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Ecological quality assessment in the Eastern Mediterranean combining live and dead molluscan assemblages

Yael Leshno [a,b], Chaim Benjamini [a], Yael Edelman-Furstenberg [b,*]

[a] Department of Geological and Environmental Sciences, Ben Gurion University of the Negev, Beer Sheva 84105, Israel
[b] Geological Survey of Israel, 30 Malchei Yisrael St., 95501 Jerusalem, Israel

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ABSTRACT

The EU directive to quantify ecological quality by deviation from pre-impacted conditions often fails to be implemented because past information is usually incomplete or missing. Molluscan death assemblages, representing long-term accumulation of shells on the sea floor, average out short-term variability and can serve as a baseline for quality assessment. AMBI, Bentix and Shannon–Wiener indices were calculated for live and dead assemblages from polluted and control stations on the highly oligotrophic Levantine shallow shelf of Israel. Bentix successfully tracked deterioration over time, from moderate EcoQS in the dead to poor in the live assemblage. Additional modification of the ecological classification of species by scoring the naturally abundant *Corbula gibba* as pollution-sensitive improved the utility of the Bentix index in monitoring in this part of the Mediterranean. This adjustment of Bentix, and use of death assemblages for an ecological baseline, should therefore be incorporated in monitoring for compliance with EU directives.

1. Introduction

Recent anthropogenic modification of marine environments has led to the decline of marine ecosystems worldwide (Magni, 2003; Lotze et al., 2006; Halpern et al., 2012). The European Union established two directives in order to better preserve and protect marine environments around the Mediterranean: the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD; 2008/56/EC). The WFD and WSFD classify marine water bodies according to their ecological health, represented by an Ecological Quality Status (high, good, moderate, poor and bad) (Mee et al., 2008; Borja et al., 2010, 2011; Van Hoey et al., 2010). The directives aim to achieve good environmental status for European water bodies by 2015 and 2020, for WFD and MSFD respectively (Borja et al., 2009a; Rice et al., 2012; Simboura et al., 2014). Chemical, sedimentological and biological indicators are used to determine an Ecological Quality Status (EcoQS) of a system (Simboura and Zenetos, 2002). The WFD and WSFD promoted the use of, and search for, new ecological quality indicators for comparative purposes (Borja and Muxika, 2005; Hering et al., 2006; Borja et al., 2009b, 2012). Following the publication of these directives, many studies have compared various benthic indices aimed at finding the method most suitable for the EcoQS assessment of a particular marine environment (e.g., Muxika et al., 2007; Simonini et al., 2009; Simboura and Argyrou, 2010; Souza et al., 2013; Simboura et al., 2014; Hutton et al., 2015; Brauko et al., 2015). The most commonly used faunal indices are the AMBI (Borja et al., 2000) and Bentix (Simboura and Zenetos, 2002) indices, which categorize species into ecological groups based on their sensitivity or tolerance to pollution, following Pearson and Rosenberg's (1978) model of species response to organic enrichment. The relative scales of other often used diversity indices, such as the Shannon–Wiener index, were standardized to correspond to the EcoQS of the WFD (e.g., Rice et al., 2012). The WFD and WSFD define the ecological status of a system as the deviation from pre-impacted reference conditions. However, in many cases information on natural conditions is incomplete or missing, and left to expert judgment in the absence of robust data (Borja et al., 2012).

A record of the composition and structure of pre-anthropogenic live communities is preserved in long-term accumulations of empty shells on the sea floor, quantified and defined as a 'death assemblage' (Kidwell, 2007). Shells from consecutive generations are mixed over time by burrowing organisms and physical reworking of shells on the sea floor, resulting in a 'time-averaged' record that can be compared to the living community. This type of record averages out variations in the live community such as subannual, seasonal and reproductive cycles, as well as multiyear variations in the living community and is a long-term baseline record of the ecosystem (see Kidwell, 2013 for review). Under natural conditions, there is good agreement in the structure and composition of species between the dead time-averaged assemblage and the living community (Olszewski and Kidwell, 2007). Rapid and severe anthropogenic changes to the living community

* Corresponding author.
E-mail addresses: yael.leshno@mail.huji.ac.il (Y. Leshno), chaim@exchange.bgu.ac.il (C. Benjamini), yael@gsi.gov.il (Y. Edelman-Furstenberg).

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('live assemblage') from the baseline time-averaged record, create a mismatch in ecological indices between the live and dead assemblages (e.g., species composition, diversity, and dominance). Therefore, the comparison of live vs. dead assemblages is a potentially valuable tool in environmental assessment (Kidwell, 2007).

The method used for creating a comparative database is to collect the dead shells left behind by the macrobenthic fauna by sieving the top of the sea floor sediment, use them to reconstruct an ecological baseline, and then compare them to the live assemblage from the same locality (Kidwell, 2007, 2013). A statistically robust baseline can be constructed for comparative purposes, overcoming the subjective bias inherent in 'expert judgment' in monitoring compliance with the WFD and MSFD.

This study compares live and dead assemblages of molluscan faunas from a monitored station near the Dan Region Wastewater Project (Shafdan), to control stations at the same depth representing the highly oligotrophic natural shallow shelf of the Levantine margin offshore Israel. The AMBI, Bentix and Shannon–Wiener (H′) indices were calculated for live and dead assemblages of molluscan faunas, to evaluate their utility as indicators of EcoQS of polluted and control stations for the Eastern Mediterranean. This study is the first application of the AMBI and Bentix indices on time-averaged molluscan death assemblages, an approach that could be widely used to determine EcoQS in environments for which there is no available reference database. This is also the first test of utility of the AMBI and Bentix indices in the Eastern Mediterranean, east of Greece.

2. Material and methods

2.1. Study area

The Levantine coast of Israel trends N-NE–S-SW in a nearly straight line over 180 km, varying in width from 10 to 25–30 km (Inman and Jenkins, 1984; Rosentraub and Brenner, 2007). The upper 120 m of the water column is stratified during most of the year and becomes mixed in winter (Herut et al., 2000; Rosentraub and Brenner, 2007). The Levantine basin is naturally oligotrophic, characterized by primary production estimated at ca. 45 gC/m^{2}y (Berman et al., 1984; Kress and Herut, 2001; Kress et al., 2004) and Chlorophyll a values that range between 0.009 and 0.4 μg/l (Yacobi et al., 1995). This extreme nutrient depletion is attributed to the westward transport of deep water as part of the anti-estuarine circulation of the Mediterranean (Coll et al., 2010). Furthermore, in the past, the Nile River was the main supplier of nutrients, until the construction of the High Aswan Dam in 1965 (Stanley, 1988; Herut et al., 2000). Since then, nutrient levels have become exceedingly low in the coastal zone (Inman and Jenkins, 1984), with negative changes to the marine food webs and on fisheries (Nixon, 2003).

On the other hand, recent urbanization of the Israeli coast reversed this trend with considerable input of nutrients to the shallow shelf via direct outfalls (EEA, 2001; Kress et al., 2004). The largest single discharge is from the Shafdan wastewater treatment plant that has been discharging sewage sludge onto the shelf at 36 m water depth since 1987 (Kress et al., 2004; Hyams-Kaphzan et al., 2009). The Shafdan produces 292,000 dry metric tons of activated sewage sludge annually, most of which is redirected to agriculture and landfill. On average 16,000 m^3/day of excess sewage sludge are discharged through a single seabed pipe line emptying 5 km offshore 15 km to the south of Tel Aviv (Kress et al., 2004) (Fig. 1). The area was not polluted prior to the activation of the outfall (Galil and Lewinsohn, 1981). Permanent control and polluted monitoring stations have been established in the area by the Israel Oceanographic and Limnological Research institute (IOLR) to the north and south of the outfall, sub-parallel to the coastline (Kress et al., 2004). Previous research in the area focused on the effects of sewage sludge on polychaetes (Kress et al., 2004) and benthic foraminifera (Hyams-Kaphzan et al., 2009), showing that the impact was localized.

