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Optimising a widely-used coastal health index through quantitative ecological group classifications and associated thresholds

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ABSTRACT

Many globally applied biotic indices, including the AMBI benthic index, are based on species' sensitivity/tolerance to anthropogenic disturbances. The AMBI scoring primarily relies on the correct assignment of both taxon stressor-sensitivities and the disturbance thresholds or bands. Using an extensive, long-term monitoring dataset from New Zealand (NZ) estuaries, we describe how the AMBI has been strengthened through quantitative derivation of taxon-specific sensitivities and condition thresholds for two key estuarine stressors [mud and total organic carbon (TOC)], and the integration of taxon richness. The results support the use of the existing AMBI condition bands but improve the ability to identify cause; 2–30% mud reflected a 'normal' to 'impoverished' macrofaunal community; 30–95% mud and 1.2–3% TOC 'unbalanced' to 'transitional'; and >3–4% TOC 'transitional' to 'polluted'. The (refined) AMBI was also successfully validated (R^2 values >0.5 for mud, and >0.4 for TOC) for use in shallow, intertidal dominated estuaries NZ-wide. Most biotic indices lack the ability to differentiate between anthropogenic disturbances, which in turn undermine their effectiveness for applied purposes. By integrating key quantitative information to an existing benthic index, these results enable more robust identification of coastal stressors and facilitate defensible management decisions.

1. Introduction

Determination of the benthic condition of shallow coastal ecosystems focuses on monitoring both biotic and abiotic sediment quality indicators (e.g. the Australian Oceans Policy, the Canadian Oceans Act and Oceans Strategy, the USA Oceans Act, the European Water and Marine Strategy Framework Directives (WFD, 2000/60/EC and MSFD, 2008/56/EC), and the New Zealand Estuary Monitoring Protocol (EMP, 2002) and Estuarine Trophic Index (Robertson et al., 2016a,b)). In particular, indicators have been developed to reflect environmental degradation associated with increased sediment mud content (Robertson et al., 2015), organic enrichment (Hyland et al., 2005; Pusceddu et al., 2009; Sutula et al., 2014; Robertson et al., 2015), and toxicity (Brady et al., 2015). Macroinfaunal communities are generally selected as the primary biotic indicator due to their functional importance, their diversity of responses and their relatively sedentary existence. To facilitate the interpretation of macroinfaunal abundance data as it relates to environmental variables, 'sensitivity' groupings have been developed for many taxa, either quantitatively (Robertson et al., 2015) or through expert opinion (e.g. Gillett et al., 2015). Comparing the relative magnitudes of each of the taxon-specific sensitivity groupings at a particular site provides an indication of where the macroinfaunal community fits along the environmental gradient(s).

The most widely used coastal biotic index, the AZTI (AZTI-Tecnalia Marine Research Division, Spain) Marine Benthic Index (AMBI), has been verified in relation to a range of abiotic variables (Borja et al., 2000), environmental impact sources (Borja and Muxika, 2005) and regions, including Europe, the United States (Borja et al., 2008; Borja and Tunberg, 2011; Teixeira et al., 2012), South America (Muniz et al., 2005), and Canada (Callier et al., 2008). The AMBI biotic coefficient (BC) is generated by

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combining weighted abundances of each of five sensitivity groupings (called ecological groups – EGs) to anthropogenic disturbance, ranging from very sensitive to very insensitive, and is then used to categorise a particular site into one of seven 'disturbance bands' (Normal to Azoic, derived by comparing the macroinfaunal response to a range of environmental variables). Hence, the two key drivers of the AMBI BC scoring approach are the correct assignment of each taxon to an EG, and the disturbance thresholds or bands used.

While such an approach clearly provides an easy-to-use, cost effective tool for assessing the condition of benthic coastal habitat, in some cases the performance of the AMBI has been limited. For example, it can perform unsatisfactorily where samples have a low number of taxa with assigned EG values (Muxika et al., 2007; Gillett et al., 2015), or where sensitivity groupings are based predominantly on the international AMBI list (http://ambi.azti.es) due to an absence of local sensitivity data (e.g. Rodil et al., 2013; Gillett et al., 2015). Local sensitivity EG data, derived through expert opinion, significantly improves the performance of AMBI when augmented with international list values (Gillett et al., 2015). AMBI performance, particularly in its role in managing coastal benthic pollution in estuaries, is also limited by its inability to differentiate between various key anthropogenic disturbance stressors such as muddiness, organic matter, oxygen conditions and toxicants, and natural disturbance such as low salinity (Baritone et al., 2012)).

The present study addressed four objectives to improve the efficacy of the AMBI in New Zealand (NZ) estuaries: (1) to determine improvements in the AMBI from the inclusion of quantitative EGs derived from NZ macrobenthic data; (2) to validate the AMBI using an independent, nationwide dataset; (3) to derive thresholds of two primary stressors, sediment muddiness and organic enrichment, to better inform the current AMBI condition bands for use in NZ estuaries; and (4) to determine the usefulness of adding species richness to the AMBI, either as M-AMBI (AMBI's multivariate extension; Bald et al., 2005; Muxika et al., 2007), or through direct integration to the abundance-weighted AMBI equation.

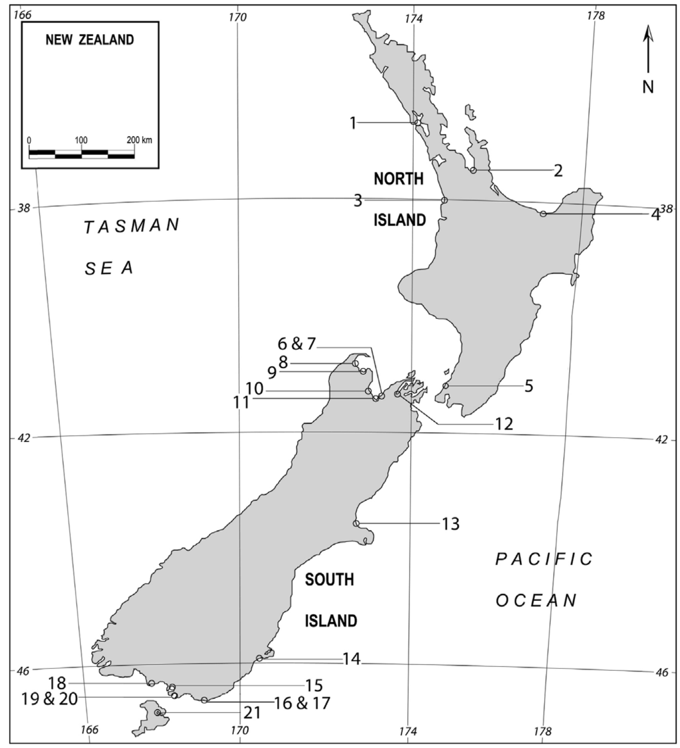

Fig. 1. Geographic locations of the 21 estuaries sampled in New Zealand used to calibrate and validate the AMBI benthic index. Refer to Table 1 for location and physical details relating to each estuary. Figure is modified from Robertson et al. (2015).

2. Materials and methods

2.1. Study locations and sampling protocol

An extensive nation-wide estuary monitoring dataset collected over 15 years by NZ regional government authorities was used in Robertson et al. (2015) to model and derive taxon-specific EGs. Here we used data from 21 of the 25 estuaries (four tidal river estuaries with minimal intertidal regions were omitted during dataset standardisation as outlined below). The present study assesses whether the inclusion of local EGs improved the efficacy of the AMBI and facilitates establishing threshold values along primary stressor gradients. Benthic datasets were standardised to minimise variance in index values by: selecting moderate-high salinity zones (>25 psu) in representative mid-low water intertidal habitat with low sediment metal concentrations (apart from rare situations where metal concentrations are naturally high due to geological activity); targeting predominantly shallow, relatively large tidal lagoon systems, dominated by intertidal habitat and perpetually open to tidal exchange (see Fig. 1 for locations of estuaries, Table 1 for relevant attributes), a type that constitutes >150 of New Zealand's 400+ estuaries (NIWA's Coastal Explorer Tool available at: http://www.niwa.co.nz/coasts-and-oceans/nz-coast/coastal-explorer).

