



Using molluscs to assess ecological quality status of soft-bottom habitats along the Atlantic coastline of the United States

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ABSTRACT

AMBI and M-AMBI are widely used biotic indices for assessing the ecological quality status of benthic macroinvertebrate communities in estuarine and coastal soft-bottom habitats. Identifying the species needed for estimating these indices, however, is both expensive and time-consuming, and requires a high degree of taxonomic expertise. The use of proxy taxa as a means of subsampling the target community may save time, resources, and the breadth of taxonomic expertise needed. Our study used macroinvertebrate benthic survey data from the Atlantic Coast of the United States to test the fidelity of molluscs as proxies of the whole community. We calculated the AMBI and M-AMBI scores for both the molluscan and whole communities and then adjusted the molluscan-only index scores to that of the whole community using the linear relationship between the two communities within a Bayesian framework. We found that the mollusc-only AMBI approach underperformed at classifying the ecological quality of the whole community, particularly regarding sample sites classified as needing remediation. The low performance of the mollusc-only AMBI approach is likely due to the dearth of molluscs with high environmental stress tolerances. In contrast, the mollusc-only M-AMBI outperformed AMBI at classifying ecological quality. The M-AMBI linear model correctly classified nearly all of the adjusted mollusc-only sample sites needing remediation. The increased efficacy of mollusc-only M-AMBI may be due to the incorporation of species richness and diversity into the index, as both metrics were highly correlated between the molluscan and whole communities. Mollusc-only M-AMBI did have some drawbacks, however, with fidelity decreasing as ecological quality decreased. Overall, our study highlights the potential utility of a mollusc-only approach for assessing the ecological quality of estuarine and coastal soft-bottom habitats.

1. Introduction

The need to translate data on benthic macroinvertebrate community composition into easily understandable scores detailing ecological quality status (EcoQS) of marine soft-bottom habitats led to the development of biotic indices (Borja et al., 2000, 2015; Diaz et al., 2004). One of the most commonly used biotic indices worldwide is the AZTI Marine Biotic Index (AMBI; Borja et al., 2019), which was originally developed in response to the European Union's Water Framework Directive (WFD; European Commission, 2000) to identify coastal and estuarine sites in need of restoration based on their EcoQS (Borja et al., 2000). In AMBI, benthic macroinvertebrate species are assigned to ecological groups (EG) of increasing tolerance to stressors (e.g., eutrophication) and an

ecological quality score is derived from the weighted abundance of each group. These scores correspond to five EcoQS classes—'High', 'Good', 'Moderate', 'Poor', and 'Bad' (Vincent et al., 2002)—that determine whether restoration action is needed. From an environmental management perspective, the boundary between 'Good' and 'Moderate' separates water bodies that require remediation from those that do not (Vincent et al., 2002; Borja et al., 2004).

The relative abundance of stress tolerant and stress sensitive taxa in a community is not, however, the only aspect of ecological quality listed under the WFD, as the directive also includes measures of community structure such as species diversity and richness (Hatton-Ellis, 2008; European Commission, 2000). To more thoroughly address the guidelines set out by the WFD, a multi-metric AMBI (M-AMBI) was developed

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by combining the AMBI scores with Shannon-Wiener diversity and species richness (Muxika et al., 2007). Unlike AMBI, M-AMBI also incorporates reference conditions to set sound targets corresponding to undisturbed ecological conditions (Gibson et al., 2000; Heiskanen et al., 2004; Borja et al., 2012). The reference conditions may be established using historical records, models, expert judgement, a site with the same habitat that represents an undisturbed condition, or by using the highest observed diversity and richness and lowest AMBI scores among the samples analyzed (Borja et al., 2008, 2012, 2019).

Identifying species for EG classification for AMBI or M-AMBI analyses can be labor intensive and requires a high degree of taxonomic expertise and familiarity of the local fauna (Ferraro and Cole, 1990; Mendes et al., 2007). The use of proxy taxa as a means of subsampling the target community may save time, resources, and the breadth of taxonomic expertise needed (e.g., Foram-AMBI, Alve et al., 2016; and the Benthic Opportunistic Polychaetes and Amphipods Index, Dauvin and Ruellet, 2007).

Because molluscs are readily identifiable, one of the dominant phyla within the shallow marine benthos, and sensitive to environmental stressors (Bresler et al., 2003; Butler et al., 2010; Dimitriou et al., 2015; Koulouri et al., 2006; Marshall et al., 2018; Moraitis et al., 2018; Smith, 2005; Stergiou et al., 1997; Zenetos, 1996), they are good proxy candidates of the whole benthic macroinvertebrate community for biotic indices such as AMBI and M-AMBI (e.g., Nerlović et al., 2011, 2012; Leshno et al., 2015; Dietl et al., 2016; Tweitmann and Dietl, 2018). Molluscs also have a high preservational potential, which allows for the accumulation of their remains within the top layer of the sediment (e.g., Kidwell, 2001, 2007). In the absence of long-term monitoring records of the living assemblage, such “death assemblages” provide an opportunity to assess temporal variability in the EcoQS of a site over decadal to centennial timescales. For example, Leshno et al. (2016) applied mollusc-only AMBI to both living and death assemblages to assess the impact of eutrophication on the EcoQS of the Levantine Basin in Israel. While Leshno et al. (2016) found that mollusc-only AMBI scores could be used to track changes in the EcoQS of the basin, they assumed that the mollusc-only AMBI scores were representative of the whole benthic macroinvertebrate community.

To test the fidelity of mollusc-only scores to the whole benthic macroinvertebrate community, Dietl et al. (2016) performed a meta-analysis on 12 European benthic macroinvertebrate community studies and compared mollusc-only index scores to those obtained from the whole community. Their results showed a strong positive correlation between mollusc-only AMBI scores to those from the whole community. After applying a correction based on the correlation between the mollusc-only and whole-community data, they showed that mollusc-only scores correctly identified 78% of the expected whole-community ecological quality statuses (Dietl et al., 2016).

It is not known, however, whether the fidelity of these results hold for other regions of the world because 1) differences in the performance of the biotic indices may be influenced by local stressors (Van Hoey et al., 2010) and 2) the relationship between mollusc-only and whole-community scores may differ across regions due to differences in species composition and geographic variation in species responses to stressors (Gillett et al., 2015). Additionally, Dietl et al. (2016) did not assess the fidelity of a mollusc-only M-AMBI index. Mollusc-only M-AMBI may prove to be a more accurate estimate of EcoQS than AMBI because of its inclusion of species richness and diversity, which improves differentiation of sites with low abundance and few taxa (Borja et al., 2003; Muxika et al., 2007). In this study, we evaluate whether molluscs can reliably be used as proxies for the whole community for both AMBI and M-AMBI along the Atlantic coastline of the United States.