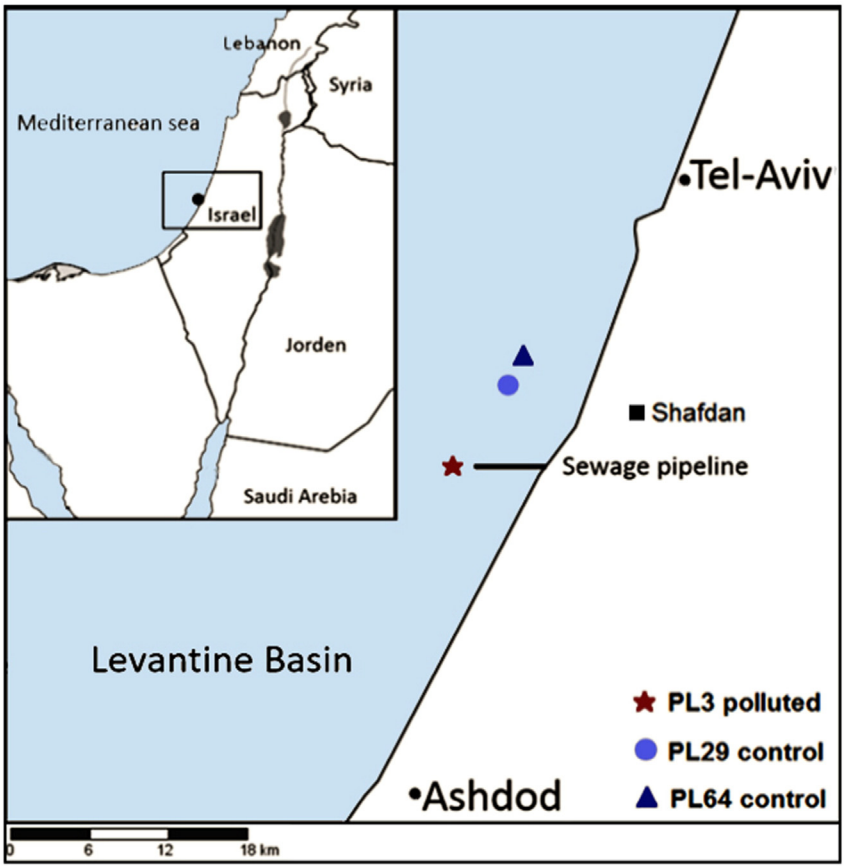

Fig. 1. Location map of the Israeli coast showing the position of the Shafdan sewage sludge outlet and polluted and control sampling stations.

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Table 1
The ecological indices and threshold values corresponding to the WFD and WSFD Ecological Quality Status (EcoQS).

Ecological indices		Ecological Quality Status (EcoQS)				
		High	Good	Moderate	Poor	Bad
AMBI	$\{(0 \times \%GI) + (1.5 \times \%GII) + (3 \times \%GIII) + (4.5 \times \%GIV) + (6 \times \%GV)\} / 100$	0.0–1.2	1.2–3.3	3.3–4.3	4.3–5.5	5.5–6
Bentix	$\{(6 \times \%GS) + (2 \times \%GT)\} / 100$	4.5–6	3.5–4.5	2.5–3.5	2.0–2.5	0
Shannon–Wiener (H′)	$-\sum(p_i \times \log2(p_i))$	>3.8	3.0–3.8	1.9–3.0	0.9–1.9	<0.9

Although sludge is continuously injected from the Shafdan, it does not accumulate on the sea floor from year to year. Presence of sludge on the seafloor near the outlet depends on the seasonal storm and current regimes and biotic consumption (Kress et al., 2004; Hyams-Kaphzan et al., 2009). The sampling program for this study was at three of IOLR's permanent sampling stations: one polluted station, PL3, located 200 m NE of the outfall at 36 m water depth; control station PL29 at 5.5 km NE of the outfall at 34 m water depth, and control station PL64, located 7 km N-NE of the outfall at 35 m water depth (Fig. 1).

2.2. Field and laboratory methods

Sampling of the polluted (PL3) and control (PL29, PL64) stations took place in the 2012 winter (Jan), spring (May–Jun), summer (July) and autumn (Nov) cruises of R/V Etziona, with the aim of capturing one year of seasonal variability. Polluted station PL3 and control station PL29 were sampled on all cruises, while the second control station PL64 was added in the spring and autumn samplings of 2012 to ensure that control station PL29 was well outside the polluted area. Three replicate sediment samples containing dead molluscs were collected from the top 1.5 cm of a GOMEX box-corer or a Van Veen Grab on each cruise at each station. At each station two replicate samples for live molluscs were collected by dragging a dredge over a 30 m seafloor transect in addition to the live molluscs collected from all box-corer or grab samples. Samples were sieved at 2 mm and specimens were identified to the species level noting whether individuals were live or dead at the time of sampling. Organic enrichment was indicated by TOC (wt.%) from short sediment cores on each cruise at each station.