Variation among sites or estuaries due to different sampling or identification techniques is considered negligible since sampling was standardised in accordance with the NZ National EMP (Robertson et al., 2002) and one expert undertook the majority of the macroinvertebrate taxonomic work. Details of the sampling protocol, including the timing, replication and number of sampling events per estuary, and the biotic and abiotic parameters measured, are presented in Robertson et al. (2015). Briefly, benthic macrofauna were sampled using a 130 mm diameter (area = 0.0133 m^2) core manually driven 150 mm into the sediment, with 10–12 randomly located replicates collected and analysed per location. Samples were sieved on a 0.5 mm mesh and retained fauna were preserved in 95% isopropyl alcohol/seawater solution. Macrofauna were identified to the lowest possible taxonomic resolution and counted.

A sediment sample (approx. 250 g from the top 20 mm at the surface) was collected for analysis from each macrofaunal sampling location and analysed for: (1) grain size distribution (% mud, sand, gravel) using wet sieving and gravimetric calculations; (2) TOC via elemental analyser (628 Series CNS, Leco); and (3) metal contaminants (total recoverable Cd, Cr, Cu, Ni, Pb and Zn) using nitric/hydrochloric acid digestion, ICP-MS (low level) USEPA 200.2 (US EPA, 2009) – see Robertson et al. (2015) for estuary-specific metal concentrations.

2.2. AMBI under three classification schemes

Of all the available benthic indices (reviewed by Borja et al., 2012), this study focused on the applicability of the AMBI biotic index for two reasons. First, the AMBI was designed to be responsive to a number of anthropogenic disturbance variables, including mud and organic enrichment, which are particularly important primary stressors of macrofaunal communities in shallow, intertidal, NZ estuaries (Robertson et al., 2015). Second, Robertson et al. (2015) used organic enrichment, grain size and macroinvertebrate abundance data to assign EGs to 99 (quantitatively for 39 and semi-quantitatively for 60) taxa according to their responses to mud content and organic enrichment. These NZEGs – labelled I, II, III, IV or V, with V assigned to the most tolerant taxa and I to the

Table 1

General features of the estuaries, harbours and embayments sampled. Abbreviations: 'Estuary type', TL = tidal lagoon, CE = coastal embayment, TRM = tidal river mouth, TRD = tidal river/delta, IB = island barrier (modified from Hume et al. (2007) to account for estuaries with significant delta areas, e.g. Freshwater); and for 'Dominant land use', P = pasture, NFS = native forest/scrub, EFS = exotic forest/scrub, Urb = urban (NIWA's Catchment Land Use for Environmental Sustainability model – CLUES 10.1).

Number code[a]	Estuary	Region	Latitude	Estuary type	Estuary area (km^2)	Intertidal area (km^2)	Catchment area (km^2)	Dominant land use	Spring tidal range (m)	AMBI calibration or validation[b]
1	Kaipara (Otamatea Arm)	Northland	36°18′ S	TL	17	6.8	614	P	2.4	Calibration
2	Firth of Thames	Waikato	37°04′ S	CE	721	85	4194	P/NFS/EFS	2.9	Validation
3	Raglan Harbour	Waikato	37°45′ S	TL	31	13.2	532	P/NFS/EFS	2.9	Validation
4	Ohiwa	Bay of Plenty	38°00′ S	TL	27	18.9	186	NFS/P	1.7	Calibration
5	Porirua Harbour	Wellington	41°06′ S	TL	8.2	2.8	171	P/NFS	1.0	Calibration
6	Nelson Haven	Tasman	41°13′ S	TL	15	8.9	129	NFS/ENS/Urb	3.6	Validation
7	Delaware	Tasman	41°09′S	TL	3.5	3.3	93	NFS/ENS/P	3.5	Validation
8	Aorere (Ruataniwha)	Tasman	40°39′ S	TRD	8.6	7.3	711	NFS	3.6	Validation
9	Motupipi	Tasman	40°50′ S	TL	1.7	1.6	41	NFS/P	3.6	Validation
10	Moutere Inlet/Delta	Tasman	41°09′ S	TL (IB)	7.6	7.2	182	EFS/P	3.6	Calibration
11	Waimea	Tasman	41°17′ S	TL (IB)	33	29.5	913	NFS/EFS/P	3.6	Calibration
12	Havelock	Marlborough	41°16′ S	TRM	8	1.6	1200	NFS/P	2.2	Calibration
13	Avon Heathcote	Canterbury	43°32′ S	TL	7	6	188	Urb/P	1.8	Validation
14	Kaikorai	Otago	45°55′ S	TL	1.5	1.3	55	P/Urb	1.7	Validation
15	New River	Southland	46°28′ S	TL	46	34.1	4314	P	2.2	Validation
16	Waikawa	Southland	46°37′ S	TL	7	5.7	237	NFS/P	2.0	Validation
17	Haldane	Southland	46°38′ S	TL	2	1.9	70	NFS/P	2.0	Calibration
18	Jacobs River	Southland	46°20′ S	TL	7.2	5.6	1527	P	1.9	Calibration
19	Awarua	Southland	46°34′ S	TL	27	21.3	50	NFS/P	2.0	Validation
20	Bluff	Southland	46°33′ S	TL	28	14.3	40	NFS/P	1.9	Validation
21	Freshwater	Stewart Island	46°54′ S	TRD	8.1	6.2	320	NFS	1.9	Validation

Table is modified from Robertson et al. (2015).

[a] Number codes correspond to numbered estuaries in Fig. 1.

[b] Estuary data used to either calibrate ($n = 307$ samples) or validate ($n = 1065$ samples) the AMBI.

Table 2
Summary of the AMBI values and their corresponding benthic condition bands (from Borja et al., 2000 and Muxika et al., 2005).

Biotic coefficient	Dominant ecological group	Benthic community health	Ecological status[a]
$0.0 < \text{AMBI} \leq 0.2$	I	Normal	High status
$0.2 < \text{AMBI} \leq 1.2$		Impoverished	Good status
$1.2 < \text{AMBI} \leq 3.3$	III	Unbalanced	Moderate status
$3.3 < \text{AMBI} \leq 4.3$	IV–V	Transitional to pollution	Poor status
$4.3 < \text{AMBI} \leq 5.0$		Polluted	
$5.0 < \text{AMBI} \leq 5.5$	V	Transitional to heavy pollution	Bad status
$5.5 < \text{AMBI} \leq 6.0$		Heavy pollution	
$6.0 < \text{AMBI} \leq 7.0$	Azoic	Azoic	

[a] European Union Water Framework Directive (WFD) 'EcoQ' bands (Borja et al., 2003).

least (narrative descriptions in Borja et al., 2000 – see Table 2 in the present paper), were assigned so that the EGs could fit directly with the AMBI biotic coefficient:

$$\text{Biotic coefficient (BC)} = \{(0 \times \%\ \text{EG I}) + (1.5 \times \%\ \text{EG II}) + (3 \times \%\ \text{EG III}) + (4.5 \times \%\ \text{EG IV}) + (6 \times \%\ \text{EG V})\}/100.$$

In the present paper we followed a similar approach to Gillett et al. (2015), whereby index performance was assessed using (1) standard international EGs exclusively, (2) NZEGs exclusively, and (3) a hybrid model amalgamating NZEGs supplemented with standard international EGs from the AMBI list (H-NZEGs), with these analyses based on the calibration dataset (Table 1). Estuaries included in the calibration dataset were selected to cover most of New Zealand and to represent the full mud/TOC gradients.