2. Materials and methods

2.1. Dataset

To evaluate the fidelity of mollusc-only AMBI and M-AMBI to the whole benthic macroinvertebrate community, we accessed data from the United States National Centers for Coastal Ocean Science (NCCOS) long-term monitoring database's National Benthic Inventory (NBI) (NCEI Accession 0202842; Balthis et al., 2019). The NBI comprises benthic abundance data from 2713 sample sites collected by NOAA and partnering institutions along the Atlantic coast of the United States since 1991 (see Balthis et al., 2019 for collection methods). We selected only samples collected using a 0.04 square meter Young-modified Van Veen grab sampler, resulting in 2661 eligible sample sites. To differentiate molluscan and non-molluscan taxa, as well as to update the species-level taxonomic determinations, if needed, we uploaded the taxonomic list for all 2661 sample sites into the World Register of Marine Species (WoRMS Editorial Board, 2021; Supplemental Material 1). Following the guidelines outlined in Borja and Muxika (2005), two options of selecting sample sites to estimate EcoQS are available. They recommended that sample sites should either contain at least three species or at least three individuals. When using sample sites with less than three species but at least three individuals, there is the potential for EcoQS misclassification by AMBI that would require expert judgment to rectify (Borja, Personal Communication; Appendix A). Hence, of the 2661 sites, we selected for analysis the 710 sample sites—ranging from New York to Louisiana—that had at least three molluscan species and three replicate samples (Supplemental Material 2; see Appendix A for results using samples with at least three molluscan individuals).

2.2. Indices

Index scores were calculated using the square-root transformed (Warwick et al., 2010; Tweedley et al., 2014; Appendix A) abundance data for each sample site using the AMBI Index Software v6.0 (Borja et al., 2012; <http://ambi.azti.es>; Species List v.Dec2020). AMBI is calculated by assigning species to five ecological groups indicating an increasing tolerance to environmental stressors (e.g., eutrophication): EGI = sensitive; EGII = indifferent; EGIII = tolerant; EGIV and EGV = second and first order opportunists, respectively (Borja et al., 2000, 2004). If a species was not included in the software's EG classification list, we assigned its EG as either: 1) the EG of the genus; 2) the EG of the majority (>50%) of its congeneric species; or 3) unassigned and therefore not included in the analysis (see Supplemental Material 3). The AMBI scores were then calculated using the equation:

$$AMBI = \frac{[(0\%EGI) + (1.5\%EGII) + (3\%EGIII) + (4.5\%EGIV) + (6\%EGV)]}{100}$$

AMBI scores range from zero to seven and correspond to five EcoQS classifications under the European Water Framework Directive ('High', $0 < AMBI < 1.2$; 'Good', $1.2 < AMBI < 3.3$; 'Moderate', $3.3 < AMBI < 4.3$; 'Poor', $4.3 < AMBI < 5.5$; 'Bad', $5.5 < AMBI < 7$; Borja et al., 2004).

M-AMBI combines three indices: AMBI; Shannon-Wiener diversity (H'); and species richness (S) (Muxika et al., 2007). Each index is standardised by subtracting its mean and dividing by its standard deviation. Then, the index scores are combined via a factor analysis and the scores placed along an orthogonal gradient from user-defined reference ('High') to highly degraded ('Bad') conditions (Pelletier et al., 2018; Sigovini et al., 2013; Gillet et al., 2019). The resulting position within Euclidean space is the M-AMBI score. M-AMBI scores range from zero to one, corresponding to the WFD EcoQS classifications

(‘High’, $0.77 < \text{M-AMBI} < 1$; ‘Good’, $0.53 < \text{M-AMBI} < 0.77$; ‘Moderate’, $0.39 < \text{M-AMBI} < 0.53$; ‘Poor’, $0.20 < \text{M-AMBI} < 0.39$; ‘Bad’, $0 < \text{M-AMBI} < 0.20$). Because the goal of our study was to assess the fidelity of a mollusc-only approach, and not to determine the ‘true’ EcoQS of the sample sites, we used the software’s default reference conditions, i.e., the highest observed diversity and richness and lowest AMBI scores across all the sample sites to set the reference conditions for M-AMBI (Borja et al., 2008, 2012, 2019; Appendix A). Note, the EcoQS and index scores are opposite for AMBI and M-AMBI, with AMBI inversely correlated and M-AMBI positively correlated with ecological quality.

2.3. Determining the relationship

To determine the relationship between the mollusc-only AMBI and M-AMBI scores, the 710 sample sites were ordered by the whole-community index scores from largest to smallest and alternately assigned to either group I or group II, ensuring an even distribution of index scores (355 sample sites in group I, and 355 in group II; Dietl et al., 2016). Second, a simple linear regression was applied to group I between the mollusc-only and whole-community index scores using the *lm* function in R (R Core Team, 2020):

$$y_i = \alpha_{\bar{x}} + \beta(x_i - \bar{x}) + \varepsilon_i, \quad \varepsilon_i \sim N(0, \sigma^2)$$

wherein y_i is the estimated whole-community index score, $\alpha_{\bar{x}}$ is the intercept, x_i is the mollusc-only index score, \bar{x} is the mean mollusc-only index score, β is the slope of the regression, and ε_i is the observation error. We evaluated the effects of three priors on parameter estimation with the knowledge that the slope must be greater than zero: one that straddled zero (normal), one that was positive and fat-tailed (log-normal), and one that was positive and had an upper bound (uniform). We found that prior choice did not affect estimates for the intercept, slope, or variance (i.e., values were essentially identical). Accordingly, we applied the uniform prior, which was the simplest option (Supplemental Material 4). Bayesian methods were used to assess the model fit, which allowed us to calculate the posterior distributions of the slope and intercept (Korner-Nievergelt et al., 2015). In addition, we performed a posterior predictive check on group I to account for uncertainty more fully in the model fit as well as the variation in the data (Gabry and Mahr, 2021). A regression coefficient of one indicates a perfect match between the mollusc-only and whole-community scores. If the slope is not one, then the mollusc-only community is either over-estimating or under-estimating the whole-community scores. Next, the regression equation created using the data in group I was applied to the mollusc-only scores in data group II to calculate the estimated whole-community index scores and corresponding standard error.

To determine the performance of the linear model in estimating the

whole-community index, we used the *sim* function in R (arm package; Gelman et al., 2020) to simulate 2000 draws from the joint posterior distribution of the intercept and slope. We then regressed the mollusc-only scores in data group II against the 2000 simulated regression equations, creating 2000 expected whole-community scores for each sample site in data group II. The EcoQS assignment for each sample site in group II was based on both the expected and observed whole-community index scores.

2.4. Adjusting the EcoQS boundaries

Previous studies have suggested adjusting the EcoQS boundaries when transforming the abundance data because the transformation shortens the AMBI scale (i.e., reduces the difference between the ‘High’ and ‘Bad’ sites) (Warwick et al., 2010). We used the model fit from the posterior predictive checks to decide whether to adjust the EcoQS boundaries for both data group I and group II using the polynomial relationship between the untransformed and transformed index scores (sensu Warwick et al., 2010). If the linear model fit was good (i.e., the simulated data under the fitted model had the same density distribution as the observed data), then we adjusted the EcoQS boundaries. If the model fit was poor, underlying assumptions about the distribution of the data (e.g., normally distributed) may not have been valid, and therefore we did not adjust the EcoQS boundaries as it may decrease the accuracy of the model’s EcoQS classifications.