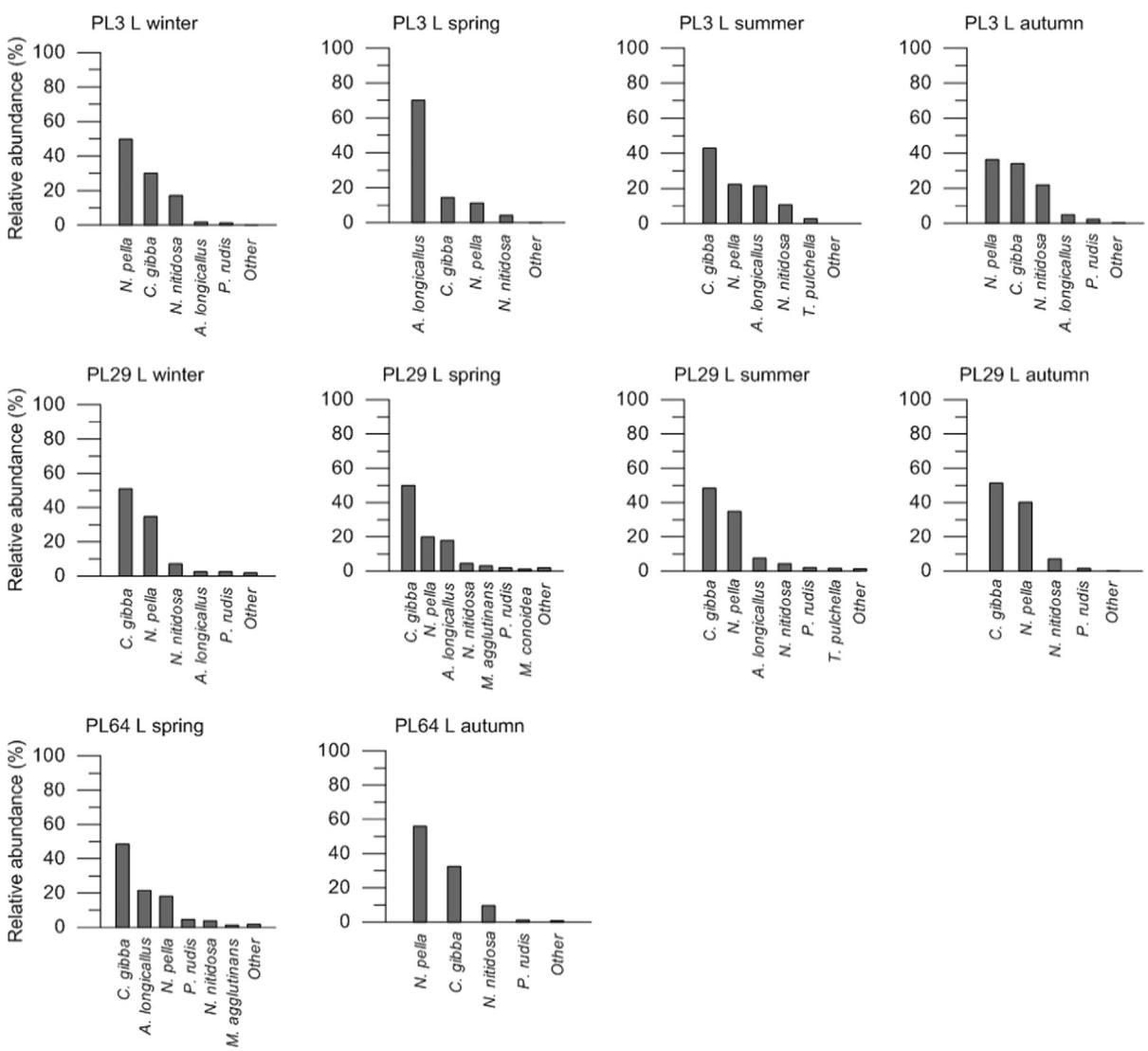

Fig. 2. Relative abundance of the dominant species (>1% of the total number of individuals) in each of the live (L) and death (D) assemblages from the polluted (PL3) and control (PL29, PL64) stations over winter, spring, summer and autumn of 2012.

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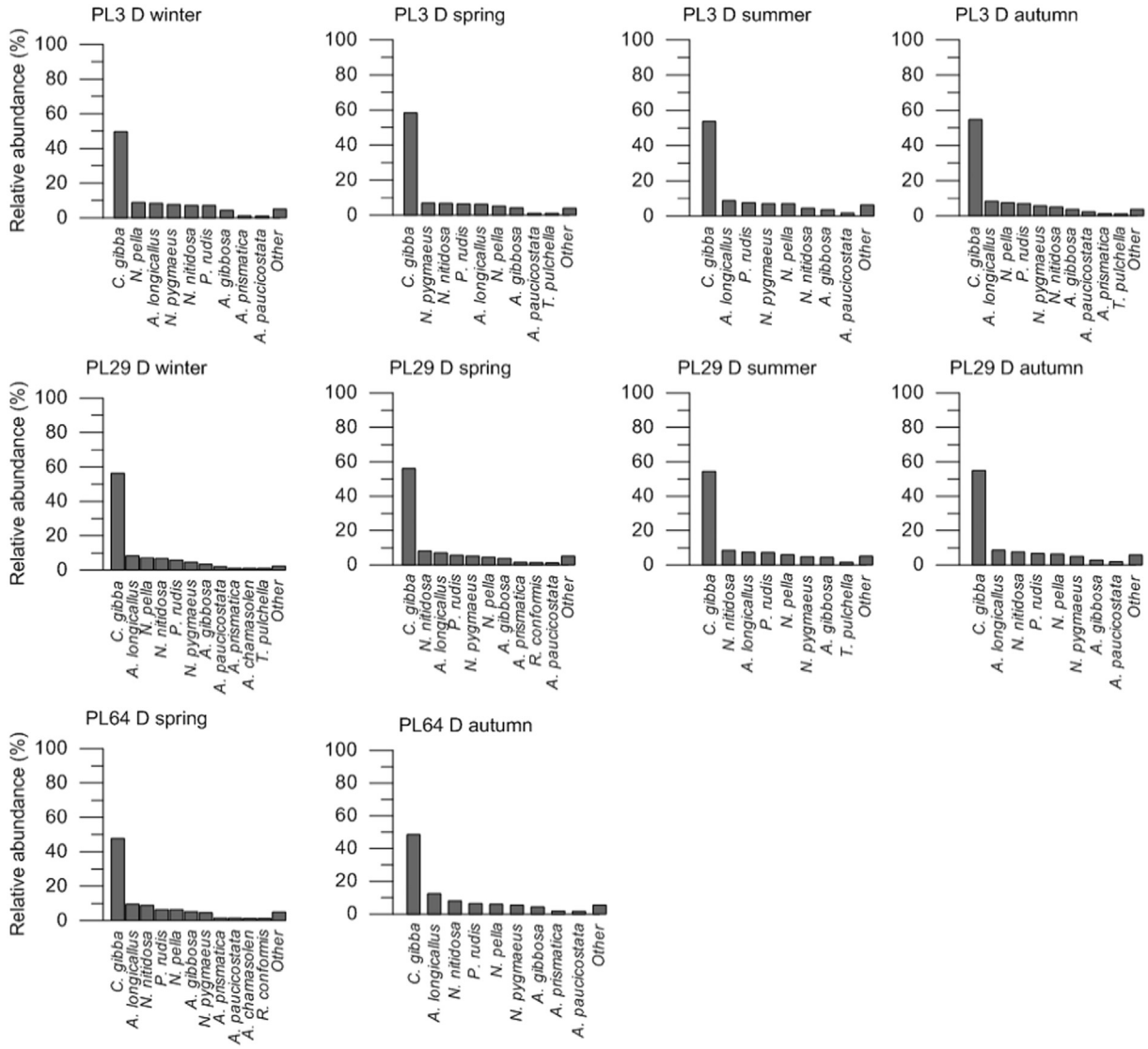

Fig. 2 (*continued*).

2.3. Data analyses

The AMBI, Bentix and Shannon–Wiener indices were calculated for each sample of the live and dead assemblages of the control and polluted stations. The calculated indices and EcoQS threshold values for each ecological index are detailed in Table 1.

AMBI classifies taxa into five ecological groups (EG) based on their sensitivity or tolerance to pollution: Group I (GI) includes very sensitive species that are present only under pristine conditions, GII includes species indifferent to pollution, GIII includes species tolerant to pollution, GIV includes second-order opportunistic species and GV includes first-order opportunistic species (Borja et al., 2000). AMBI ranges from 0 (high ecological status) to 7 (bad status) based on the relative proportion of the five ecological groups in each sample. AMBI was calculated using the free AZTI software v5.0 available from http://www.azti.es.

Bentix (Simboura and Zenetos, 2002) is based on AMBI, but reduces the number of ecological groups to group sensitive (GS, equivalent to AMBI's GI and GII), and group tolerant (GT, equivalent to GIII, GIV and GV of AMBI). Bentix scales range from 2 to 6, with high values reflecting good and low values reflecting bad EcoQS. Bentix was calculated using the Bentix add-in software for Excel v.1.1, available from www.hcmr.gr. Bentix is independent of habitat type, sample size and taxonomic sampling effort, and is considered a robust and effective tool (Simboura and Argyrou, 2010).

The Shannon–Wiener index (H′) is a common measure of species diversity (Salas et al., 2006) that combines the relative abundance of species and the number of taxa found (Magurran, 2004). However, the index has a limited relative scale that makes the interpretation of empirical values difficult (Magurran, 2004). To incorporate H′ within the WFD and MSFD, threshold values were determined to reflect EcoQS, where low values correspond to poor and high values to good environmental status (e.g., Dolven et al., 2013).

All three ecological indices were calculated on the raw abundance data of species and values were averaged for triplicate samples of dead and duplicate samples of the live assemblage at each station and season. An EcoQS was assigned to each mean value according to the threshold values for each index (Table 1). PRIMER-E v.6 of the Plymouth Marine Laboratory (Clarke and Warwick, 1994) was used for the calculation of Shannon–Wiener index and statistical analysis. Student T-test was used to test for differences between live and dead assemblages and between control and polluted stations within each assemblage for all indices. P-values were corrected using the False Discovery Rate (FDR) (Benjamini and Hochberg, 1995) to account for multiple significance testing. Bray–Curtis similarity coefficient was calculated based

Table 2
Bentix, AMBI and Shannon–Wiener (H′) average values and their associated Ecological Quality Status (EcoQS) for the polluted (PL3) and control (PL29, PL64) sampling stations of the live (L) and dead (D) assemblages, over four seasons of 2012 (winter, spring, summer and autumn) from the Israeli Mediterranean shelf.