2.3. AMBI validation

The ability of the AMBI to characterise macrobenthic condition in relation to sediment mud and organic carbon content was then validated using an independent dataset. Spanning the majority of NZ, this dataset was comprised of 1065 samples collected from 13 estuaries (Fig. 1, Table 1). The AMBI performance was evaluated based on the degree of variability (via regression coefficients) in Biotic Coefficient (BC) scores explained by mud and TOC between the calibration and validation datasets.

2.4. M-AMBI

A weakness of the standard abundance-weighted AMBI is that it does not consider taxon richness (i.e. number of taxa per sample). A common approach used to incorporate richness (and Shannon–Wiener (log2) diversity) into the AMBI is to apply its more recently developed multivariate derivative, the M-AMBI (Bald et al., 2005; Muxika et al., 2007). This method compares monitoring results with reference conditions, to derive an M-AMBI value that expresses the relationship between observed values and reference condition values. In this study, M-AMBI coefficients were calculated using a software tool developed by AZTI-Tecnalia (http://ambi.azti.es/ – currently version 5.0; Borja et al., 2012), although alternative approaches are also available (Sigovini et al., 2013). According to Sigovini et al. (2013), for each sample M-AMBI closely approximates this value:

$$\text{M-AMBI} = (\text{Richness} + \text{Shannon Diversity} + \text{AMBI-BC})/3.$$

However, because both abundance and richness are expected to better inform a biotic index through inclusion of sensitivity groupings, we introduced a richness metric for each EG directly into the AMBI BC equation in the same way as abundance:

$$\text{Richness-integrated AMBI BC} = \{((0 \times (\%\ \text{EG I} + \%\ \text{Richness EG I}))\textit{nonumber} + (1.5 \times (\%\ \text{EG II} + \%\ \text{Richness EG II})) + (3 \times (\%\ \text{EG III} + \%\ \text{Richness EG III})) + (4.5 \times (\%\ \text{EG IV} + \%\ \text{Richness EG IV})) + (6 \times (\%\ \text{EG V} + \%\ \text{Richness EG V}))\}/200$$

2.5. Statistical analyses

To evaluate the AMBI index under each EG classification scheme, associated AMBI BC scores were plotted against gradients of mud and TOC, with resulting R^2 values used to compare the strength of each classification scheme to discern macrobenthic condition along each stressor gradient. Comparisons of R^2 values between calibration and validation datasets were also used to evaluate the ability of the AMBI to characterise benthic condition in estuarine sites beyond those initially used to calibrate the index. As an alternative statistic, we also reported residual standard error (RSE), the error of the modelled residuals. In addition, a 10-fold cross validation was performed on the combined calibration/validation dataset ($n = 1372$ samples), which enabled the assessment of model performance (i.e. accuracy) by identifying potential fluctuations in R^2 values. For example, selecting a different set of estuaries for model calibration or validation may result in R^2 value shrinkages (i.e. reduced model accuracy). Finally, R^2 values derived from scatterplots of index coefficients versus mud and TOC gradients were used to evaluate the efficacy of the M-AMBI and the alternative Richness-integrated AMBI.

To derive "classification" thresholds of mud and TOC that defined boundaries in AMBI coefficients, regression tree models were selected due to their ease-of-use and strong performance, particularly for large datasets with correlated causal variables, simulating non-linear relationships and ranking causal variables (e.g. De'ath and Fabricius, 2000). Trees were developed through binary recursive partitioning, an iterative process that splits the data into groups, and then continues splitting each group into more homogeneous groups. Each group is characterised by a typical value of the response variable attributable to that split, the number of observations in the group, and the values of the explanatory variables that define it. The ctree() function in R (Development core team, 2015 – 3.0.3 GUI 1.63 Snow Leopard build 6660) package 'party' (https://cran.r-project.org/web/packages/party) was used to generate trees, with a maximum split number of 3 and $P < 0.05$ as the stopping criteria; box plots indicating variances rather than bootstrapped confidence intervals (CIs) were employed because the latter are often considered too narrow (Brenden et al., 2008). The robustness of classification threshold estimates was also assessed for log-transformed mud and TOC data, since this should not affect the identified estimates (Qian and Cuffney, 2012). In addition, potential "breakpoint-based" thresholds (beyond any classification thresholds that were identified herein) were assessed using segmented (or piecewise) regression (Muggeo, 2012). Segmented regression analysis allows evaluation of a segmented linear response composed of two functions that differ in their slopes but their lines converge at the breakpoint. This was undertaken using the 'segmented' package (https://cran.r-project.org/web/packages/segmented) and associated CIs calculated using its confint.segmented() function within R.

The classification and breakpoint-based threshold types both provide ecologically meaningful but different information. While classification thresholds identify groups of data points with differing properties (Toms and Villard, 2015), breakpoint-based thresholds indicate the point of maximum benthic deterioration, or the position where conditions could be expected to improve as stressor influence is reduced (Sutula et al., 2014). These thresholds were used to assess the applicability of existing AMBI benthic condition/ecological status bands (Table 2) to the present dataset. Initial results were based on both replicate- and site-level data. Replicate-level data were reported to accurately assess the stressor–response relationship given the variation in stressor levels within sites; site-level data were reported using site averages ($n = 10$ in most cases). Site-level data were included to counter potential intra-site (replicate) spatial dependencies that may inflate R^2 values, and thus invalidate statistical inference at that scale (Dormann, 2007).

3. Results

In total, 176 macroinvertebrate taxa were recorded from 1372 samples. Of these, 100 taxa were assigned an EG using the standard international AMBI list, 99 taxa based on NZEGs alone, and 159 taxa were classified under the H-NZEGs scheme (Table 3).

3.1. Application of AMBI under three classification schemes

The ability of the AMBI to detect changes in macrobenthic condition relative to sediment muddiness and organic enrichment was substantially enhanced when supported by quantitative NZEGs, and was even more sensitive when hybridised with international EGs where NZEGs were lacking (Fig. 2, panels left to right). Under each classification scheme, the variability in the AMBI score explained by mud and TOC was lowest and non-significant using only standard international EGs (mud $R^2 = 0.002$; $P = 0.34$,

Table 3
Summary of the number of samples classified, and taxa assigned and not assigned an ecological grouping using three classification schemes.

AMBI EG scheme	Samples classified (%)[a]	*n* taxa assigned	*n* taxa not assigned	*n* taxa with contrasting EGs[b]
Standard International	98.1	100	76	NA
New Zealand (NZEGs)	100	99	77	44
Hybrid New Zealand (H-NZEGs)	100	159	17	NA
	Total samples: 1372	Total taxa: 176		

[a] Samples with more than 20% of the individuals not assigned to an EG were not included in subsequent analyses, as per AMBI guidelines (Borja and Muxika, 2005).
[b] Number of taxa with classifications differing between NZEGs and standard international list. NA means not applicable.

