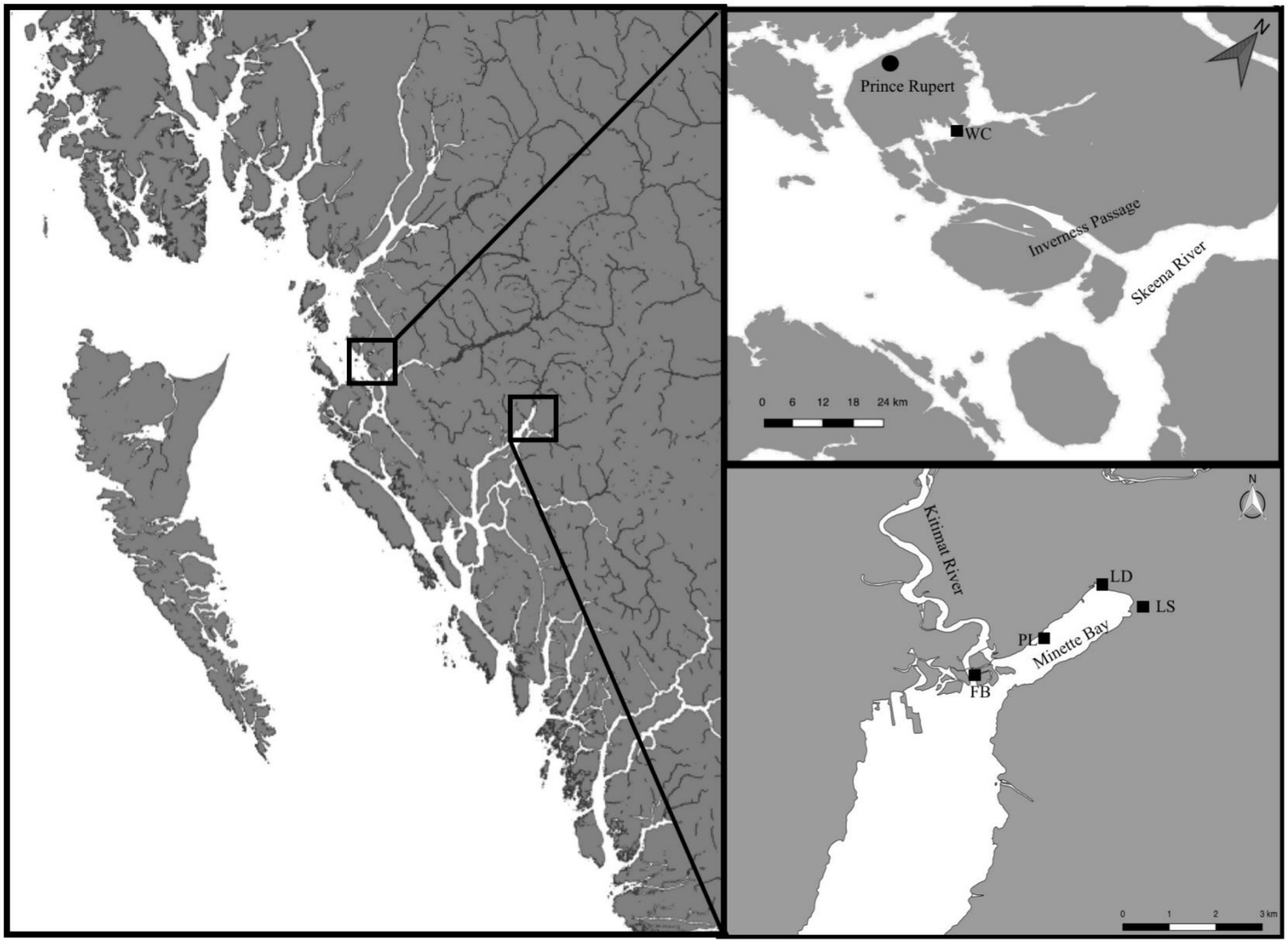

Figure 1. Map of intertidal mudflats sampled during summer 2017 near Kitimat and Prince Rupert, British Columbia, Canada. WC: Wolfe Cove, LS: Log Sort, LD: Log Dump, and FB: Foxy Beach. Mudflat near Prince Rupert in the Skeena River Estuary (WC: Wolfe Cove 54.242433, -130.273033) had high macrofaunal 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).