Live and dead assemblages	AMBI	AMBI std.	AMBI EcoQS	Bentix	Bentix std.	Bentix EcoQS	H′	H′ std.	H′ EcoQS
PL3 D winter	2.65	0.24	Good	2.93	0.12	Moderate	2.67	0.27	Moderate
PL3 D spring	3.02	0.21	Good	2.86	0.13	Moderate	2.32	0.26	Moderate
PL3 D summer	2.88	0.19	Good	2.89	0.11	Moderate	2.58	0.27	Moderate
PL3 D autumn	2.93	0.05	Good	2.76	0.08	Moderate	2.49	0.06	Moderate
PL29 D winter	2.95	0.02	Good	2.71	0.03	Moderate	2.41	0.11	Moderate
PL29 D spring	2.95	0.30	Good	2.74	0.05	Moderate	2.46	0.18	Moderate
PL29 D summer	2.91	0.11	Good	2.80	0.10	Moderate	2.43	0.12	Moderate
PL29 D autumn	2.96	0.10	Good	2.69	0.12	Moderate	2.42	0.14	Moderate
PL64 D spring	2.60	0.26	Good	2.90	0.32	Moderate	2.82	0.25	Moderate
PL64 D autumn	2.74	0.21	Good	2.81	0.20	Moderate	2.67	0.16	Moderate
PL3 L winter	1.43	0.10	Good	2.05	0.00	Poor	1.60	0.23	Poor
PL3 L spring[a]	2.75	–	Good	2.00	–	Poor	1.31	–	Poor
PL3 L summer	2.52	0.49	Good	2.13	0.18	Poor	1.87	0.26	Poor
PL3 L autumn	1.75	0.29	Good	2.09	0.01	Poor	1.85	0.18	Poor
PL29 L winter	2.44	0.11	Good	2.13	0.05	Poor	1.68	0.02	Poor
PL29 L spring	2.80	0.28	Good	2.08	0.02	Poor	1.95	0.22	Poor
PL29 L summer[a]	2.45	–	Good	2.15	–	Poor	1.82	–	Poor
PL29 L autumn	1.97	0.57	Good	2.06	–	Poor	1.42	0.08	Poor
PL64 L spring	2.95	0.35	Good	2.18	0.13	Poor	1.98	0.05	Poor
PL64 L autumn	1.59	0.24	Good	2.08	0.04	Poor	1.51	0.16	Poor

[a] Replicate excluded due to small sample size.

on values of the three benthic indices for each sample and an nMDS ordination was plotted for the live and dead assemblages from all sampling stations and seasons. The R statistic of analysis of similarity (ANOSIM) was used to test for differences between the live and dead assemblages and nested polluted and control stations within each assemblage. Draftsman plot and Pearson correlation coefficient were used to test for correlations between the three benthic indices, TOC and the abundance of the most dominant species.

3. Results

3.1. The molluscan fauna and TOC

In this study, 26 families of bivalves and 20 families of gastropods were identified from the polluted and control stations (full species listing in Table 1 of Leshno et al., 2015). The molluscan fauna was strongly dominated by bivalves, most prominently by the cosmopolitan myoid, *Corbula gibba*. Fig. 2 illustrates the relative abundance of the most abundant species (comprising >1% of the total number of individuals) of the live and dead assemblage at the polluted (PL3) and control (PL29, PL64) stations. The composition of species in the live assemblage remained the same between the control and polluted stations, but the relative abundance of a few species changed seasonally. The most abundant species in the live assemblage were *C. gibba*, *Nuculana pella*, *Abra longicallus* and *Nucula nitidosa*. The death assemblage showed high agreement in taxonomic composition within and between sampling stations and seasons, dominated by the species *C. gibba*, *A. longicallus*, *Pitar rudis*, *N. nitidosa*, *N. pella*, *Anadara gibbosa* and one gastropod species, *Nassarius pygmaeus* (Fig. 2).

TOC (wt.%) measured from sediment cores at PL3 was 2.0% in winter, decreased to 1.1% in spring, and increased in summer and autumn to a maximum of 3.5%. TOC at control stations PL29 and PL64 were fixed at 0.3–0.5% throughout the year.

3.2. Benthic ecological indices

Table 2 lists the mean values of AMBI, Bentix and Shannon–Wiener and their corresponding EcoQS for the live and dead assemblages for each station and season. The AMBI and Bentix ecological group (EG) classification of the dominant species (>1% of the total number of individuals) of the live and dead assemblage is presented in Table 3. The complete list of EG scores for all species is included in Appendix A. AMBI classified the live and dead assemblage in all cases as good, with values ranging from 1.59 to 3.02 for all stations and seasons (Fig. 3). Bentix values varied between 2.00 and 2.18 for all samples of the live assemblage, equivalent to a poor EcoQS. Bentix values of the death assemblage were significantly higher than that of the live assemblage ($t_{(46)} = 18.93$, $p < 0.01$), varying between 2.69 and 2.93, assigning it to a moderate EcoQS (Fig. 4) (Note the reversal of numerical scale for EcoQS between the Bentix and AMBI indices). Likewise, the Shannon–Wiener classified the live assemblage as poor for all seasons and stations (1.31–1.98), while samples of the death assemblage had a significantly higher ($t_{(46)} = 11.85$, $p < 0.01$) moderate EcoQS (2.32–2.82, Table 2). The Bentix and Shannon–Wiener indices showed a significant difference in EcoQS between the live and dead assemblages, while AMBI

Table 3
Ecological index scores and mollusc feeding modes for the dominant species (>1% of the total number of individuals) of the live and death assemblages.

Species	Bentix EG	AMBI EG	Live/dead	% LA	% DA	Feeding mode	Reference
Abra longicallus	2	3	L and D	13	8	Mixed feeder	WoRMS (2014)
Abra prismatica	2	1	D	0	1	Surface deposit feeder	Todd (2001) and WoRMS (2014)
Acanthocardia paucicostata	2	1	D	0	1	Filter feeder	MarLIN (2006)
Anadara gibbosa	1	1	L and D	<1%	4	Suspension feeder	Todd (2001)
Corbula gibba	2	4	L and D	44	54	Suspension feeder	WoRMS (2014)
Nassarius pygmaeus	1	2	D	0	6	Predator	Todd (2001)
Nucula nitidosa	2	1	L and D	8	7	Deposit feeder	WoRMS (2014)
Nuculana pella	2	1	L and D	30	7	Deposit feeder	Todd (2001)
Pitar rudis	1	2	L and D	3	7	Suspension feeder	Todd (2001)
Tellinella pulchella	1	1	L and D	<1%	1	Deposit feeder	Todd (2001)