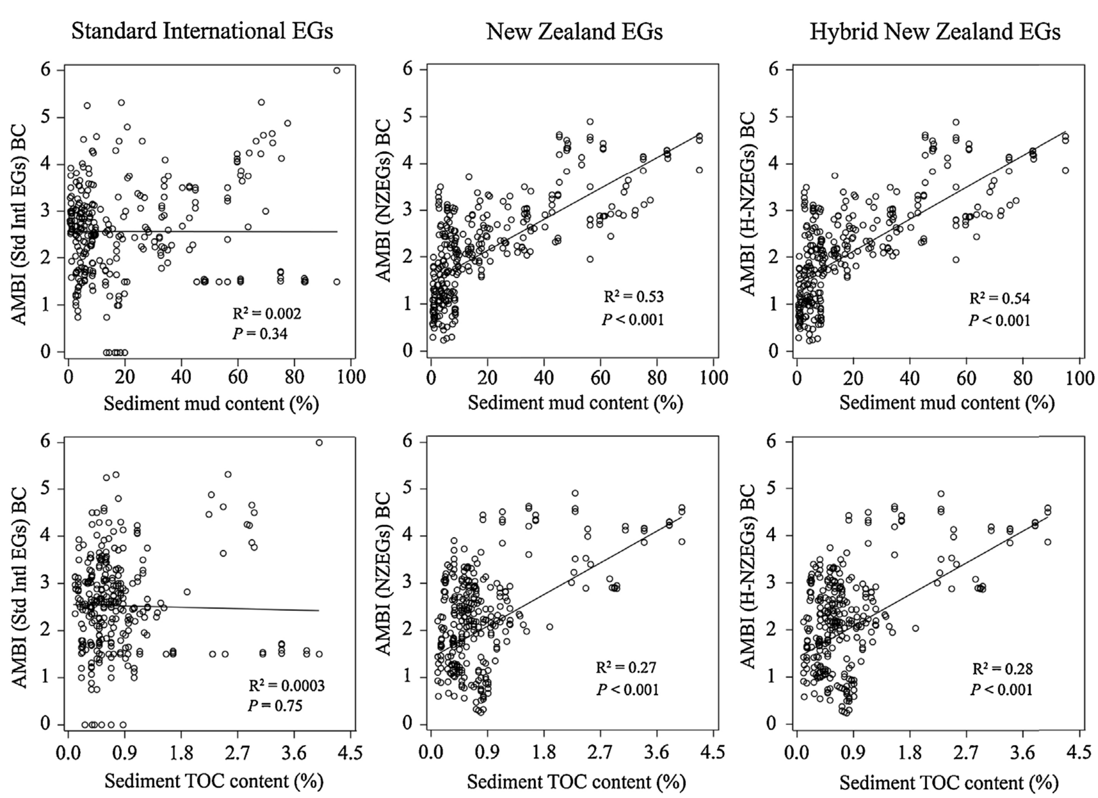

Fig. 2. AMBI BC values under three ecological group classification schemes: standard international EGs (left panels), NZEGs (middle panels) and Hybrid NZEGs (right panels); each regressed against sediment mud and total organic carbon gradients. Replicate-level calibration data ($n = 307$) was used to generate each plot.

Table 4
Summary of regressions between the response of the AMBI (under each classification scheme) to mud and TOC gradients using replicate (n = 307) and site (n = 31) level data.

Classification scheme	Abiotic gradient	Replicate-level		Site-level	
		R^2	Parameter significance	R^2	Parameter significance
Standard International EGs	Mud	<0.01	0.34	0.04	0.15
	TOC	<0.01	0.75	0.11	0.64
NZEGs	Mud	0.53	<0.001	0.57	<0.001
	TOC	0.27	<0.001	0.35	<0.001
Hybrid NZEGs	Mud	0.54	<0.001	0.51	<0.001
	TOC	0.28	<0.001	0.42	<0.001

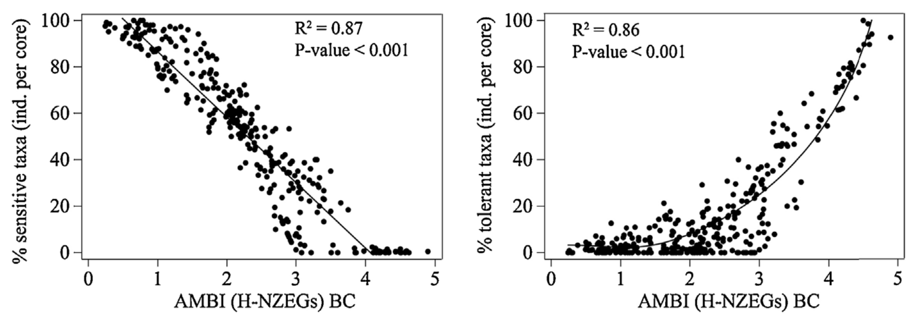

Fig. 3. AMBI (H-NZEGs) coefficients plotted against the proportion of (left) sensitive taxa ($\sum$%EG I & II) and (right) tolerant taxa ($\sum$%EG IV & V) using the calibration dataset (n = 307).

and TOC R^2 = 0.0003; P = 0.75), but significantly increased using the NZEGs (mud R^2 = 0.53; P < 0.001, and TOC R^2 = 0.27; P < 0.001) and Hybrid NZEGs (H-NZEGs) schemes (mud R^2 = 0.54; P < 0.001, and TOC R^2 = 0.28; P < 0.001). In addition, R^2 values were of similar magnitude between replicate- and site-level data (Table 4), indicating that any spatial dependencies operating at the replicate level were unlikely to invalidate results at that scale. All subsequent analyses in this paper are based on the replicate level H-NZEGs scheme data because it provided the best model fit.

Regression analyses revealed a linear increase in BC scores (i.e. progressively declining abundances of sensitive taxa and increasing abundances of tolerant taxa; Fig. 3) with increasing mud and TOC concentrations across all locations. According to Fig. 2, variability in coefficients, and the overall number of data points under all classification scenarios, was greatest in situations where sediment mud and TOC concentrations were approximately <15% and <1%, respectively.

3.2. Integration of taxon richness

For the present calibration dataset, the performance of the M-AMBI was less successful than the standard AMBI in classifying macrofaunal condition along the mud (R^2 = 0.13; P < 0.001) and TOC (R^2 = 0.10; P < 0.001) gradients. To provide a more effective approach, taxon richness within each EG was introduced directly into the standard abundance-weighted equation. Taxon abundance and richness varied considerably across the H-NZEGs biotic coefficient (Fig. 4). The inclusion of taxon richness (hereafter denoted 'R-H-NZEGs') resulted in a reduction in the uncertainty of AMBI scores along both stressor gradients (Fig. 5), with variability in scores explained by mud and TOC each increasing by 0.04. This was linked to an observed decrease in the variability at the lower concentrations of mud (approx. <15%) and TOC (approx. <1%), where previously the standard abundance-weighted AMBI indicated the poorest fit.

3.3. AMBI validation

When tested using independent data (>1000 observations), the ability of both AMBI coefficients (i.e. H-NZEGs and R-H-NZEGs) to delineate macrobenthic condition along primary stressor gradients was not compromised (Fig. 6). For example, with only a slight decrease (i.e. 0.10 for H-NZEGs, and 0.05 for R-H-NZEGs) in the level of variability explained, and negligible differences in regression

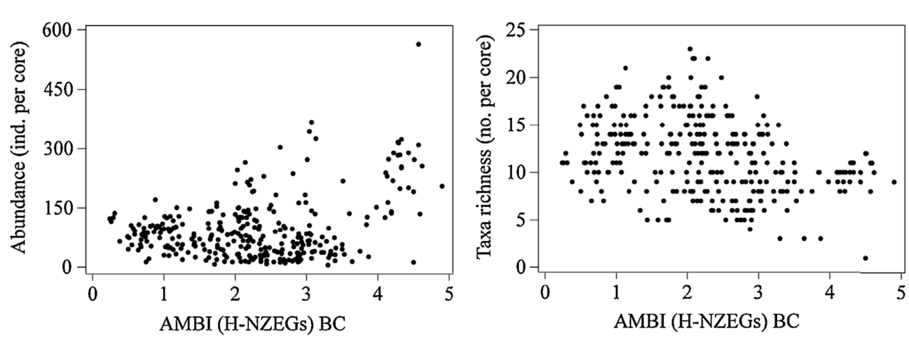

Fig. 4. Relationship between the AMBI (H-NZEGs) coefficient and community-level metrics, abundance and taxon richness, based on calibration data (n = 307 per plot). Taxon abundances were greatest and most variable in Biotic Coefficient (BC) values >2, and taxon richness greatest and most variable in BC 1–3 but declined and variability decreased either side of this range.