2.5. Evaluating fidelity

We calculated confusion matrices (Townsend, 1971; Goutte and Gaussier, 2005) for each of the EcoQS classifications in all 2000 simulations to visualize the fidelity and uncertainty of the estimated whole-community index scores. Additionally, the precision, recall, and F_1 scores were calculated for each EcoQS (Fig. 1). Precision is the fraction of sites correctly classified as a specific EcoQS (e.g., ‘High’) among all the sites given that classification. In other words, precision is the probability that a ‘High’ site classified by the model actually belongs in the ‘High’ EcoQS classification. Recall, also known as sensitivity, is the fraction of sites within a specific EcoQS that were correctly recovered (i.e., the probability that a ‘High’ site was correctly recovered as ‘High’ by the model). An F_1 score is a measurement of accuracy that accounts for both precision and recall (i.e., F_1 is the harmonic mean of precision and recall), and thus provides more information on the classification power of the linear model than sensitivity (Goutte and Gaussier, 2005). Lastly, we combined the 2000 confusion matrices, precision, recall, and F_1 scores of the EcoQS and calculated the average and standard deviations. Importance was placed on the linear model’s ability to capture the

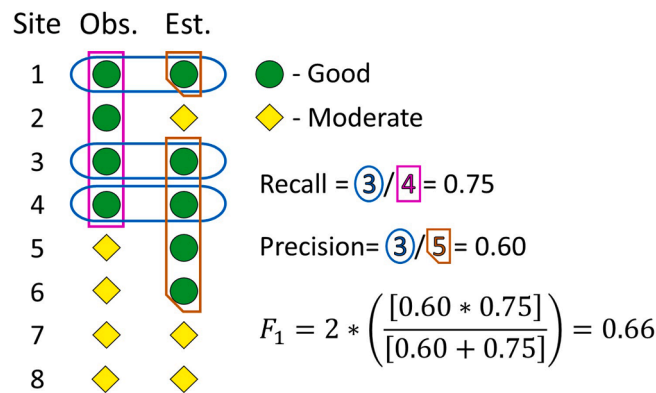


Fig. 1. Hypothetical fidelity calculations for sample sites classified as ‘Good’ EcoQS. Recall = the fraction of observed (Obs.) whole-community sites recovered as ‘Good’ by the adjusted mollusc-only model; Precision = the fraction of estimated (Est.) whole-community sites correctly classified as ‘Good’; $F_1 = 2 * ([\text{Precision} * \text{Recall}] / [\text{Precision} + \text{Recall}])$.

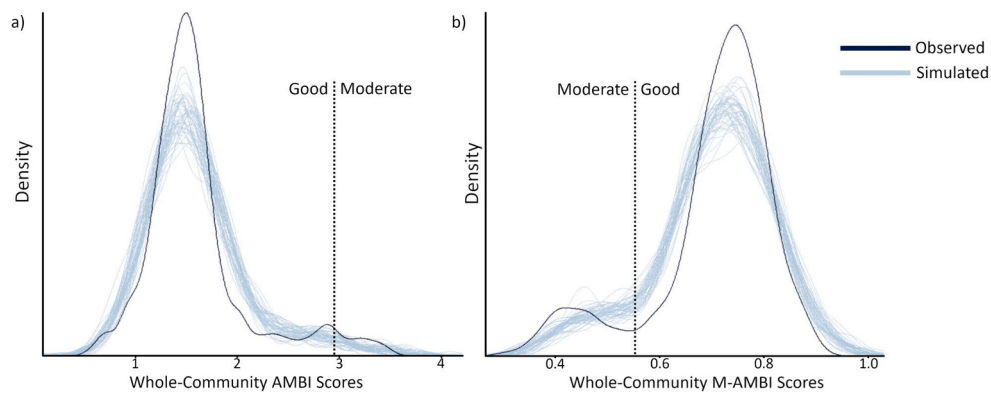


Fig. 2. The posterior predictive checks for both AMBI (a) and M-AMBI (b) using square-root transformed abundance data. The black line represents the density distribution of the observed whole-community scores from group I, the grey lines represent the simulated whole-community scores under the model fit, and the dotted vertical lines represent the Good-Moderate remediation boundary.

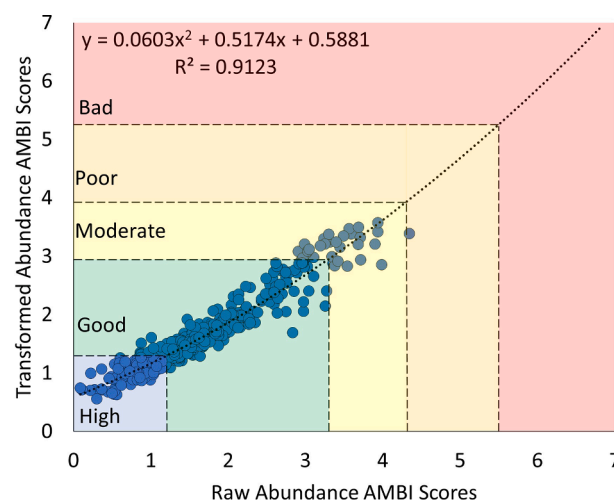


Fig. 3. The polynomial relationship between the raw abundance AMBI scores and the square-root transformed abundance AMBI scores, with the dashed lines showing their respective EcoQS boundaries.

boundary between ‘Good’ and ‘Moderate’, which separates sites that require remediation from those that do not, with ‘Moderate,’ ‘Poor,’ and ‘Bad’ sites requiring remediation (Vincent et al., 2002).

3. Results

3.1. EcoQS boundaries

The AMBI posterior predictive check of the square-root transformed data indicated a good model fit, with the density distribution of the simulated whole-community AMBI scores matching the distribution of the observed scores (Fig. 2a). Therefore, we adjusted the AMBI EcoQS boundaries in subsequent analyses to: ‘High’, $0 < \text{AMBI} < 1.30$; ‘Good’, $1.30 < \text{AMBI} < 2.95$; ‘Moderate’, $2.95 < \text{AMBI} < 3.93$; ‘Poor’, $3.93 < \text{AMBI} < 5.26$; ‘Bad’, $5.26 < \text{AMBI} < 7$ (Fig. 3). The M-AMBI posterior predictive check indicated that the density distribution of the simulated whole-community M-AMBI scores did not match the observed scores, with the simulated scores over-estimating the observed scores for the degraded sites (i.e., M-AMBI scores < 0.53 ; Fig. 2b). Therefore, we did not adjust the M-AMBI EcoQS boundaries in subsequent analyses (see Table A3).

3.2. AMBI

The whole-community AMBI scores were positively correlated ($R^2 =$

0.649 ± 0.002) with the mollusc-only scores in group I (Fig. 4a). The estimated slope, however, was not one ($\beta = 0.561 \pm 0.022$), meaning mollusc-only AMBI scores both underestimated and overestimated the whole-community scores (overestimated < 2.6 AMBI $>$ underestimated).

The linear model correctly recovered 91% of ‘Good’ whole-community sites from group II (recall = 0.906 ± 0.030 ; $F_1 = 0.825 \pm 0.010$). Furthermore, 76% of the ‘Good’ estimated whole-community sites assigned by the linear model did belong in the ‘Good’ classification (precision = 0.759 ± 0.007). More than half of the ‘High’ and ‘Moderate’ sites ($F_1 = 0.258 \pm 0.062$ and 0.115 ± 0.033 , respectively), however, were incorrectly classified by the linear model as belonging to another EcoQS classification. In addition, although the linear model accurately classified nearly 100% of sites that would not require remediation, it only classified 21% of the sites that would have required action (precision = 0.963 ± 0.001 and 0.213 ± 0.041 , respectively). The linear model was unable to recover $\sim 92\%$ of sites that would have required remediation (recall = 0.079 ± 0.027 ; Table 1, Fig. 4b).