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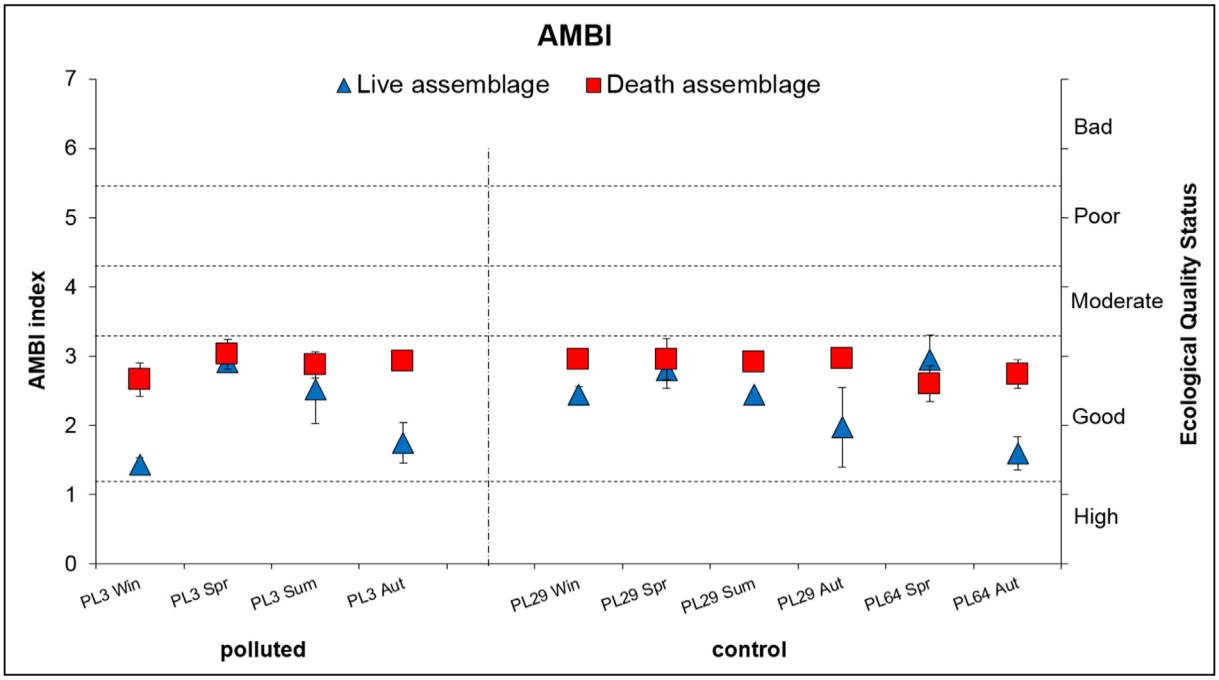

Fig. 3. AMBI values and associated Ecological Quality Status (EcoQS) of the live and dead assemblages at the polluted (PL3) and control (PL29, PL64) stations. Both the live and dead assemblages score a good EcoQS.

showed no significant difference. Within the good status, the live assemblage generally had higher values than the dead assemblage (Table 4). The three benthic indices failed to show variation of EcoQS between the polluted and control stations and seasons within the live assemblage (Table 4).

An nMDS plot based on values of the three benthic indices showed a high degree of separation between the live and dead assemblages; live assemblages from all stations and seasons were widely scattered while the death assemblages plotted more closely to one another (Fig. 5). Overall variations in benthic indices values between the live and dead assemblages were statistically significant (ANOSIM, $R = 0.99$, $p < 0.05$), but variations between polluted and control stations nested within each assemblage were not statistically significant (ANOSIM, $R = 0.018$, $p > 0.05$). Draftsman plot and Pearson correlation

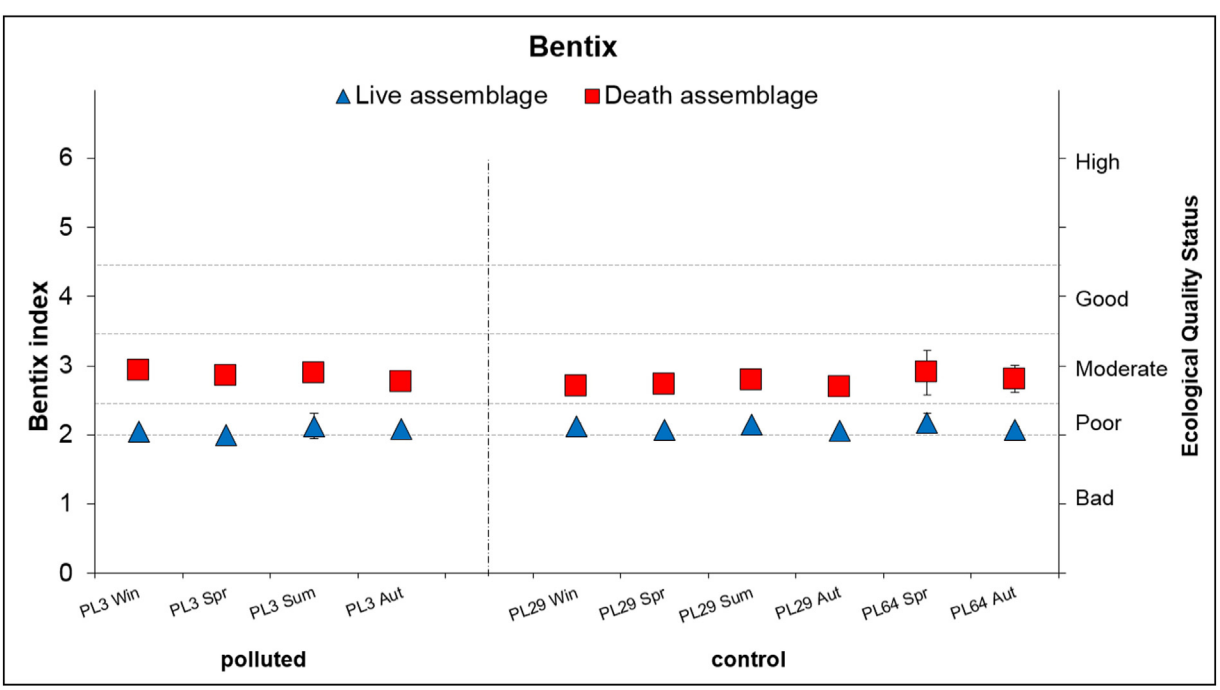

Fig. 4. Bentix values and associated Ecological Quality Status (EcoQS) of the live and death assemblages at the polluted (PL3) and control (PL29, PL64) stations. The live assemblages are all classified as poor while the death assemblages are classified as moderate EcoQS.

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Table 4
Summary of statistical analysis for the three indices for the live and dead assemblages and the control and polluted stations.

	AMBI	Bentix	Shannon–Wiener
Live assemblage	Good	Poor	Poor
Control vs. polluted	$t(16) = 01.21$, $p = 0.24$	$t(16) = -0.72$, $p = 0.48$	$t(16) = -0.10$, $p = 0.92$
Death assemblage	Good	Moderate	Moderate
Control vs. polluted	$t(28) = 0.24$, $p = 0.81$	$t(28) = 1.62$, $p = 0.12$	$t(28) = -0.28$, $p = 0.79$
Live–dead assemblages	$t(20) = 4.38$, $p < 0.01$	$t(45) = 21.15$, $p < 0.01$	$t(46) = 11.85$, $p < 0.01$

p-values were adjusted according to the False Discovery Rate (FDR).

coefficient show a strong significant positive correlation between the Bentix and Shannon–Wiener indices (Fig. 6). Standardization of AMBI and Bentix to a common scale of EQR values resulted in similar Pearson correlation coefficient values.

3.3. Bentix ecological grouping for the Levantine shelf

The relative abundance of tolerant vs. sensitive Bentix ecological groups in the live as compared to the death assemblage is presented in Fig. 7. *C. gibba*, the most common species in nearly all samples, was the dominant species of the GT in the death assemblage, whereas the deposit feeder *N. pella* was the most abundant GT species in the live assemblage (Fig. 7). The relative abundance of the tolerant species (GT, score 2) increased significantly in the live assemblage as compared to the dead ($t_{(47)} = -87.04$, $p < 0.01$), attributed to an increase in the relative abundance of *N. pella* in the live assemblage (Table 3, Fig. 2).