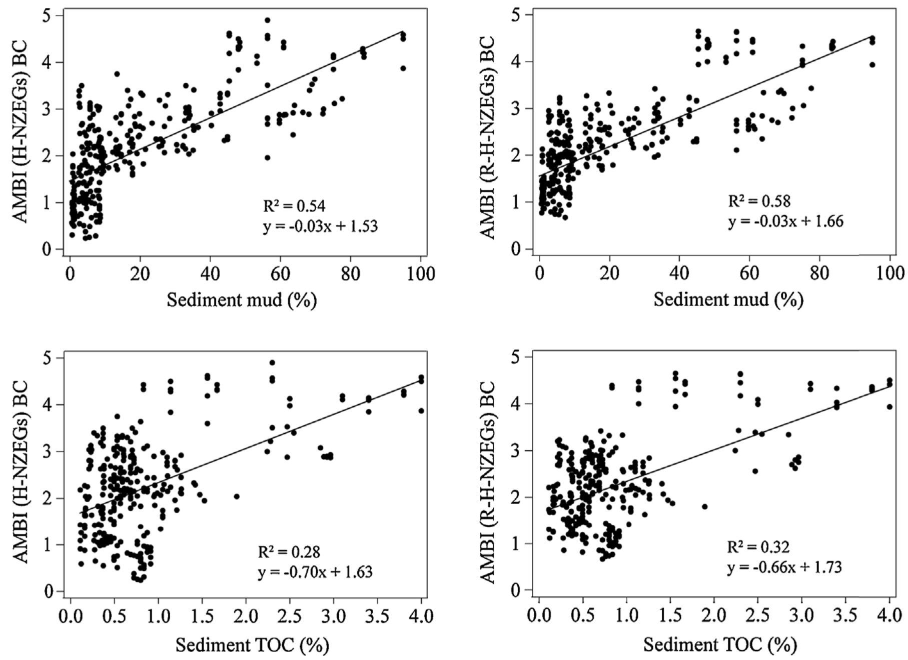

Fig. 5. Relationship between the AMBI coefficient based on the (left) standard abundance-weighted AMBI equation and (right) richness-integrated AMBI equation, using the calibration dataset ($n = 307$ per plot).

line slopes and intercepts, the influence of mud on both the AMBI indices was highly correlated between the calibration/validation datasets. Further, for TOC, R^2 values for H-NZEGs and R-H-NZEGs increased by 0.06 and 0.12, respectively (with slightly steeper regression line slopes apparent for the validation dataset in both cases). This indicated that relations between AMBI scores and TOC were effectively strengthened when data were introduced from sites independent of those used to calibrate the index. Finally, model accuracy was not reliant on a specific combination of calibration/validation data as indicated by the similar R^2 values (and residual standard error estimates) among all associated models, including those based on 10-fold cross validation performed on the combined calibration/validation dataset (Table 5).

3.4. Thresholds of macrobenthic condition

Regression tree analysis was effective in identifying classification threshold values of mud and TOC that delineated macrobenthic condition for both H-NZEGs and R-H-NZEGs coefficients (Fig. 7). Interpreting the trees, which individually explained >95% of the total variance, starting from their apices, the first three abiotic splits were attributed to mud at various concentrations (0–41.8%),

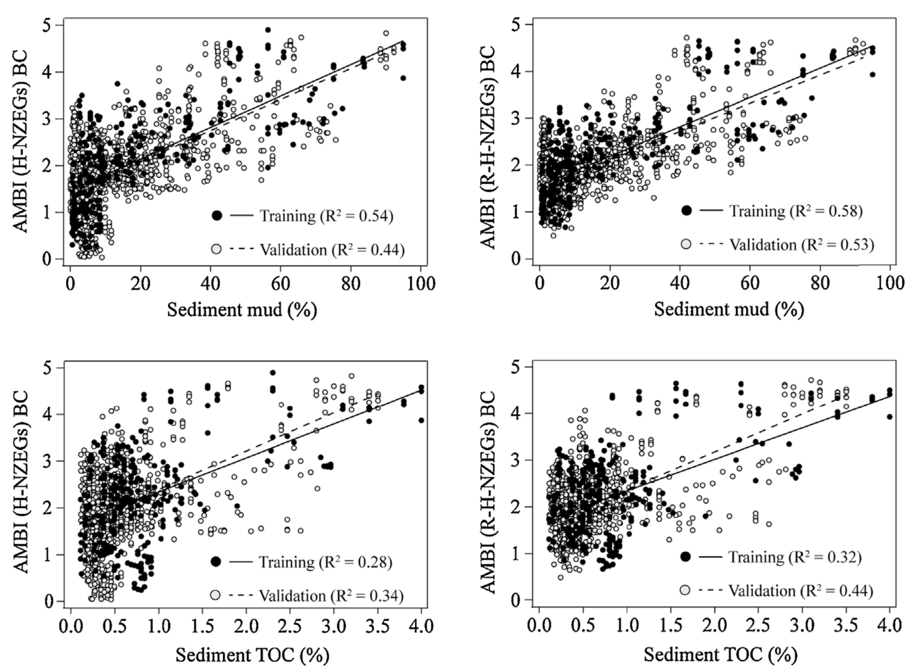

Fig. 6. Response of the AMBI (H-NZEGs) (left) and AMBI (R H-NZEGs) (right) coefficient in terms of mud and TOC gradients. Plots included calibration ($n = 307$) and validation datasets ($n = 1065$).

Table 5
Summary of regressions between the response of the AMBI (H-NZEGs) and AMBI (R H-NZEGs) coefficient to mud and TOC gradients using the calibration (Cal), validation (Val) and the combined (Comb) calibration/validation datasets. Residual standard error (RSE) and degrees of freedom (df) also reported for each model. CV = cross validation.

Index	Stress gradient	Dataset used	Model equation	R^2	R^2 (10-fold CV)[b]	RSE	df
H-NZEGs	Mud	Cal	$y = 0.033x + 1.531$	0.54		0.71	305
		Val	$y = 0.032x + 1.484$	0.44		0.68	1063
	TOC	Comb[a]	$y = 0.032x + 1.491$	0.48	0.47	0.69	1370
		Cal	$y = 0.708x + 1.635$	0.28		0.89	305
R-H-NZEGs	Mud	Val	$y = 0.856x + 1.488$	0.34	0.33	0.74	1063
		Comb[a]	$y = 0.812x + 1.522$	0.33		0.78	1370
	TOC	Cal	$y = 0.030x + 1.665$	0.58	0.55	0.60	305
		Val	$y = 0.029x + 1.597$	0.53		0.51	1063
		Comb[a]	$y = 0.029x + 1.608$	0.55	0.40	0.53	1370
		Cal	$y = 0.666x + 1.739$	0.32		0.76	305
		Val	$y = 0.796x + 1.584$	0.44		0.56	1063
		Comb[a]	$y = 0.760x + 1.618$	0.41		0.61	1370

[a] Combined calibration/validation dataset ($n = 1372$ samples).
[b] R^2 value obtained through 10-fold cross validation.

