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A new diatom-based multimetric index (MMI-D) for ecological health monitoring in the Tropical Rift Valley Lake (Lake Hawassa, Ethiopia)

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Multimetric assessment is one of the important tools for diagnosing, detecting and measuring impairment in ecosystem function in lentic ecosystems. It enhances detection capabilities across a broader variety of stressors and offers a more complete picture of ecological conditions than single metrics or biological indicators. In this context, a diatom-based multimetric index (MMI-D) has been developed to evaluate the ecological health of Lake Hawassa. Physicochemical and benthic diatom sampling was conducted at nine sites along the lakeshore, representing varying levels of human disturbance, from February to November in 2015 and 2016. The sampling sites were classified a priori into three categories: minimally disturbed (three sites), moderately disturbed (three sites) and highly disturbed (three sites). This classification, was based on a clustering analysis using the percent disturbance score (PDS). Of the 24-diatom candidate metrics, only ten were chosen as core metrics for the development of MMI-D, based on redundancy analysis, reaction to environmental conditions, percent discriminatory efficiency (%DE) and box plots. The newly established MMI-D clearly distinguished between reference and non-reference sites, and between the lake's three clusters. The MMI-D's performance was validated using independent data sets from lakes Hawassa and Ziway and it demonstrated the best capability for discrimination between different disturbance levels. MMI-D 2-stage Least Squares (2SLS) regression analysis revealed an inverse but robust connection with the PDS, indicating its responsiveness to Lake Hawassa habitat quality degradation ($n = 9$, $R^2 = 0.921$, $p < 0.001$). The MMI-D revealed a high %DE (95.1%) and a negative but significant connection with nutrients, total suspended solids (TSS), and turbidity ($R^2 > 0.6$; $p < 0.05$). Generally, it can be concluded that this index is a powerful tool that could assist endusers by providing a practical method for measuring the ecological quality of Lake Hawassa.

Keywords: benthic diatoms, ecological quality, Lake Ziway, redundancy analysis, validation

Introduction

Lake Hawassa is one of the most threatened Rift Valley lakes because of human pressure related to adverse watershed, land use, urban development and industrial expansion. For example, in the 1980s, there was no sign of pollution on the land adjacent to Lake Hawassa since there were few recreational activities and minimal urban development (Kibret 1985). Nowadays, recessionary farming, deforestation, urbanisation, recreational activities and industrial expansion are among the stressors that have affected the lake's water quality. The effect of these multiple stressors has resulted in a deterioration of the lake's ecology. The effects have been physical (modified shoreline, less riparian vegetation cover, numerous manmade structures in and around the littoral zone), chemical (an increasing concentration of nutrients) (Wondmagegn 2019), metallurgical (a high bioaccumulation of trace metals) (Nigussie et al. 2010) and biological (a proliferation of pollution tolerant, macroinvertebrate assemblages) (Aklilu 2011).

Lakes of sub-Saharan Africa, including Lake Hawassa, are utilised for a variety of purposes that are uncommon in developed countries. Such purposes include waste

disposal, laundry washing, cattle watering, and personal hygiene (Wondmagegn and Mengistou 2023) which according to Revenga et al. (2005) have caused a decline in overall ecological functioning and altered the species abundance and richness of biological communities (Strayer 2006). Changes in benthic organisms in response to human pressures are effective tools for monitoring the biological integrity of East African waterbodies (Masese et al. 2013). Besides, Odountan et al. (2019) recently advocated that benthic indices and metrics be used for biomonitoring lakes in West Africa and developing countries. Therefore, best management practice to ensure the sustainable function of the lake ecosystem should include the biological monitoring of aquatic organisms to evaluate changes in chemical and physical components of the aquatic environment. Such action will not only enhance the evaluation of deterioration, but also its remediation (Masese et al. 2013; Stribling and Dressing 2015a; Stribling and Dressing 2015b).

In earlier times, indicator organisms (metrics) were used for biomonitoring practice based on their response to human perturbation in their community structure. Later, ecologists developed a single biotic index to get a better description

of the human disturbance to aquatic ecology than the indicator organisms (Barbour et al. 1996). In recent years, a single biotic index is being replaced by a multimetric biotic index since a single biotic index can respond to limited stressors which may affect the accuracy of the assessment (Wang et al. 2015). Therefore, the multimetric approach is a more robust water quality assessor of ecosystem integrity and is able to respond to various stressors at once (Schoolmaster et al. 2012; Wang et al. 2015). This method uses an aggregation of individual community metrics that comprise benthic biological elements for the development of a single composite multimetric index (De la Rey et al. 2004). Its use is recommended for monitoring lakes like that of Lake Hawassa which is exposed to multiple stressors (Wondmagegn and Mengistou 2020). A composite multimetric index can potentially reflect multiple effects of human impact on the structure and function of an aquatic ecosystem (Barbour et al. 1999; Menetrey et al. 2011). It compares the biological metrics of minimally disturbed to highly disturbed sites in the water body (Stoddard et al. 2006; Whittier et al. 2007).

The application of the diatom-based multimetric index in lake biomonitoring is quite recent and only a few studies have been conducted in tropical regions (Phiri et al. 2007; Wang et al. 2015; Chen et al. 2017) but not Ethiopian lakes such as Lake Hawassa. Since the water quality of Lake Hawassa has been affected by several stressors, a multimetric approach is highly recommended (Wondmagegn et al. 2019) for obtaining accurate results from the response of diatoms to the potential stressors of the littoral regions of the lake. Therefore, the objective of this study was to develop a diatom-based multimetric index (MMI-D) of the biotic integrity of Lake Hawassa, and to validate its capability of discriminating between reference and non-reference sites in the lake.