Bentix status did not change between the polluted and control stations of the live assemblage, but seasonal variation in the live relative abundances of *N. pella* and *C. gibba* showed a faunal response to the sewage sludge. The relative abundances of *N. pella* and *C. gibba* and TOC values are plotted against seasons of 2012 at the polluted (PL3) and control (PL29) stations (Fig. 8). The live relative abundance of *C. gibba* was positively correlated to the benthic indices and negatively correlated to TOC values (Fig. 6). At the polluted station, *C. gibba* abundance changed from 50% in winter, 14% in spring as TOC decreased, and 43% in summer when TOC built up, and decreased to 34% in autumn when TOC continued to increase. The relative abundance of *C. gibba* at control station PL29 remained high year-long (ca. 50%) and is not correlated to the slight changes in TOC (Figs. 2, 6 and 8). In contrast to *C. gibba*, *N. pella* showed a strong significant negative correlation with the benthic indices and a positive correlation with TOC (Figs. 6 and 8). The live relative abundance of *N. pella* at PL3 was synchronized with seasonal variation in TOC values, as abundance decreased from 50% in winter to 11% in spring, and increased to 22% in summer and 36% in spring (Fig. 8). In station PL29 the relative abundance of *N. pella* was high (35–40%), but decreased in spring (20%).

The natural high abundance of *C. gibba* (Fig. 2) and its low negative correlation to TOC values (Figs. 6 and 8) suggest that the species tolerant EG classification should be revised for the Levant, and scored as GS instead of GT (Table 3). Fig. 9 shows the Bentix values calculated based on a modified EG classification of *C. gibba* to GS. Overall, the modified Bentix values correspond to higher EcoQS for both the live and dead assemblages, and the live assemblage shows a significant difference in EcoQS between the control (good status) and the polluted (moderate status) stations ($t_{(11)} = 2.96$, $p < 0.01$) (Fig. 9).

4. Discussion

The ecological quality status of a polluted and two control stations in the Shafdan area was assessed by three benthic indices. The death assemblage showed a persistent composition and rank order of species (Fig. 2) with no significant differences in EcoQS between sampling stations and seasons (Table 2, Figs. 3 and 4). This showed that the expectation of averaging out seasonal, reproductive or short-term events that may have influenced the species composition and relative abundance was met. Human-generated ecological changes are often more rapid and intense than natural processes (Kidwell, 2007, 2015). Such rapid anthropogenic changes are marked by a decrease in the input rate of

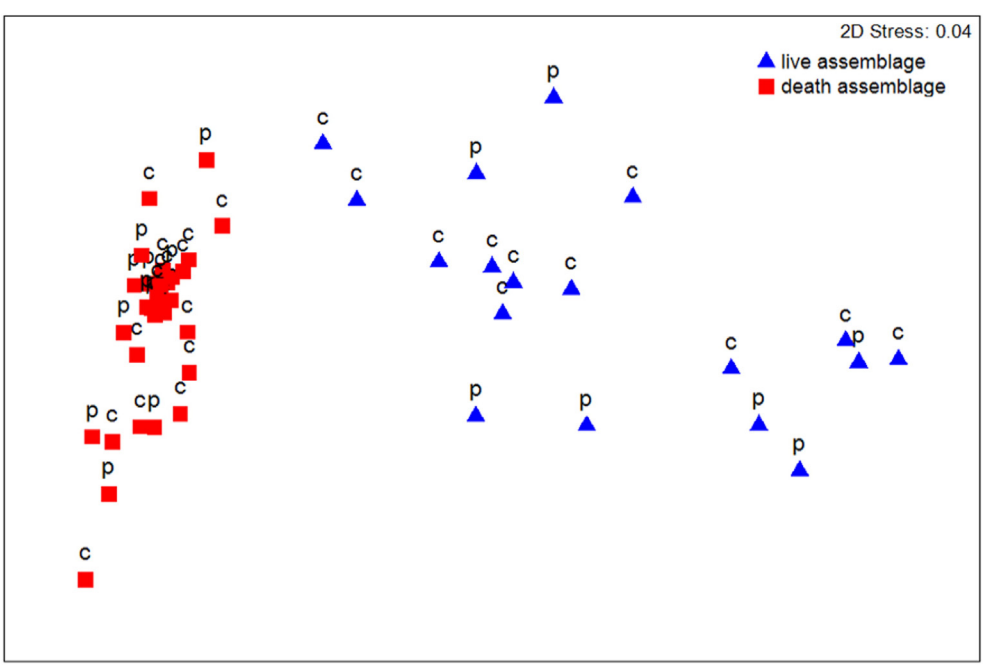

Fig. 5. Multidimensional scaling (nMDS) based on the values of the three benthic indices calculated for the live and death assemblages from the control (c) and polluted (p) stations.

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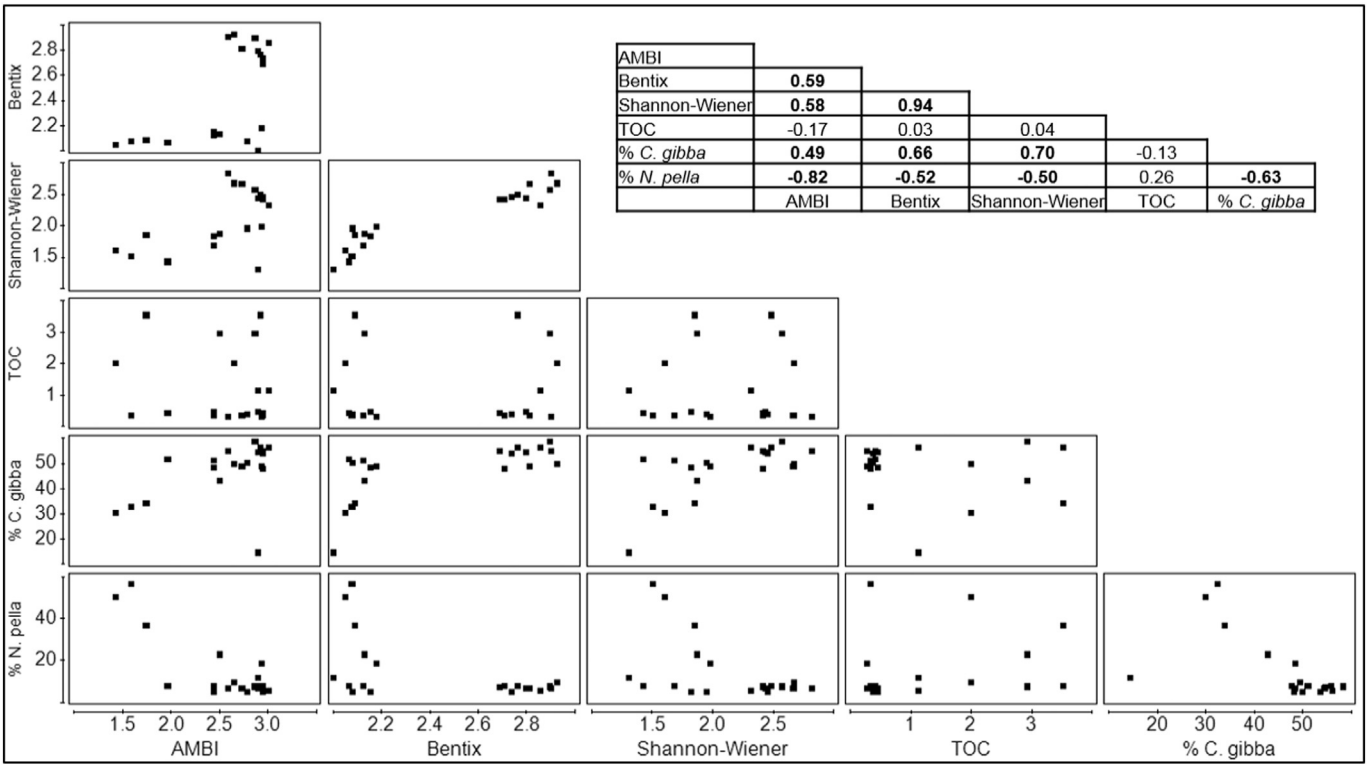