3.3. M-AMBI

The whole-community M-AMBI scores had a strong positive correlation ($R^2 = 0.841 \pm 0.001$) with the mollusc-only scores in group I (Fig. 5a). The estimated slope was not one ($\beta = 0.814 \pm 0.019$), again indicating that the mollusc-only M-AMBI scores overestimated the

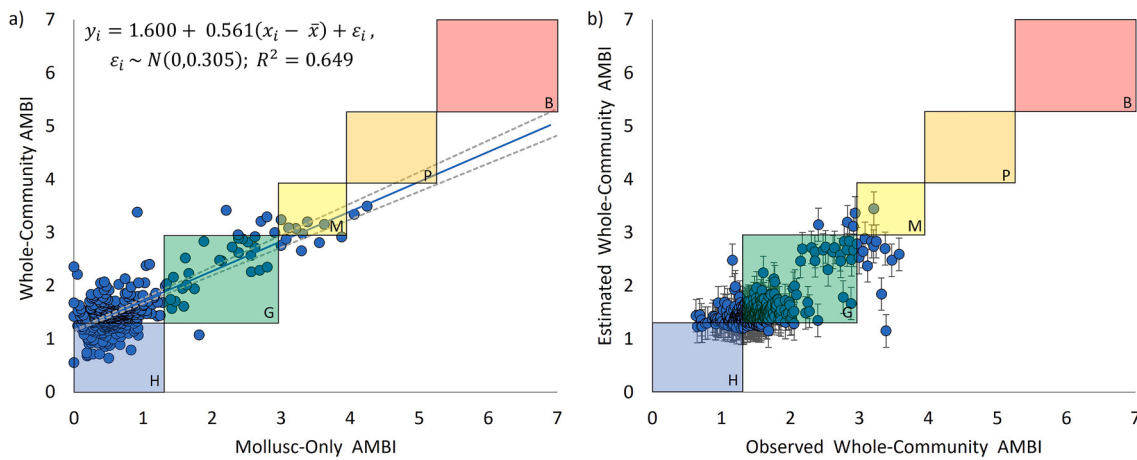


Fig. 4. a) Linear regression between the whole-community and mollusc-only AMBI scores. The dashed lines represent the 95% confidence region of the linear regression. b) Comparison between the estimated whole-community AMBI and the observed whole-community AMBI scores. The error bars represent one standard deviation. The boxes indicate the EcoQS classification; blue = ‘High’ (H), green = ‘Good’ (G), yellow = ‘Moderate’ (M), orange = ‘Poor’ (P), red = ‘Bad’ (B).

whole-community scores, with the overestimation becoming more pronounced the lower the M-AMBI score.

The linear model correctly classified the majority of ‘High’, ‘Good’, and ‘Moderate’ whole-community sites from group II ($F_1 = 0.803 \pm 0.010$, 0.887 ± 0.003 , and 0.809 ± 0.022 , respectively). The estimated whole-community ‘Poor’ sites were correctly classified for 93% of sites (precision = 0.926 ± 0.136), but the model was unable to recover the

Table 1
Fidelity metric scores for both AMBI and M-AMBI.

AMBI	Precision	Recall	F ₁ Score
High	0.414 ± 0.034	0.194 ± 0.063	0.258 ± 0.062
Good	0.759 ± 0.007	0.906 ± 0.030	0.825 ± 0.010
Moderate	0.213 ± 0.041	0.079 ± 0.027	0.115 ± 0.033
Poor	NA	NA	NA
No Remediation	0.963 ± 0.001	0.988 ± 0.001	0.976 ± 0.000
Remediation	0.213 ± 0.041	0.079 ± 0.027	0.115 ± 0.033
M-AMBI	Precision	Recall	F ₁ Score
High	0.798 ± 0.007	0.808 ± 0.022	0.803 ± 0.010
Good	0.873 ± 0.008	0.903 ± 0.007	0.887 ± 0.003
Moderate	0.884 ± 0.018	0.746 ± 0.020	0.809 ± 0.019
Poor	0.926 ± 0.136	0.427 ± 0.031	0.580 ± 0.039
No Remediation	0.973 ± 0.001	1.000 ± 0.001	0.986 ± 0.001
Remediation	0.998 ± 0.007	0.778 ± 0.012	0.875 ± 0.006

majority of whole-community ‘Poor’ sites (recall = 0.427 ± 0.031). The estimated whole-community M-AMBI also performed well with regards to the remediation boundary by correctly recovering 100% of the sites that would not require remediation (recall = 1.000 ± 0.001) and 78% of the sites that would require remediation (recall = 0.778 ± 0.012). Furthermore, nearly 100% of the sites classified as needing remediation would have required action (precision = 0.998 ± 0.007) (Table 1, Fig. 5b).

4. Discussion

Our results demonstrate that estimated-whole community AMBI scores derived from the adjusted mollusc-only data only performed optimally at recovering the observed whole-community EcoQS classifications for the ‘Good’ sites (Table 1; Fig. 4). In contrast, the M-AMBI linear model performed exceptionally well at recovering nearly all of the EcoQS classifications of the observed whole-community sample sites, except for the ‘Poor’ sites (Table 1; Fig. 5). M-AMBI also outperformed AMBI with respect to correctly classifying the sites on both sides of the Good-Moderate remediation boundary (Table 1), highlighting the potential utility of a mollusc-only M-AMBI model for EcoQS classification of coastal and estuarine soft-bottom habitats along the Atlantic coastline of the United States. Below we discuss the advantages and limitations of a mollusc-only approach and explore how it may help set site-specific

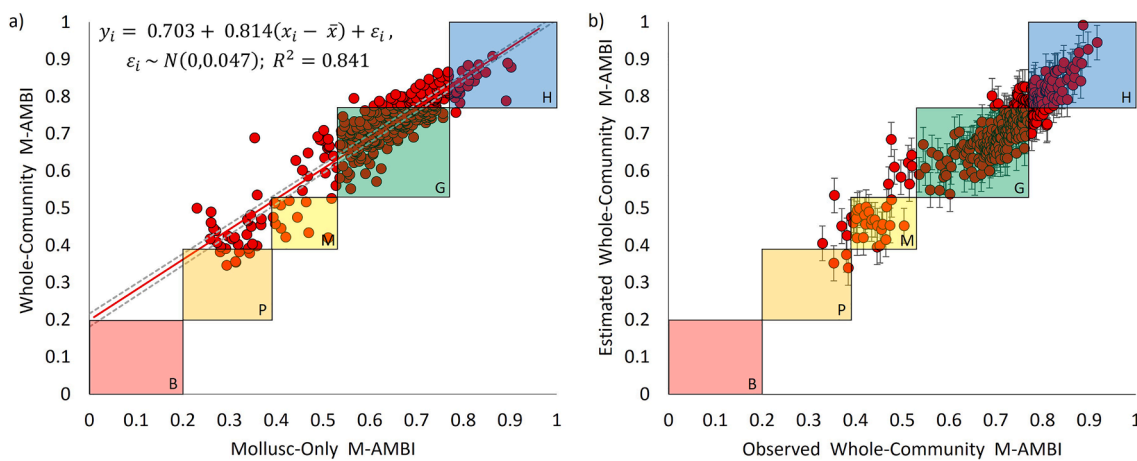


Fig. 5. a) Linear regression between the whole-community and mollusc-only M-AMBI scores. The dashed lines represent the 95% confidence region of the linear regression. b) Comparison between the estimated whole-community M-AMBI and the observed whole-community M-AMBI scores. The error bars represent one standard deviation. The boxes indicate the EcoQS classification; blue = ‘High’ (H), green = ‘Good’ (G), yellow = ‘Moderate’ (M), orange = ‘Poor’ (P), red = ‘Bad’ (B).

geohistorical baselines.