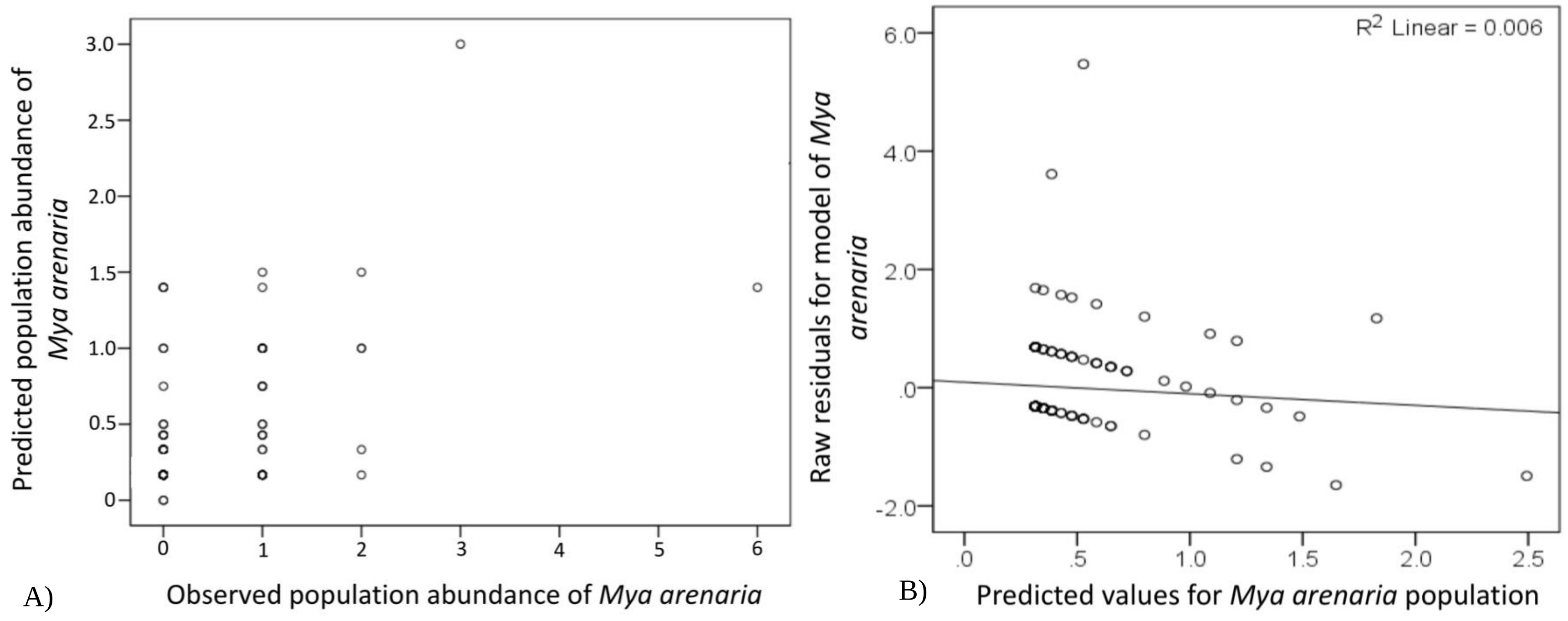

Figure 2: Model output for general linearized model of *Mya arenaria*. A) The relationship between predicted and observed population counts of *M. arenaria* at Kitimat, BC. Predicted values are based on burrow counts. B) The relationship between model residuals and model predicted values for the linear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