	AMBI	Bentix	Shannon-Wiener	TOC	% *C. gibba*
AMBI					
Bentix	**0.59**				
Shannon-Wiener	**0.58**	**0.94**			
TOC	-0.17	0.03	0.04		
% *C. gibba*	**0.49**	**0.66**	**0.70**	-0.13	
% *N. pella*	**-0.82**	**-0.52**	**-0.50**	0.26	**-0.63**

Fig. 6. Draftsman plot and Pearson correlations between the benthic indices (AMBI, Bentix and Shannon–Wiener), TOC and the relative abundance of the species *Corbula gibba* and *Nuculana pella*. Significant correlation values are in bold.

new shells into the death assemblage creating a time lag between the composition and structure of the live assemblage and that of the complementary dead assemblage (Kidwell, 2007, 2009, 2015). The moderate Bentix and Shannon–Wiener status and the good AMBI status of the Eastern Mediterranean death assemblages represent a record of the marine fauna before (or shortly after) the onset of pollution in the

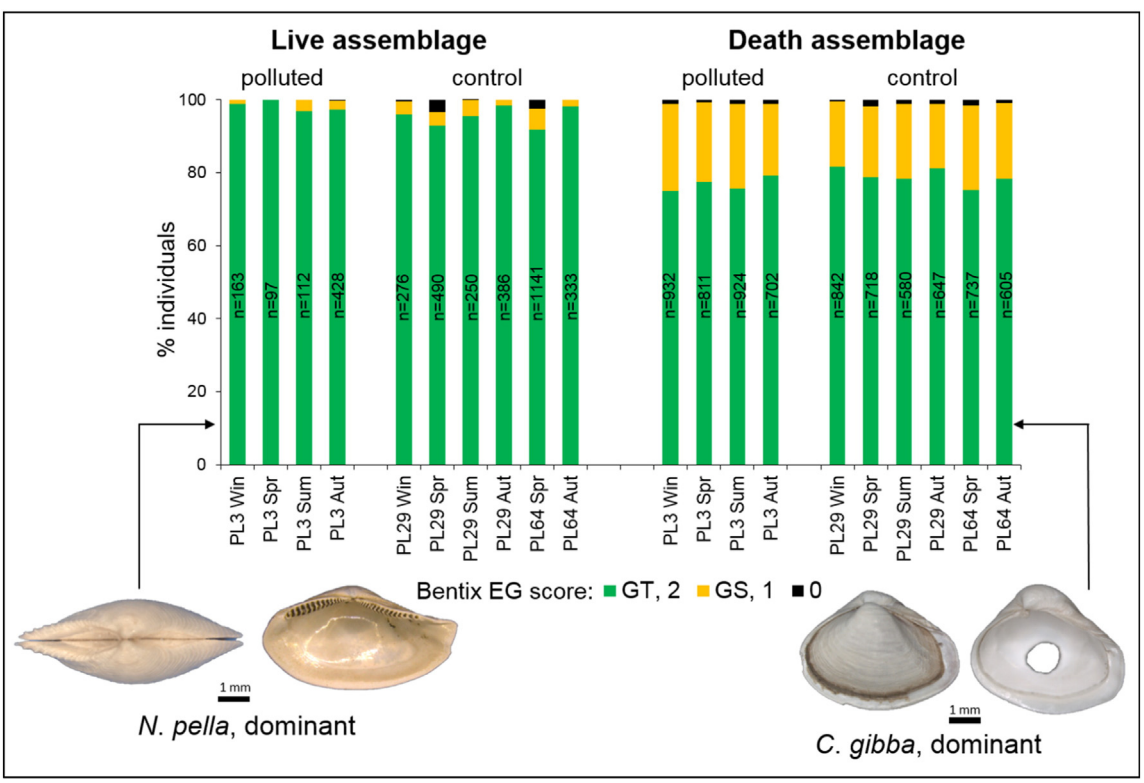

Fig. 7. The relative abundance of Bentix tolerant (GT, score 2) and sensitive (GS, score 1) ecological groups of the live and death assemblages of the control and polluted stations. Species with no known ecological information are given the score 0. The dominant species of GT in the live and death assemblage are the suspension feeder *Corbula gibba* and the deposit feeder *Nuculana pella*, respectively.

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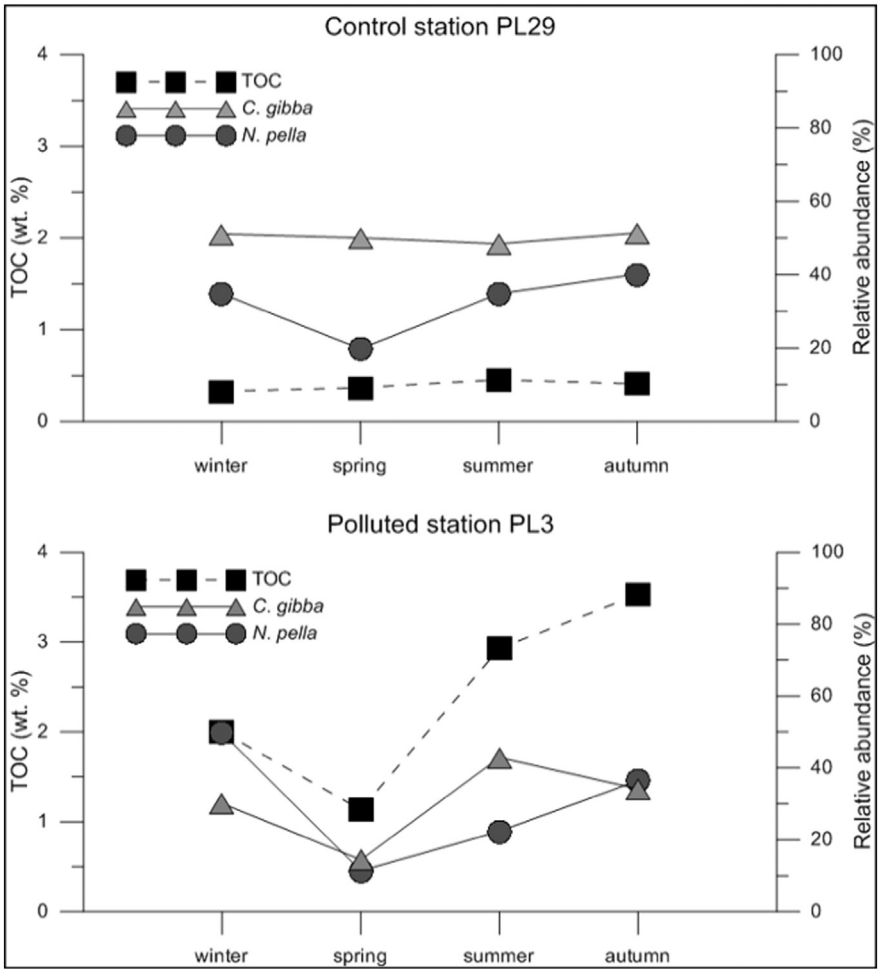

Fig. 8. TOC (wt.%) and relative abundance of *Corbula gibba* and *Nuculana pella* in the live assemblage for the four seasons of 2012 at the polluted (PL3) and control (PL29) stations.

area. Therefore, the change in EcoQS values of Bentix and Shannon–Wiener indices, from moderate in the dead to poor in the live assemblages (Tables 2 and 4, Fig. 4), corresponds to a change over time. This temporal change was detected by the multivariate analysis of indices, which showed a significant difference between live and dead assemblages across all stations and seasons (Fig. 5). The changes were also