4.1. AMBI

Our mollusc-only AMBI linear model appears to have recovered a lower percentage of the observed whole-community sites than the model in Dietl et al. (2016) for their European dataset. However, the difference in performance likely can be explained by the different methodologies used for assessing fidelity. Dietl et al. (2016) focused on model sensitivity (recall) and whether the model over- or under-estimated the ecological quality across all the EcoQS classifications. They were able to correctly recover the EG classification of 78% of the observed whole-community sites, with 13% over-estimating the EcoQS of the sample sites, and 9% being under-estimated. In contrast, our study assessed the classification power for each EcoQS separately, and the Bayesian approach we used provided additional opportunity to assign credibility to our estimates.

To compare the fidelity between our study and Dietl et al. (2016), we re-calculated the AMBI scores from the European dataset they used and evaluated the fidelity of the mollusc-only AMBI linear model using the methods outlined in this study (Appendix B). For the European dataset, the mollusc-only AMBI linear model was able to correctly classify and recover the majority of the 'Good' sites (precision = 0.792 ± 0.014 , recall = 0.843 ± 0.093), but was unable to classify or recover any of the 'Moderate' and 'Poor' sites (precision = 0.000 ± 0.000 , recall = 0.000 ± 0.000 for both EcoQS classifications). When assessing the remediation boundary, the linear model was able to recover 92% of the sites that would not require remediation action, but only 3% of sites that would require remediation action (recall = 0.918 ± 0.058 and 0.028 ± 0.071 , respectively), which is consistent with results observed in our study (99% and 8% for no remediation and remediation, respectively; Table 1). Therefore, our results are consistent to those discussed in Dietl et al., (2016), with mollusc-only AMBI underperforming with respect to classifying degraded sample sites.

A primary drawback of a mollusc-only AMBI approach may be the unequal distribution of molluscan species in the AMBI EGs (Dietl et al., 2016). The EG of a species is determined by its tolerance to increasing nutrients levels (Grall and Glémarec, 1997). Molluscs, in general, are highly sensitive to elevated nutrient levels, which leads to a right-skewed EG classification distribution with only 9% of all molluscan species in the AMBI software falling in the 3rd–5th EG (Appendix C). EGIII includes species that are tolerant to elevated nutrients, with their population growth stimulated by increased organic matter. EGIV and EGV are second- and first-order opportunistic species, respectively, which are often small-sized, short-lived, and well-adapted to life in oxygen-reduced sediments (Grall and Glémarec, 1997). Several nutrient tolerant species are evident in our dataset such as the blue mussel *Mytilus edulis* (EGIII), the dwarf surf clam *Mulinia lateralis* (EGIV), and the transverse ark clam *Anadara transversa* (EGIV). Yet, roughly 90% of the species in our dataset belong to EGI and EGII. Species in these EGs are either sensitive to elevated nutrient levels (with growth and reproduction stunted by increased organic matter; EG I) or indifferent to nutrient enrichment and generally found in low densities (EG II; Grall and Glémarec, 1997). The dearth of molluscs in the tolerant EGs biases mollusc-only AMBI scores to the lower score ranges (corresponding to 'High' and 'Good' EcoQS).

4.2. M-AMBI

The mollusc-only M-AMBI approach outperformed AMBI, particularly with classifying the degraded sites (Table 1). M-AMBI lessened the EG bias of molluscs by incorporating estimates of species diversity and richness, which are the two most important parameters in the calculation of M-AMBI (Muxika et al., 2007; Sigovini et al., 2013). In other words, even if the AMBI score does not differ between two sample sites, small changes in species richness and diversity can distinguish them. We

found a strong correlation in species diversity and richness between the molluscan and the whole community, which, given the parameters' importance in the calculation of M-AMBI, likely contributed to the observed high fidelity of the mollusc-only M-AMBI scores (Appendix D).

Even though mollusc-only M-AMBI performed well at estimating the EcoQS of the whole-community, decreasing fidelity was evident in the more degraded sites (Fig. 5). Three potential hypotheses may help explain this pattern. First, the decreased fidelity may be a result of the assumption of normality in the linear model we used. The whole-community scores appear to follow a bimodal distribution, which was smoothed out by the linear model, causing the model to over-estimate the ecological quality of the degraded sites (Fig. 2). Second, the decreased fidelity may be due to the observed EG bias, wherein the dearth of molluscs in the nutrient-tolerant ecological groups inhibits the ability of the model to detect the degraded sample sites, causing the mollusc-only M-AMBI scores to overestimate the whole-community scores. Third, the bimodal distribution of the whole-community M-AMBI scores may be due to the dearth of degraded sites in our dataset (11% of whole-community sample sites), which itself may be a constraint of needing at least three molluscan species within each sample site. Of course, the bimodal distribution could also be a combination of all three. Nonetheless, given the strong correlation in species diversity and richness between the molluscan and whole community estimates in our study, molluscs are reasonably good proxies for EcoQS when M-AMBI is used.

4.3. Limitations of a mollusc-only approach

Given that molluscs have an EG bias towards stress sensitivity, our decision to restrict the sample sites to those with at least three molluscan species may have missed the 'Poor' and 'Bad' EcoQS sites (although this bias is potentially lessened when including sites with at least three individuals and less than three species; see Appendix A). Indeed, when we expand the selection of our sites to those containing at least three replicate samples and at least three species regardless of phylum classification (resulting in 920 sample sites from the original 2661, compared to 710 used in this study), there is an increase in the number of 'Poor' and 'Bad' sites classified by M-AMBI (from 13 to 151; Appendix E). From the perspective of an environmental manager, this increase in sites suggests the mollusc-only approach may not be sufficient for distinguishing Poor and Bad sites from one another. On the other hand, if an environmental manager is concerned with simply determining whether resources need to be allocated towards remediation action (i.e., a site is 'Moderate' or worse), then a mollusc-only M-AMBI can provide a cost and time effective tool for assessing EcoQS.

The observed precision scores of the remediation boundaries (Table 1) highlight the effectiveness of a mollusc-only M-AMBI approach. Precision scores represent the credibility of the model's EcoQS assignment; that is, the probability that a site assigned to a particular classification is correct. Our M-AMBI precision scores indicate a nearly 100% probability that a site classified as requiring remediation by the linear model would indeed require remediation, and a 97% probability that a site classified as not requiring remediation would not (Table 1). Thus, our results indicate a mollusc-only M-AMBI model, once adjusted for the whole-community, is a reliable tool for assessing the need for remediation in soft-bottom coastal and estuarine habitats along the Atlantic coastline of the United States.

4.4. Comparison to other proxy approaches

A mollusc-only approach is not the only time- and cost-saving tool available to environmental managers. For instance, the benthic opportunistic polychaetes amphipods index (BOPA) is a proxy-based approach that utilizes a subset of the species available in a benthic sample (Dauvin and Ruellet, 2007). BOPA has been shown to be an appropriate index for assessing the EcoQS of the whole benthic macroinvertebrate community

under normal marine salinity conditions, although its fidelity decreases in transitional (30–0.5 ppt) and freshwater systems (Dauvin and Ruellet, 2007). The index was later adapted to transitional and freshwater systems by adding taxa from the Hirudinea and Oligochaeta, creating the Benthic Opportunistic Annelida Amphipods index (BO2A) (Dauvin and Ruellet, 2009; de-la-Ossa-Carretero and Dauvin, 2010). The EG distribution for the Annelida is normally distributed, encompassing more stress-tolerant ecological groups than molluscs (Appendix C), which may allow BO2A to outperform a mollusc-only approach in distinguishing ‘Poor’ and ‘Bad’ sites.