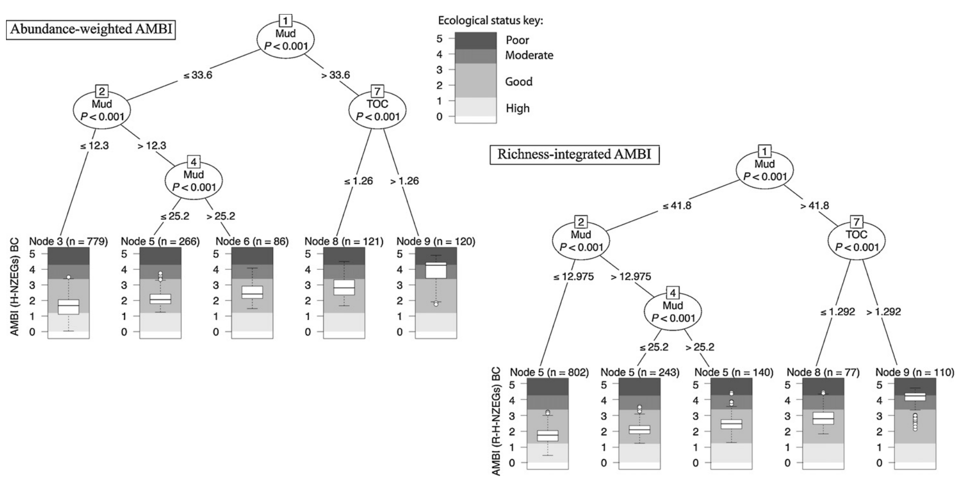

Fig. 7. Regression trees explaining the (left) abundance-weighted and (right) richness-integrated Hybrid AMBI (NZEGs) biotic coefficients in terms primary sedimentary stressors, muddiness and organic enrichment (TOC). Descending from each tree's apex, threshold values of the dominant abiotic (explanatory) variable partition the AMBI (Hybrid NZ-EGs) scores into five relatively distinct groups (see Nodes at bottom of trees). In each node, the lines extend the range, the box extends from the 25th to the 75th percentile and the central line represents median (50th percentile). Open circles identify outliers. With both trees based on the combined calibration and validation datasets ($n = 1372$), the abundance-weighted AMBI tree (left) and richness-integrated tree (right) explained 98.8% and 99.4% of the total variance, respectively.

culminating in three relatively distinct groups based on the responses of each coefficient (see respective nodes at bottom of each tree). Within each of these three nodes, median scores increased, indicating decreasing abundances and richness of sensitive taxa (EG I & II) and increasing abundances and richness of tolerant taxa (EGs IV & V) as sediment muddiness increased. In terms of benthic condition, coefficients in each node ranged from 'Normal' (BC: 1.2–3.3) to 'Transitional to pollution' (BC 3.3–4.3), according to existing AMBI condition bands (Table 2). TOC concentrations were only used as a split criterion if mud content was very high (33.6 and 41.8%, respectively), indicating organic enrichment rather than muddiness as the primary stressor for that split. Sites where sediment TOC contents exceeded 1.2% had median scores corresponding to the 'Transitional to pollution' condition band. None of these classification thresholds varied when mud and TOC data were log-transformed.

In addition to the trees, piecewise regression performed on the TOC distribution data suggested an additional breakpoint in the AMBI for both coefficients at ~3% TOC (breakpoint $P < 0.05$, for both H-NZEGs and R-H-NZEGs) (Fig. 8), with the majority of scores beyond this threshold fitting the 'Polluted' (BC: >4.3) condition band. Organic carbon rather than muddiness was used as the explanatory variable to search for additional breakpoints because regression trees indicated that TOC had the strongest association with the AMBI in elevated mud/TOC situations, and was therefore likely to be responsible for any consequent breakpoints.

4. Discussion

Given the increasing global trend of coastal degradation linked to sedimentation and eutrophication (Halpern et al., 2008), the AMBI is routinely employed worldwide because it provides a cost-effective means of assessing the health of coastal resources and environments for management. Here, the efficacy and robustness of the AMBI were improved through integration of local ecological group information (Robertson et al., 2015), through the addition of taxon richness and the derivation of thresholds that delimit benthic condition along primary estuarine stressor gradients.

Of the three ecological group (EG) classification schemes, the strongest AMBI biotic coefficient (BC) comprised a hybrid of predominantly local NZEGs supplemented by international EGs where no local sensitivity data were available (i.e. the H-NZEGs). There are three primary reasons for this result. Firstly, the AMBI performs better when the assessment accounts for an increasing percentage of taxa in a sample (Borja and Muxika, 2005; Muxika et al., 2007) and the H-NZEGs accounted for the greatest number of taxa in the schemes assessed. Notably though, differences in fit between the H-NZEGs and NZEGs schemes (0.01 R^2 differential) were negligible,

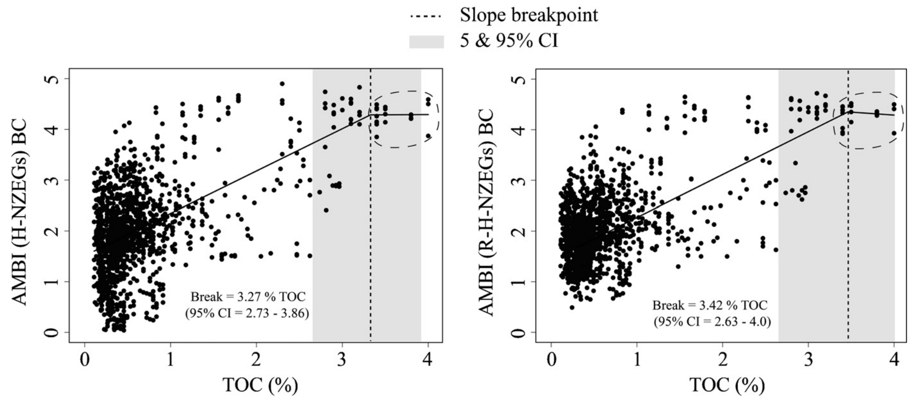

Fig. 8. Piecewise regression of the (left) abundance-weighted and (right) taxon richness-integrated AMBI (H-NZEGs) biotic coefficients relative to sediment total organic carbon content using the combined training and validation datasets ($n = 1372$). All data points inside dashed ellipses on both plots belong to New River and Jacobs River Estuaries (Estuary code 15 and 18, respectively; Table 1). Slope breakpoints and confidence intervals obtained using the 'segmented' package in R.

indicating that local classifications alone were of greater influence on the AMBI than the hybrid approach. Secondly, in developing the local NZEGs, we accounted for the differences in sensitivity of macrobenthic taxa along environmental gradients (see references in Bolnick et al., 2012) and/or throughout their area of occurrence (Zettler et al., 2013), both of which are poorly represented in the international AMBI list (Zettler et al., 2013). Therefore the local NZEGs were more appropriate for use in NZ estuaries because they were developed to represent the sensitivities of a comprehensive suite of indicator taxa in these systems. Finally, because the NZEGs target the primary stressors identified in shallow NZ estuaries they are likely to characterise the benthic condition more accurately than the international AMBI, which targets disturbance along a broader (i.e. not stressor-specific) environmental gradient.

The present results also demonstrated that when underpinned by the standard international EGs alone, the AMBI was inadequate in tracking macrobenthic condition along key stressor gradients in New Zealand estuaries. This is likely due to only 56 of the 100 taxa classified using the global list sharing the same classification in the NZEGs. Inconsistencies in EG classification worldwide may be attributable to some form of genetic predisposition to stress among functionally similar species (i.e. variation within genera) developed through exposure to differing levels of stressor intensity over time (Brown-Peterson et al., 2005; Watanabe et al., 2007; Lilja et al., 2008). This seems a plausible explanation given New Zealand's relative geographic isolation, more recent colonisation (early 1800s) and hence shorter history of anthropogenic pollution. Alternatively or additionally, inconsistencies in EG classification worldwide may be explained by the fact that the global list of EGs was assigned through expert judgement, so classifications will largely depend on specific experience/knowledge with respect to a particular taxon (Keeley et al., 2012; Zettler et al., 2013), whereas the NZEGs were derived using quantitative means. This point highlights that without the integration of defensible local classifications (preferably quantitatively derived), the AMBI is likely to perform poorly, as was reflected in a previous unsuccessful attempt to apply the AMBI to two upper North Island (NZ) estuaries (Rodil et al., 2013). It is therefore inadvisable to rely solely on the global list when applying the AMBI to new locations outside those for which it was originally developed, namely predominantly European estuaries (Grémare et al., 2009; Gillett et al., 2015).