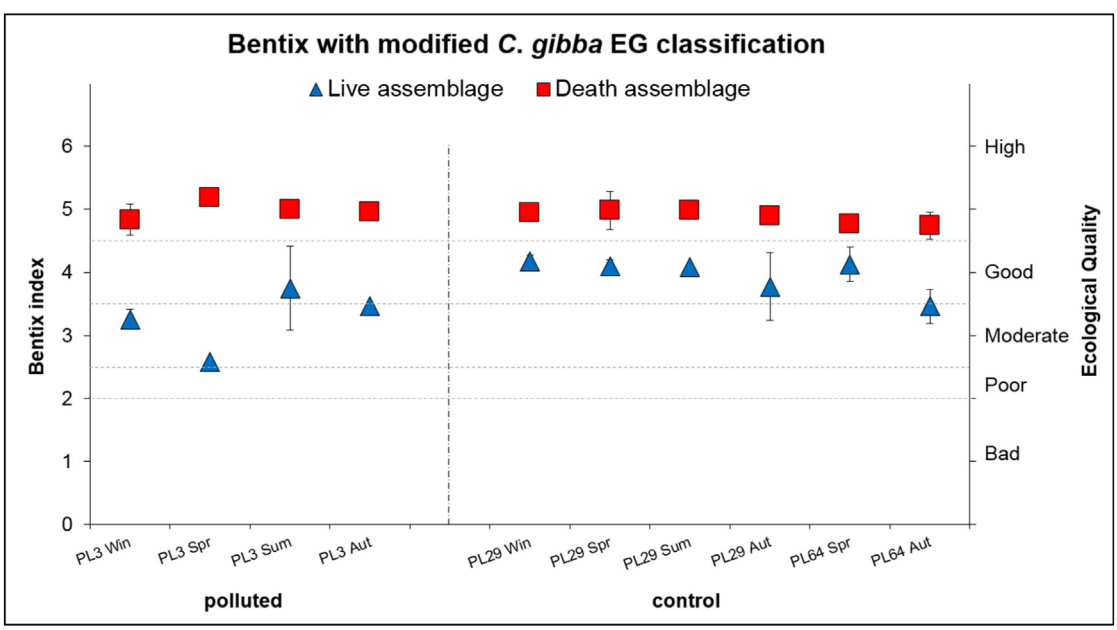

Fig. 9. Bentix values and EcoQS of the live and death assemblages at the polluted (PL3) and control (PL29, PL64) stations based on a modified EG classification of the dominant species *Corbula gibba*, from GT to GS. After modification, Bentix successfully differentiates between the control (good) and polluted (moderate) stations.

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supported by multivariate analysis of species abundance that showed mismatch between the live and dead assemblages (Table 5 and Fig. 8 in Leshno et al., 2015).

Bentix proved more sensitive than AMBI in distinguishing the ecological quality of the live and the dead assemblages (Table 4). Bentix showed significantly different EcoQS for the live and dead assemblages, while AMBI classified both assemblages as good (Table 2, Figs. 3 and 4). Other studies also show that Bentix is more sensitive since it was designed for the Eastern Mediterranean (Simboura and Reizopoulou, 2008; Simboura and Argyrou, 2010). The poor performance of AMBI relative to Bentix was due to a discrepancy in species classification between the two indices (Table 3). In particular, the bivalve species *N. pella* was classified by AMBI as GI (sensitive), despite the strong negative correlation between *N. pella* abundance and EcoQS (Fig. 6). This caused AMBI to be biased towards a healthier EcoQS assessment, obscuring the impact of the pollution. On the other hand, *N. pella* was classified by Bentix as GT (Table 3), indicating the species is tolerant to the pollution, and there was a strong negative correlation between the relative abundance of *N. pella* and EcoQS (Fig. 6), showing that Bentix correctly captured the EcoQS.

Most of the dominant species found dead were also found live (Table 3, Fig. 2), but the relative abundance of Bentix pollution-tolerant species (GT) showed an increase in the live assemblage as compared to the dead (Fig. 7), decreasing live EcoQS (Table 2, Fig. 4). The increase in GT species is explained by changes in feeding modes between the live and dead assemblages. There was a decrease in abundance of suspension feeding bivalves such as *A. gibbosa*, *P. rudis* and *Acanthocardia paucicostata* in the death assemblage, while the abundances of the deposit feeding bivalves *N. pella* and *N. nitidosa* and mixed feeder *A. longicallus* increased in the live assemblage (Leshno et al., 2015). The bivalves of the death assemblage were strongly dominated by suspension feeders (75%), while the live assemblage from the polluted station showed a decrease in suspension feeders and an increase in deposit feeders (Leshno et al., 2015). The change in feeding habits is attributed to the increase in the live abundance of *N. pella* (Table 3), that had a low positive correlation to TOC levels at the polluted station (Figs. 6 and 8). *N. pella* is an opportunistic, deep burrowing, deposit feeding bivalve (WoRMS, 2015) that can act as an indicator for anthropogenic pollution.

Bentix was originally designed for the oligotrophic ecosystem of the Eastern Mediterranean, giving equal weight to the tolerant and opportunistic groups of species (Simboura and Argyrou, 2010). However, high densities of tolerant species can overshadow local anthropogenic influences and underestimate EcoQS. Indeed, for the Israeli shelf of the Levantine basin, Bentix sensitivity is reduced for the live assemblages due to the pre-existing high abundance of some tolerant and opportunistic species (GT). *C. gibba* is a shallow infaunal suspension feeder that is known for its ability to also survive under relatively low oxygen conditions, allowing it to develop dense populations in areas of excess organic matter (Hrs-Brenko, 2006). *C. gibba* is highly abundant in the death assemblage of both the control and polluted stations (Fig. 2) and therefore was common to the ecosystem also prior to the Shafdan activation. High levels of organic matter at the polluted station lead to an increase in the abundance of live deposit feeding species, but the high abundance of *C. gibba* obscured this effect. Moreover, under conditions of particularly high organic overload, as in autumn when TOC values exceeded a threshold value, live *C. gibba* decreased in abundance (Figs. 6 and 8). Apparently, either oxygen depletion or excess organic matter does limit population growth under more nutrient-rich conditions. However, the relative abundance of the deposit feeder *N. pella* was found to be more sensitive to organic overload, tracking pollution more effectively than *C. gibba* (Figs. 6 and 8).

The annual cycle at the polluted station is anoxia in summer and dispersion of sludge and aeration in winter (Kress et al., 2004; Hyams-Kaphzan et al., 2009). In the year of sampling, anoxic conditions failed to develop due to the early onset and unusual frequency of storms and strong current regimes. The live assemblages failed to track the seasonal changes in EcoQS at the polluted station (Leshno et al., 2015). The three indices originally also failed to show a significant difference between the polluted and control stations. By modifying the EG classification of *C. gibba* from tolerant to sensitive it became possible to successfully differentiate EcoQS between the control and polluted stations (Fig. 9). Moreover, the baseline record preserved in the death assemblage correctly showed the ongoing multiannual impact when compared to the live molluscan fauna near the sewage sludge outlet. Incorporation of the death assemblage in calculation of three benthic indices pointed out that the modified Bentix is the most suitable index for the study area.

This study represents the first use of combined live and dead assemblages in a WFD and MSFD monitoring program. Adjustment to the Bentix database for the naturally tolerant benthic fauna of the highly-oligotrophic Israeli shelf improves its accuracy and enables its use in monitoring of oligotrophic shallow shelves around the Mediterranean. The application of benthic indices to death assemblages, a thus far overlooked source of information, is a vital step in verifying the ecological state of assumed pristine locations. Application of modified Bentix incorporating death assemblages identifies trends of anthropogenic changes to the ecosystem. It is a reliable method of evaluating ecological conditions before the onset of human activity and should be incorporated in future WFD and MSFD monitoring procedures in the Mediterranean.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2016.01.014.

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