An alternative to using a taxonomic subset of the available species is to derive the AMBI and M-AMBI values from higher taxonomic levels than species, such as the Family level (Forde et al., 2013; Tweedley et al., 2014; Sánchez-Moyano et al., 2017). Identifying individual specimens to the Family level requires a lower degree of taxonomic familiarity than identifying to the species level. As is the case for BO2A, the Family-level approach has been shown to be an appropriate index for assessing changes in EcoQS temporally and spatially within estuaries and may outperform a mollusc-only approach in distinguishing ‘Poor’ and ‘Bad’ sites due to the retention of stress-tolerant non-molluscan taxa (Forde et al., 2013).

One advantage of a mollusc-only approach over both BO2A and the Family-level approach is its potential to be applied to the geohistorical record (Dietl et al., 2016). Contained within the same benthic samples used for assessing the EcoQS of the living benthos are the shells of dead molluscs. These whole or fragmented remains, which are typically discarded after a sample is processed for its living assemblage, provide an expanded temporal perspective relative to the living component due to the accumulation of dead individuals over time and their persistence in the sediment (Kidwell, 2008). When both the dead and living components are retained, each benthic sample has the potential to provide a view of the EcoQS history of a site.

4.5. Geohistorical mollusc-only M-AMBI

M-AMBI scores are calculated based on the orthogonal distance of sample sites from a user-defined reference condition, which represents an undisturbed or least-disturbed condition (Borja et al., 2012). The three recommended methods for setting reference conditions include: using historical data (hindcasting), using data from pristine or least-disturbed sites, and modelling (Borja et al., 2012). The use of historical data is often advised against due to the difficulty in finding accurate information on benthic community composition with enough temporal resolution to cover the impact of environmental changes (Grémare et al., 1998; Hagberg and Tunberg, 2000; Labruno et al., 2007; Borja et al., 2012). The geohistorical record of molluscs may provide an alternative solution (Dietl et al., 2016; Leshno et al., 2016; Twestmann and Dietl, 2018).

Molluscs possess high preservational potential because of their hard shells, allowing for death assemblages of their remains to accumulate within the top layers of the sediment after they die. Previous studies have demonstrated that unless human activities (e.g., eutrophication) have impacted the communities, death assemblages match their living counterparts in taxonomic similarity and rank-abundance (e.g., Kidwell, 2001, 2007). Therefore, death assemblages provide an opportunity to obtain long-term, site-specific data on molluscan benthic community composition, which can be used to create geohistorical baselines to compare to the living community (e.g., Albano et al., 2016; Casey et al., 2014; Dietl and Smith, 2017; Korpanty and Kelley, 2014; Leshno et al., 2015; Lockwood and Chastant, 2006; Martinelli et al., 2017; Tomašových et al., 2020; Tyler and Kowalewski, 2017; Weber and Zuschin, 2013; Wingard, 2017). In the absence of long-term monitoring of the living assemblage, death assemblages of molluscs could be used by

environmental managers to assess the timing of degradation or duration of time a site was in a degraded state (i.e., ‘Moderate’ or worse). But, before a geohistorical mollusc-only M-AMBI approach can be implemented, future work is needed to examine the effects of potentially biasing factors associated with the formation of death assemblages (e.g., increased richness and evenness due to time-averaging, and different preservation potentials among taxa; Olszewski and Kidwell, 2007; Kidwell and Orr, 2013) on M-AMBI scores (Smith et al., 2020).

5. Conclusions

Identifying species for determining the EcoQS of coastal and estuarine soft-bottom habitats is especially labor intensive and requires a high degree of taxonomic familiarity. The use of proxy taxa as a means of subsampling the target community may save time, resources, and the breadth of taxonomic expertise needed. We assessed the use of molluscs as proxies for the whole macroinvertebrate community for the widely used biotic indices AMBI and M-AMBI. We determined that the mollusc-only AMBI approach, after scores were adjusted to the whole community, underperformed in recovering the EcoQS of the whole community, which may be due to the dearth of molluscs in the stress tolerant EGs. The mollusc-only M-AMBI approach, on the other hand, performed well, especially regarding the Good-Moderate remediation boundary. The increased efficacy of mollusc-only M-AMBI may be due to the incorporation of species richness and diversity into the calculation, with both species richness and diversity being highly correlated between the molluscan- and whole community. When the high preservation potential of molluscs is considered, a mollusc-only M-AMBI approach has the potential to establish site-specific, geohistorical baselines to guide remediation efforts. There are limitations, however, to a mollusc-only approach, with fidelity of the model decreasing as ecological quality decreases, likely due to the dearth stress-tolerant mollusc species. Overall, high fidelity of the mollusc-only M-AMBI to the whole macroinvertebrate community, the dominance of molluscan taxa within the shallow marine benthos, and the reduced taxonomic expertise needed, suggest that molluscs may provide a cost- and time-effective means of assessing EcoQS for coastal and estuarine soft-bottom habitats along the Atlantic coastline of the United States.

CRediT authorship contribution statement

Matthew J. Pruden: Methodology, Writing - original draft, Formal analysis, Investigation, Data curation, Visualization. **Gregory P. Dietl:** Methodology, Supervision, Writing - original draft, Writing - review & editing, Conceptualization. **John C. Handley:** Methodology, Software, Writing - review & editing, Conceptualization. **Jansen A. Smith:** Methodology, Writing - review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Methodological decisions

The purpose of our study was not to determine the ‘true’ AMBI and M-AMBI scores, but rather to assess the fidelity of a mollusc-only approach in general. Therefore, there were methodological decisions made that affected the individual sample site AMBI and M-AMBI scores, but not the overall conclusions drawn from our analyses.

Our first decision was to select stations that contained at least three molluscan species. Borja and Muxika (2005) recommended selecting stations with either at least three species and/or at least three individuals. When there are few individuals and very few species AMBI may misclassify the EcoQS. For example, if there are only three individuals of one species in a sample, and that species belongs to EGI, then AMBI will classify the sample site as ‘High’. A site with low diversity and richness, however, may be indicative of disturbed conditions. In such cases, expert judgement is needed when classifying sites with very few species using AMBI (Borja, Personal Communications). The EcoQS misclassification bias is less problematic for M-AMBI due to the inclusion of diversity and richness in the calculation (Muxika et al., 2007). Because the objective of our study was to test the fidelity of both a mollusc-only AMBI and M-AMBI approach, and we did not have the taxonomic expertise across the entire geographic area studied needed to rectify the EcoQS misclassification by AMBI, we chose to select sites with at least three molluscan species.