Because the standard AMBI lacks a richness metric, the M-AMBI was developed, which incorporates terms for both taxon richness and Shannon–Wiener diversity. The M-AMBI was tested in this study but was shown to perform poorly, revealing only weak relations along both environmental gradients. Such failures of the M-AMBI have also been reported elsewhere e.g. Southern California (Teixeira et al., 2012). For this reason, we explored another approach through the integration of a richness term directly into the equation used to calculate coefficients. That is, richness for each EG was introduced into the weighted AMBI BC equation in the same way as abundance. This modified index provides a more accurate representation of the combined abundance and richness results than in the M-AMBI where the richness metric excludes sensitivity groupings. This computationally simple inclusion resulted in a reduction in the 'noise' in AMBI scores at lower mud and TOC concentrations. It thereby renders the AMBI a more powerful tool in both natural and disturbed situations, at least within NZ estuaries.

To assess the fit of existing AMBI condition bands to the combined calibration/validation dataset, regression trees were employed to identify classification thresholds that grouped AMBI scores based on their relations to mud and TOC. Coefficients based on both the abundance weighted (H-NZEGs) and richness-integrated (R-H-NZEGs) equations ranged from ~0 to 5, with tree-based classification thresholds delineating benthic condition accurately with respect to existing bands. The trees identified sediment mud content as the dominant abiotic driver of benthic condition up to ~30% mud, beyond which (i.e. in the ~30–95% mud range) TOC became the focal stressor with disturbance thresholds at ~1.2% and 3% TOC. Such thresholds correspond to the AMBI disturbance bands as follows: 2% to ~30% mud reflected a 'normal' to 'impoverished' macrofaunal community, or 'high' to 'good' ecological status; ~30% mud to 95% mud and TOC ~1.2–3% reflected an 'unbalanced' to 'transitional to pollution' macrofaunal community, or 'good' to 'moderate' ecological status; and >3–4% TOC reflected a 'transitional to pollution' to 'polluted' macrofaunal community, or 'moderate' to 'poor' ecological status. The latter shift, which coincides with relevant thresholds of TOC calculated for estuaries in other parts of the globe (e.g. Hyland et al., 2005; Sutula et al., 2014), is likely to be linked with oxygen depletion and buildup of toxic by-products (ammonia and sulphide) associated with the breakdown of organic materials (Hyland et al., 2005).

The identification of these mud/TOC macrofaunal response thresholds provides strong evidence that subtle shifts in mud and TOC concentrations represent significant ecological changes. For example, in this study a shift from 'good' to 'moderate' corresponded to loss of a high value, sensitive bivalve (*Tellina liliana*, formally *Macomona liliana*) and polychaete (*Scoloplos cylindrifer*), both of which play a key role in ecosystem functioning (organic matter recycling) in soft sediment habitats (Karlson et al., in press). Meanwhile, a shift from 'moderate' to 'poor' was accompanied by the replacement of widely tolerant taxon (e.g. the surface deposit-feeding gastropod snail, *Amphibola crenata*, and the surface predating anthozoan, *Anthopleura aureoradiata*) with fewer highly tolerant, highly mobile taxon (e.g. surface scavenging crab, *Helice crassa*, and subsurface deposit-feeding polychaete worm, *Scolecolepides* spp.). Clearly, ensuring such detrimental changes are anticipated and understood is vital for effective ecosystem-level management decisions. This can be achieved if the AMBI/mud/TOC output is supplemented with information pertaining to the ecological function of particular taxa lost or gained at a site over time. We also recommend that any future refinements to the AMBI include the output of such information.

In conclusion, the main strengths of the AMBI biotic index are its continual development, informed through research on a global scale; its international application at the forefront of environmental management, e.g. in many European countries, America, Africa, Asia and Oceania; that comparisons can be made with benthic condition relative to various stressor levels around the world; and its ecologically defensible foundation. In this study, the AMBI was further strengthened by the addition of quantitative information. In order to provide confidence in the AMBI's applicability, and because indices tend to be sensitive to local conditions (Stephens et al., 2015), we also calibrated and validated it for use in shallow estuaries NZ-wide, where it was shown to be a robust proxy of stress relating to the two dominant issues affecting macrobenthic communities in these systems, sediment mud content and organic enrichment. In terms of the management of shallow estuaries, this research provides a valuable approach to better determine the abiotic stressor/ecological response relationship at a fine spatial scale (sites within estuaries). This leads to a more accurate diagnosis of likely causes of degradation than current approaches where the possible stressors can only be attributed generically to "anthropogenic disturbance". At a broad spatial scale (estuary-wide) it demonstrates that by measuring mud and TOC concentrations alone a low cost means of screening an estuary for likely biotic condition is achievable.

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References

Bald, J., Borja, A., Muxika, I., Franco, J., Valencia, V., 2005. Assessing reference conditions and physico-chemical status according to the European Water Framework Directive: a case-study from the Basque Country (Northern Spain). Mar. Pollut. Bull. 50, 1508–1522.

Baritone, E., Rosati, I., Reizopoulou, S., Basset, A., 2012. Linking classification boundaries to sources of natural variability in transitional waters: a case study of benthic macroinvertebrates. Ecol. Ind. 12, 105–122, http://dx.doi.org/10.1016/j.ecolind.2011.04.014.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., et al., 2012. Why intraspecific trait variation matters in community ecology. Trends. Ecol. Evol. 26, 183–192.

Borja, A., Basset, A., Bricker, S., Dauvin, J., Elliot, M., Harrison, T., Marques, J., Weisberg, S., West, R., 2012. Classifying ecological quality and integrity of estuaries. In: Wolanski, E., McLusky, D. (Eds.), Treatise on Estuarine and Coastal Science. , pp. 125–162.

Borja, A., Bricker, S.B., Dauer, D.M., Demetriades, N.T., Ferreira, J.G., Forbes, A.T., Hutchings, P., Jia, X., Kenchington, R., Carlos Marques, J., Zhu, C., 2008. Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Mar. Pollut. Bull. 56, 1519–1537.

Borja, A., Franco, J., Muxika, I., 2003. Classification tools for marine ecological quality assessment: the usefulness of macrobenthic communities in an area affected by a submarine outfall. In: ICES C 2003/Session J-02, Tallinn, Estonia, Sept 24–28, pp. 1–10.

Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Mar. Pollut. Bull. 40, 1100–1114.

Borja, A., Muxika, I., 2005. Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality. Mar. Pollut. Bull. 50, 787–789.

Borja, A., Tunberg, B., 2011. Assessing benthic health in stressed subtropical estuaries, eastern Florida, USA using AMBI and M-AMBI. Ecol. Ind. 11, 295–303.

Brown-Peterson, N.J., Larkin, P., Denslow, N., King, C., Manning, S., Brouwer, M., 2005. Molecular indicators of hypoxia in the blue crab *Callinectes sapidus*. Mar. Ecol. Prog. Ser. 286, 203–215.

Brady, J.P., Ayoko, G.A., Martens, W.N., Goonetilleke, A., 2015. Development of a hybrid pollution index for heavy metals in marine and estuarine sediments. Environ. Monit. Assess. 306 (187), 1–14.

Brenden, T.O., Wang, L., Su, Z., 2008. Quantitative identification of disturbance thresholds in support of aquatic resource management. Environ. Manag. 42, 821–832.