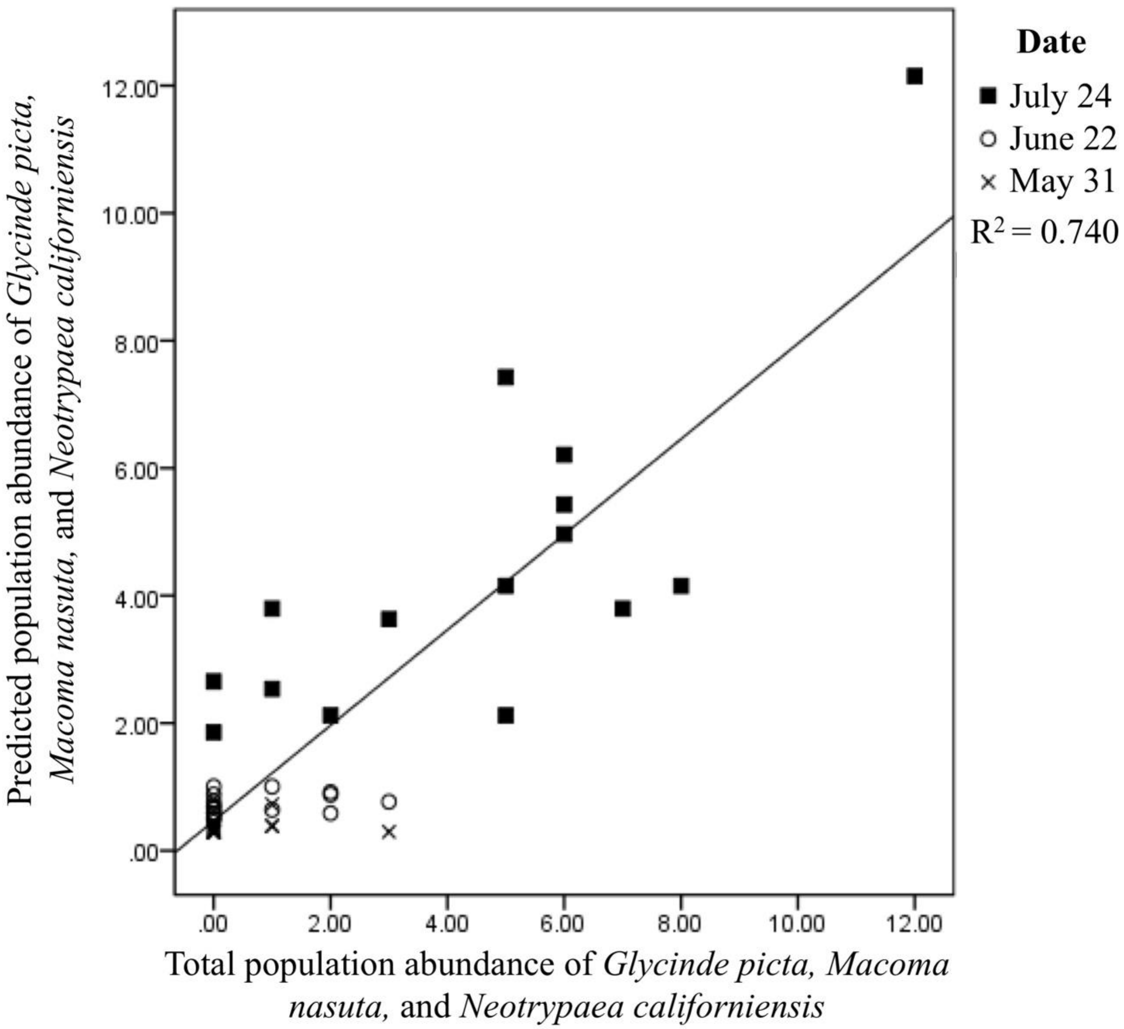

Figure 3. Observed values of *Glycinde picta, Macoma nasuta* and *Neotrypaea californiensis* versus predicted values from other burrow openings at Wolfe Cove. Invertebrate populations were counted by excavating and collecting all specimens from a 1 m^2 pit to a depth of 20 cm, while burrow openings were counted visually on the surface during low tide in the summer of 2017.