When we selected sites with at least three molluscan individuals, the number of sample sites increased from 710 to 767, and the number of mollusc-only sample sites classified as needing remediation (‘Moderate’ or worse) by M-AMBI increased by 61% (Table A1). The number of sites classified as needing remediation by AMBI decreased slightly by 9%, with just two sites, which were already straddling the boundary between ‘Moderate’ and ‘Good’, shifting between EcoQS classifications (Table A1). The fidelity of our mollusc-only M-AMBI also increased ($F_1 = 0.915 \pm 0.001$ for sites that require remediation when using at least three molluscan individuals (Table A2) versus $F_1 = 0.875 \pm 0.006$ for sites that require remediation when using at least three molluscan species; Table 1). In the case of AMBI, while the fidelity did increase, the uncertainty (i.e., standard deviation) also increased ($F_1 = 0.447 \pm 0.138$ for sites that require remediation when using at least three molluscan individuals (Table A1) versus $F_1 = 0.115 \pm 0.033$ for sites that require remediation when using at least three molluscan species; Table 1). These results suggest that when an environmental manager either has the requisite taxonomic expertise or is using M-AMBI, the ability of a mollusc-only approach to detect sites requiring remediation can be improved by using samples with as few as three molluscan individuals.

Next was our decision to transform the abundance data using a square-root transformation. Relative abundance is often overly sensitive to the dominance of a few high-abundance taxa whose abundances can also vary greatly between sample sites, which may result in misleading

AMBI calculations (Warwick et al., 2010). Transforming the relative abundance data can reduce the difference in abundance, reducing the variability of AMBI scores both within and between sample sites (Tweedley et al., 2014). Indeed, within our study the general linear relationship between the mollusc-only and whole-community index scores did not change, but the variability within the dataset did decrease, increasing the model’s overall performance (i.e., the linear relationships did not change while the standard deviation decreased after transforming the abundance; Table A3).

Our decision to adjust the EcoQS boundaries after transforming the abundance data also affected the individual sample site M-AMBI scores. Prior studies have suggested adjusting the EcoQS boundaries on the AMBI scale dependent on the data transformation used, as the transformation will shorten the distance between the ‘High’ and ‘Bad’ conditions, especially when using the AMBI software’s default reference conditions (Warwick et al., 2010; Tweedley et al., 2014). We caution, however, against adjusting the EcoQS boundaries until the fit of the model is assessed. If the model’s simulated data (i.e., the data generated under the model’s assumptions) do not match the density distribution of the observed data, there may be underlying information about the data (e.g., environmental variation) that is not being captured by the model and adjusting the EcoQS boundaries may exacerbate the misclassification. For example, the posterior predictive check for M-AMBI suggested the model was over-estimating the ecological quality of the degraded sites, and indeed if we had adjusted the EcoQS boundaries we would have misclassified a higher proportion of the degraded sites ($F_1 = 0.810 \pm 0.003$ for sites that require remediation under the adjusted EcoQS boundaries, versus $F_1 = 0.907 \pm 0.004$ for sites that require remediation under the original EcoQS boundaries; Table A4).

Lastly, we used the default reference conditions within the AMBI software (i.e., maximum richness and diversity, and minimum AMBI scores) to calculate our M-AMBI scores. When calculating the M-AMBI scores of a location to inform remediation decisions, the reference conditions should be defined independently for that specific habitat, given that reference conditions will vary spatially and between different environments (Borja et al, 2008; Borja and Tunberg, 2011; Borja et al., 2012). Our data span a variety of habitats (from estuarine to marine), creating a salinity gradient that may have amplified the difference in species richness and diversity between the reference conditions and the sample sites, as water bodies with lower salinity tend to have fewer species. However, as stated previously, the objective of our study was to assess the fidelity of a mollusc-only approach, not to determine the ‘true’ EcoQS of the sample sites. Both the mollusc-only and whole-community M-AMBI scores were calculated using the same methodologies, and therefore any potential exaggeration in the difference in species richness and diversity would be the same for both data sets. We used the default conditions within the software only to facilitate calculations, which was sufficient for the purpose of this study.

Table A1

Number of EcoQS classifications for sample sites with at least three molluscan species (sp.) and with at least three molluscan individuals (ind.).

	AMBI		M-AMBI	
	≥ 3 sp.	≥ 3 ind.	≥ 3 sp.	≥ 3 ind.
High	622	630	60	51
Good	65	116	530	523
Moderate	19	18	59	78
Poor	4	3	59	92
Bad	0	0	2	23

Table A2

Fidelity metric scores for both AMBI and M-AMBI for sample sites with at least three molluscan individuals (767 sample sites).

AMBI	Precision	Recall	F ₁ Score
High	0.505 ± 0.044	0.156 ± 0.056	0.231 ± 0.061
Good	0.761 ± 0.012	0.923 ± 0.030	0.833 ± 0.007
Moderate	0.520 ± 0.022	0.445 ± 0.212	0.451 ± 0.120
Poor	0.000 ± 0.000	0.000 ± 0.000	NA
No Remediation	0.963 ± 0.013	0.976 ± 0.011	0.969 ± 0.001
Remediation	0.535 ± 0.023	0.427 ± 0.215	0.447 ± 0.138

M-AMBI	Precision	Recall	F ₁ Score
High	0.749 ± 0.025	0.686 ± 0.010	0.716 ± 0.008
Good	0.853 ± 0.002	0.908 ± 0.011	0.880 ± 0.005
Moderate	0.677 ± 0.012	0.686 ± 0.024	0.681 ± 0.017
Poor	0.801 ± 0.055	0.551 ± 0.015	0.652 ± 0.016
No Remediation	0.973 ± 0.001	0.995 ± 0.002	0.986 ± 0.000
Remediation	0.974 ± 0.008	0.864 ± 0.007	0.915 ± 0.001

Table A3

Comparison of the linear regressions between untransformed and square-root transformed abundance using uniform priors (SD = Standard Deviation).

AMBI	Untransformed Abundance				Square-root Transformed Abundance			
	Mean	SD	5.5%	94.5%	Mean	SD	5.5%	94.5%
α_x	1.608	0.025	1.572	1.644	1.600	0.016	1.574	1.626
β	0.577	0.028	0.533	0.621	0.561	0.022	0.526	0.596
σ	0.475	0.016	0.399	0.450	0.304	0.011	0.286	0.322

M-AMBI	Untransformed Abundance				Square-root Transformed Abundance			
	Mean	SD	5.5%	94.5%	Mean	SD	5.5%	94.5%
α_x	0.637	0.003	0.632	0.642	0.703	0.002	0.699	0.707
β	0.794	0.023	0.757	0.831	0.814	0.019	0.784	0.844
σ	0.059	0.002	0.055	0.062	0.046	0.002	0.043	0.049

Table A4

Fidelity metrics of the mollusc-only M-AMBI scores if the EcoQS boundaries were adjusted for the abundance transformation.

M-AMBI	Precision	Recall	F ₁ Score
High	0.643 ± 0.056	0.789 ± 0.026	0.706 ± 0.025
Good	0.937 ± 0.003	0.938 ± 0.012	0.937 ± 0.005
Moderate	0.405 ± 0.039	0.429 ± 0.030	0.416 ± 0.033
Poor	0.907 ± 0.008	0.657 ± 0.062	0.761 ± 0.045
No Remediation	0.962 ± 0.002	0.984 ± 0.001	0.973 ± 0.001
Remediation	0.896 ± 0.007	0.783 ± 0.013	0.836 ± 0.008

Appendix B. Fidelity of a mollusc-only AMBI approach using European taxa

Table B1

Fidelity metrics of the mollusc-only AMBI data from [Dietl et al. \(2016\)](#).