Callier, M.D., McKindsey, C.W., Desrosiers, G., 2008. Evaluation of indicators used to detect mussel farm influence on the benthos: two case studies in the Magdalen Islands, Eastern Canada. Aquaculture 278, 77–88.

De'ath, G., Fabricius, K.E., 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. Ecology 81, 3178–3192.

Dormann, C.F., 2007. Effects of incorporating spatial autocorrelation into the analysis of species distribution data. Global. Ecol. Biogeogr. 16, 129–138.

Gillett, D.J., Weisberg, S.B., Grayson, T., Hamilton, A., Hansen, V., Leppo, E.W., Pelletier, M.C., Borja, A., Cadien, D., Dauer, D., Diaz, R., Dutch, M., Hyland, J.L., Kellogg, M., Larsen, P.F., Levinton, J.S., Llansó, R., Lovell, L.L., Montagna, P.A., Pasko, D., Phillips, C.A., Rakocinski, C., Ranasinghe, J.A., Sanger, D.M., Teixeira, H., Van Dolah, R.F., Velarde, R.G., Welch, K.I., 2015. Effect of ecological group classification schemes on performance of the AMBI benthic index in US coastal waters. Ecol. Ind. 50, 99–107.

Grémare, A., Labrune, C., Vanden Berghe, E., Amouroux, J.M., Bachelet, G., et al., 2009. Comparison of the performances of two biotic indices based on the MacroBen database. Mar. Ecol. Prog. Ser. 382, 297–311, http://dx.doi.org/10.3354/meps08030.

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map of human impact on marine ecosystems. Science 319, 948–952.

Hume, T.M., Snelder, T., Weatherhead, M., Liefting, R., 2007. A controlling factor approach to estuary classification. Ocean Coast. Manag. 50, 905–929.

Hyland, J., Balthis, L., Karakassis, I., Magni, P., Petrov, A., Shine, J., Vestergaard, O., Warwick, R., 2005. Organic carbon content of sediments as an indicator of stress in the marine benthos. Mar. Ecol. Prog. Ser. 295, 91–103.

Karlson, A.M.L., Niemand, C., Savage, C., Pilditch, C.A., 2016. Density of key-species determines efficiency of macroalgal uptake by intertidal benthic communities. PLOS ONE (in press).

Keeley, N.B., Macleod, C.K., Forrest, B.M., 2012. Combining best professional judgement and quantile regression splines to improve characterisation of macrofaunal responses to enrichment. Ecol. Indic. 12, 154–166.

Lilja, K., Prevodnik, A., Garderström, J., Elfwing, T., Tedengren, M., Bollner, T., 2008. Regional differences in mRNA responses in blue mussels within the Baltic proper. Comp. Biochem. Physiol. C 148, 101–106.

Muggeo, V.M.R., 2012. Segmented: Segmented Relationships in Regression Models with Breakpoints/Changepoints Estimation. R Package Version 0.2-9.3, http://cran.r-project.org/web/packages/segmented/.

Muniz, P., Venturini, N., Pires-Vanin, A.M.S., Tommasi, L.R., Borja, A., 2005. Testing the applicability of a Marine Biotic Index (AMBI) to assessing the ecological quality of soft-bottom benthic communities, in the South America Atlantic region. Mar. Ecol. Prog. Ser. 50, 624–637.

Muxika, I., Borja, Á., Bald, J., 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Mar. Ecol. Prog. Ser. 55, 16–29.

Muxika, I., Borja, A., Bonne, W., 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecol. Ind. 5, 19–31.

Pusceddu, A., Dell'Anno, A., Fabiano, M., Danovaro, R., 2009. Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status. Mar. Ecol. Prog. Ser. 375, 41–52.

Qian, S.S., Cuffney, T.F., 2012. To threshold or not to threshold? That's the question. Ecol. Ind. 15, 1–9.

R Development Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, Available at: http://www.R-project.org (accessed 09.09.15).

Robertson, B.M., Gillespie, P.A., Asher, R.A., Frisk, S., Keeley, N.B., Hopkins, G.A., Thompson, S.J., Tuckey, B.J., 2002. Estuarine Environmental Assessment and Monitoring: A National Protocol. Part A. Development, Part B. Appendices, and Part C Application, Prepared for supporting Councils and the Ministry for the

Environment, Sustainable Management Fund Contract No. 5096 Part A 93p. Part B. 159p. Part C. 40p plus field sheets.
Robertson, B.P., Gardner, J.P.A., Savage, C., 2015. Macrobenthic–mud relations strengthen the foundation for benthic index development: a case study from shallow, temperate New Zealand estuaries. Ecol. Ind. 58, 161–174.
Robertson, B.M., Stevens, L., Robertson, B.P., Zeldis, J., Green, M., Madarasz-Smith, A., Plew, D., Storey, R., Hume, T., Oliver, M., 2016a. New Zealand Estuary Trophic Index Screening Tool 1. Determining Eutrophication Susceptibility Using Physical and Nutrient Load Data, Prepared for Envirolink Tools Project: Estuarine Trophic Index, MBIE/NIWA Contract No: CO1X1420. 47p.
Robertson, B.M., Stevens, L., Robertson, B.P., Zeldis, J., Green, M., Madarasz-Smith, A., Plew, D., Storey, R., Hume, T., Oliver, M., 2016b. New Zealand Estuary Trophic Index Screening Tool 2. Determining Monitoring Indicators and Assessing Estuary Trophic State, Prepared for Envirolink Tools Project: Estuarine Trophic Index, MBIE/NIWA Contract No: CO1X1420. 68p.
Rodil, I.F., Lohrer, A.M., Hewitt, J.E., Townsend, M., Thrush, S.F., Carbines, M., 2013. Tracking environmental stress gradients using three biotic integrity indices: advantages of a locally-developed traits-based approach. Ecol. Ind. 34, 560–570.
Sigovini, M., Keppel, E., Tagliapietra, D., 2013. M-AMBI revisited: looking inside a widely-used benthic index. Hydrobiologia 717, 41–50.
Stephens, P.A., Barlow, J., Pettorelli, N., Whittingham, M.J., Cadotte, M.W., 2015. Management by proxy? The use of indices in applied ecology. J. Appl. Ecol. 52, 1–6.
Sutula, M., Green, L., Cicchetti, G., Detenbeck, N., Fong, P., 2014. Thresholds of adverse effects of macroalgal abundance and sediment organic matter on benthic habitat quality in estuarine intertidal flats. Estuar. Coasts, http://dx.doi.org/10.1007/s12237-014-9796-3.
Teixeira, H., Weisberg, S.B., Borja, A., Ranasinghe, J.A., Cadien, D.B., Velarde, R.G., Lovell, L.L., Pasko, D., Phillips, C.A., Montagne, D.E., Ritter, K.J., Salas, F., Marques, J.C., 2012. Calibration and validation of the AZTI's Marine Biotic Index (AMBI) for Southern California marine bays. Ecol. Ind. 12, 84–95.
Toms, J.D., Villard, M.A., 2015. Threshold detection: matching statistical methodology to ecological questions and conservation planning objectives. Avian Conserv. Ecol., http://dx.doi.org/10.5751/ACE-00715-100102.
US EPA, 2009. Monitoring Guidance for the National Estuary Program, EPA 842-B-92-004., pp. 75.
Watanabe, H., Takahashi, E., Nakamura, Y., Oda, S., Tatarazako, N., Iguchi, T., 2007. Development of a *Daphnia magna* DNA microarray for evaluating the toxicity of environmental chemicals. Environ. Toxicol. Chem. 26, 669–676.
Zettler, M.L., Proffitt, C.E., Darr, A., Degrader, S., Devisee, L., et al., 2013. On the myths of indicator species: issues and further consideration in the use of static concepts for ecological applications. PLoS ONE 8 (10), e78219.