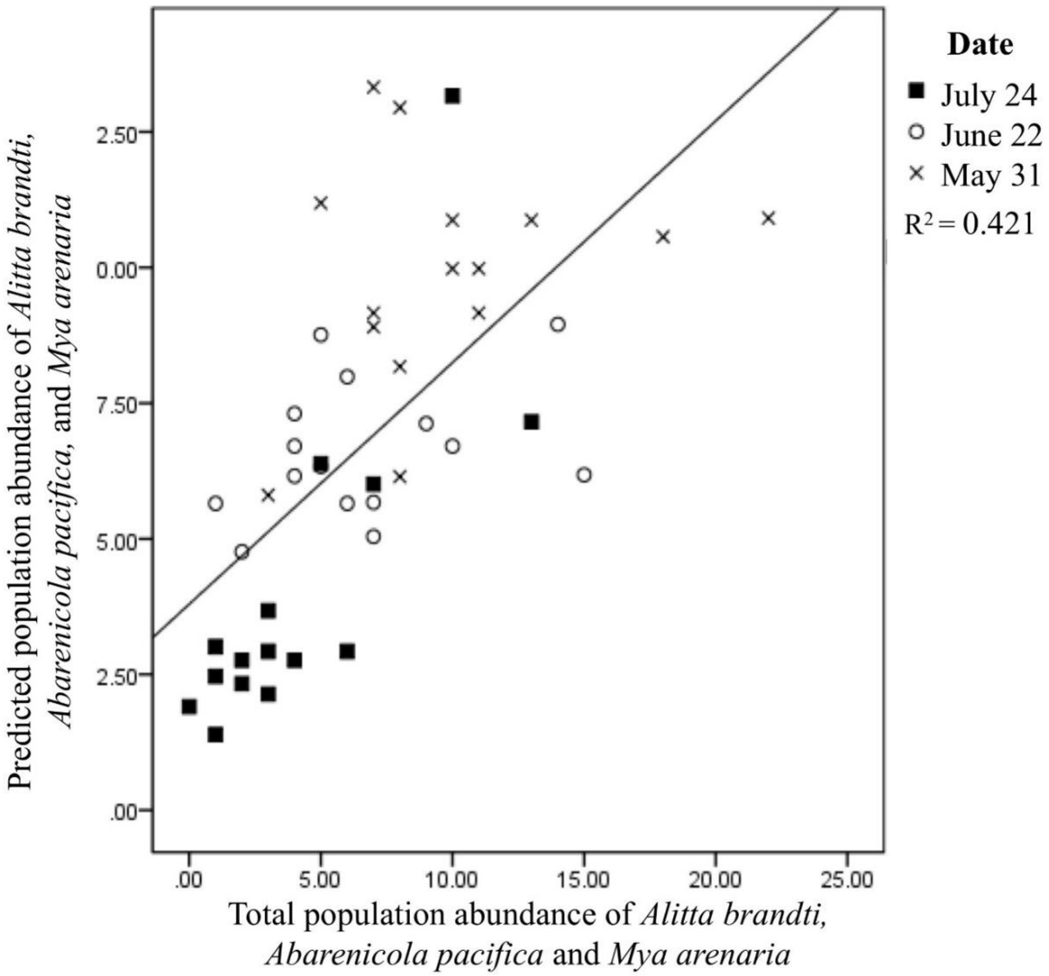

Figure 4. Observed values of *Alitta brandti*, *Abarenicola pacifica*, and *Mya arenaria* populations versus predicted values using lugworm burrows and other burrow openings as predictors at Wolfe Cove. Invertebrate populations were counted by excavating and collecting all specimens from a 1 m^2 pit to a depth of 20 cm, while burrow openings were counted visually on the surface during low tide in the summer of 2017.

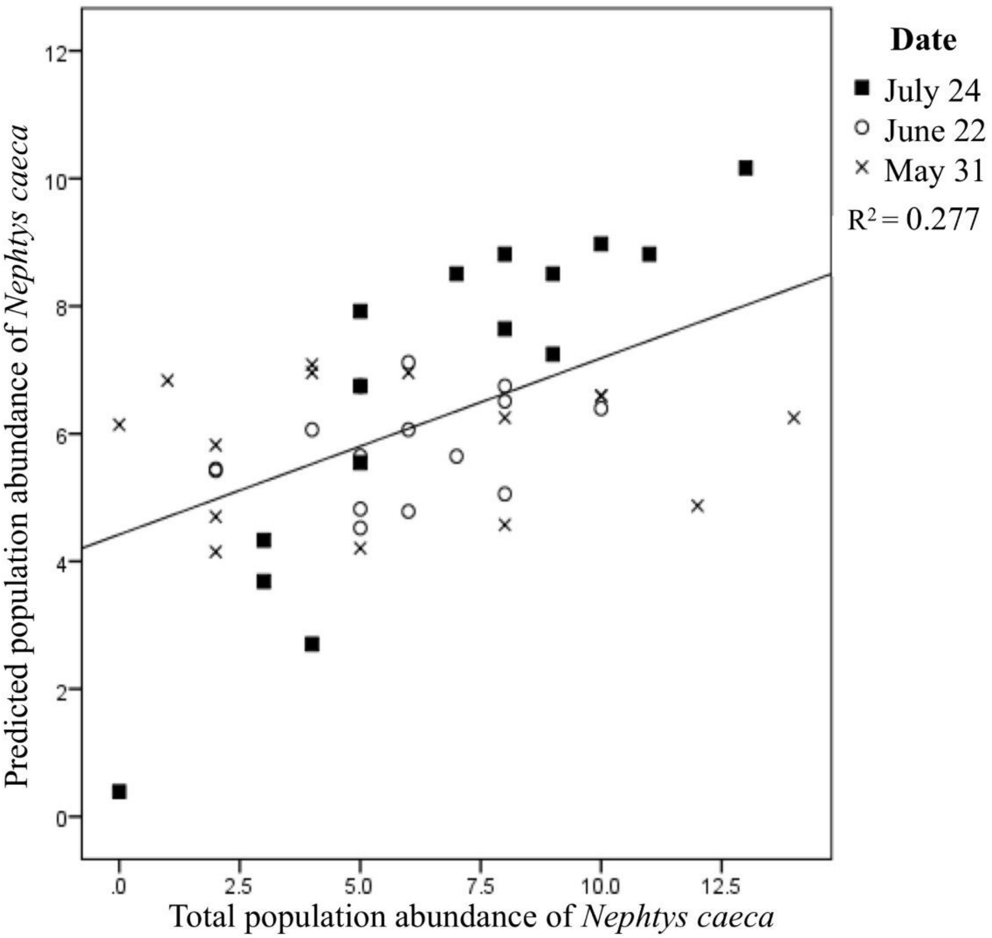

Figure 5. Observed values of the *Nephtys caeca* population versus predicted abundance using lugworm burrows at Wolfe Cove. *N. caeca* individuals were counted by excavating and collecting all specimens from a 1 m^2 pit to a depth of 20 cm, while burrow openings were counted visually on the surface during 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, α = 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