AMBI	Precision	Recall	F ₁ Score
High	0.572 ± 0.193	0.425 ± 0.174	0.447 ± 0.076
Good	0.792 ± 0.014	0.843 ± 0.093	0.814 ± 0.046
Moderate	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Poor	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
No Remediation	0.883 ± 0.007	0.918 ± 0.058	0.889 ± 0.029
Remediation	0.022 ± 0.028	0.028 ± 0.071	0.160 ± 0.015

Appendix C. Ecological grouping distributions of major benthic macro-faunal invertebrate taxa

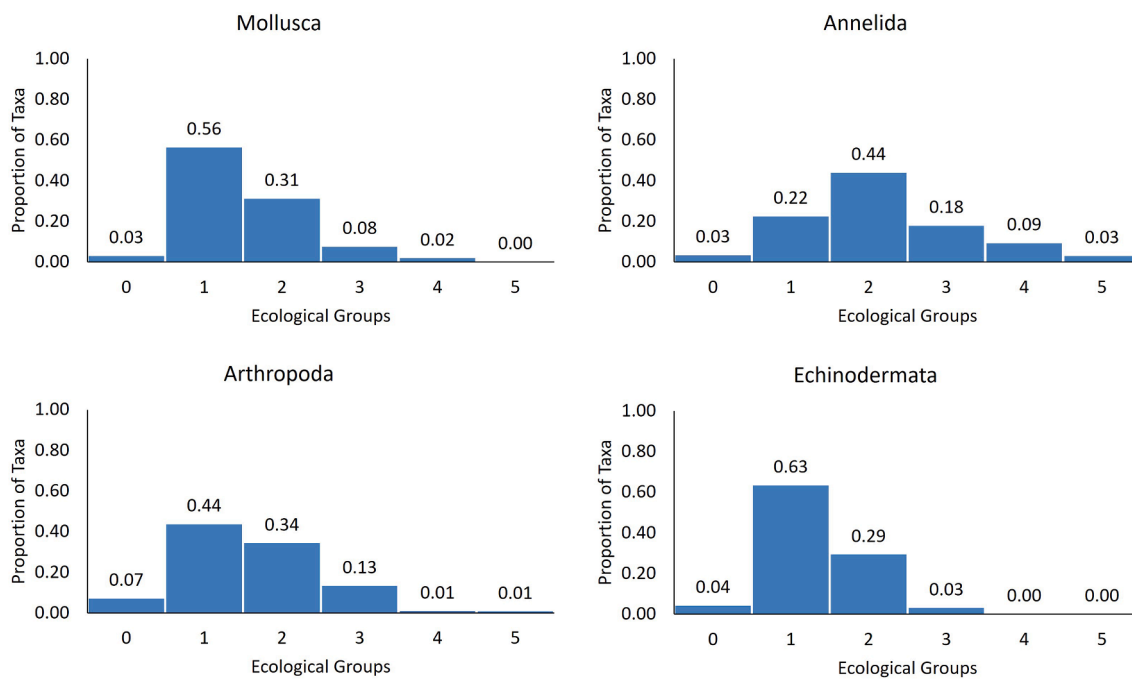


Fig. C1. Distribution of major macroinvertebrate taxa from the AMBI Index Software v6.0 (Borja et al., 2012; <http://ambi.azti.es>) by ecological groupings.

Appendix D. Relationship between molluscan and whole-community species richness and diversity

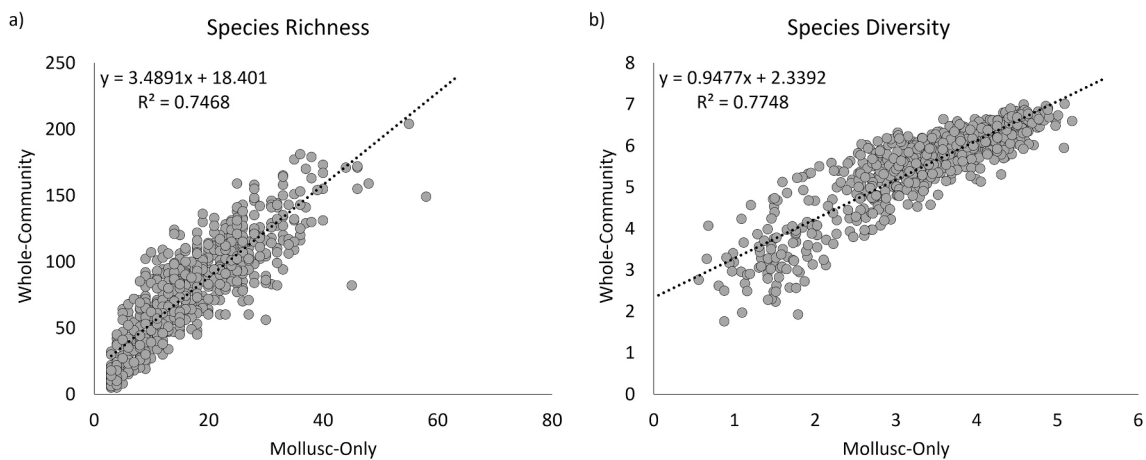


Fig. D1. Linear regression between whole-community and mollusc-only species richness (a) and species diversity (b) using square-root transformed abundance data.

Appendix E. Density distributions of whole-community AMBI and M-AMBI scores

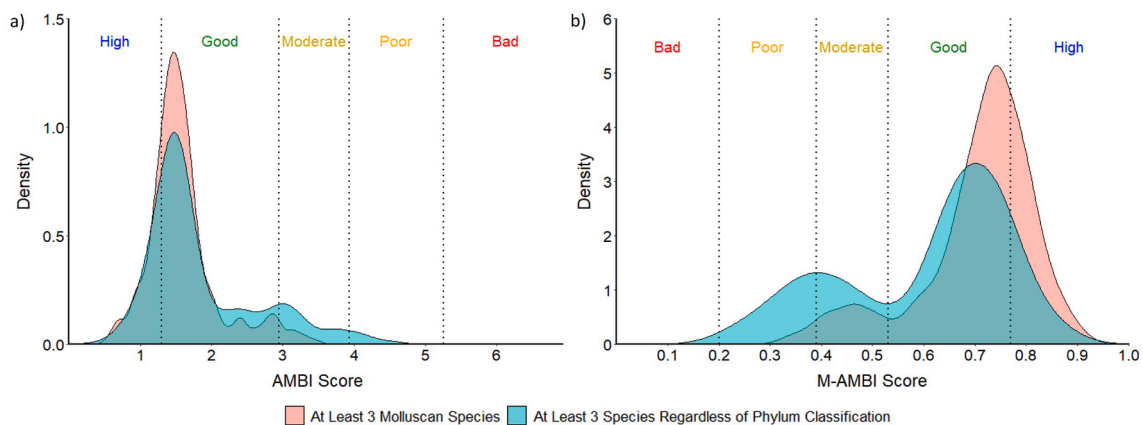


Fig. E1. Density distributions of whole-community AMBI (a) and M-AMBI (b) scores for sites with at least three molluscan species and those with at least three species regardless of phylum classification. Note the greater number of degraded sites ('Poor' and 'Bad') identified by AMBI and M-AMBI when selecting sample sites with at least three species regardless of phylum classification.

Appendix F. Supplementary materials

Supplementary materials 1–4 to this article can be found online at <https://doi.org/10.1016/j.ecolind.2021.107910>.

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