Materials and Methods

Description of the study area

Lake Hawassa lies 275 km south of Addis Ababa in the main Ethiopian rift (MER), at a surface elevation of 1686 m asl (6°33'–7°33' N and 38°22'–38°29' E; Figure 1) (Welcomme 1972). While the lake has no visible outlet, a UN Geothermal Survey has indicated the possibility of groundwater flowing away from the lake on its southwestern and northern sides, which may account for significant water loss. This outflow could help explain the relatively low alkalinity of Hawassa compared to the more saline Lake Shala and Lake Abiyata, both of which are also endorheic lakes (Makin et al. 1975).

Tikur Wuha, the only perennial river feeding the lake, drains the vast swamps of the Wendo Genet area, which drains the highlands on the east. The surface area of the lake is about 92 km² (Makin et al. 1975), 16 km long, up to 8 km wide, and has an estimated volume of 1.3 billion m³. The maximum and mean depth of the lake is about 22 m and 11 m, respectively.

Lake Hawassa is ecologically very important and is home to eight species of fish (Dadebo 2000; Tekle-Giorgis et al. 2017), pelicans, storks, herons, hammerkops, sea eagles and kingfishers. There is a small-scale fish market

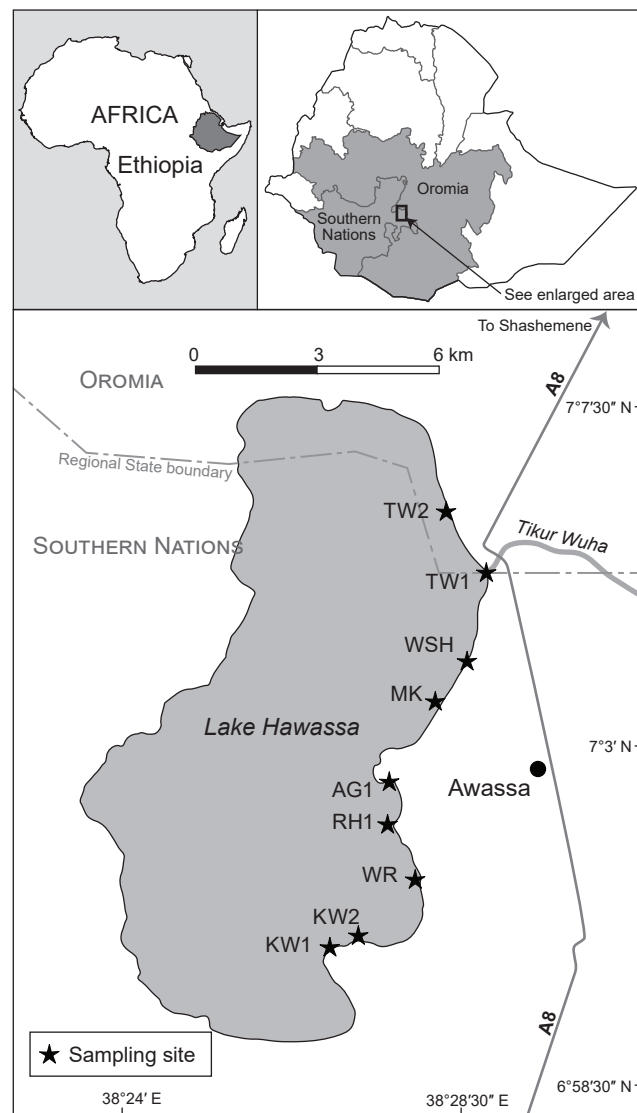


Figure 1: Map showing the location of Lake Hawassa and the sampling sites included in this study (adapted from Wondmagegn et al. 2019). Note: AG1 refers to Amora Gedel-1; MK is Mekilt; RH1 denotes referral Hospital; KW1 and KW2 are Kuyu Wata-1 and Kuyu Wata-2, respectively; TW1 and TW2 indicate Tikur Wuha-1 and Tikur Wuha-2; WR stands for Worancha; and WSH represents Wabie Shebelle Hotel

on the shore at Amora Gedel. The lake also supports large mammals including hippopotamuses (Wondmagegn et al. 2019).

Sampling site selection

The sampling sites were selected according to their exposure to anthropogenic activities. Habitat quality was determined by percent disturbance score (Table 1 and Figure 1). So, KW1, KW2 and WSH were categorised as reference groups. The remaining sites were classified as non-reference groups and included: MK, TW1, TW2 as moderately disturbed sites and WR, RH1 and AG1 as highly disturbed sites (Table 1) according to Wondmagegn et al. (2019). All the

physicochemical and biological sampling was conducted on these categorised sites with three replications.

Sampling of environmental parameters

A combined portable HQ40D multimeter was used to measure *in situ* physicochemical parameters, including temperature (T), pH, conductivity (EC) and dissolved oxygen (DO). The turbidity was measured with an OAKTON turbidimeter (T-100). Moreover, the concentrations of nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), soluble reactive phosphate (SRP) and total phosphorus (TP) were analysed spectrophotometrically at the Limnological Laboratory of Addis Ababa University. Nitrate concentrations were measured using the sodium salicylate method (Robarge et al. 1983), while ammonium levels were assessed with indophenol blue method (APHA 1995). Soluble reactive phosphate (SRP) was determined using the ascorbic acid method (APHA 1999). Nitrite concentrations were measured through the reaction of sulfanilamide with N-naphthyl-(1)-ethylenediamine dihydrochloride (APHA 1995). Total phosphorus (TP) and silica (SiO_2) were quantified using the persulfate digestion method and the molybdosilicate method, respectively (APHA 1999).

Chlorophyll a concentration was estimated using the method of Talling and Driver (1963). At each site, 200–500 ml of lake water was filtered through Whatman GF/F filters. The filters were then folded in aluminium foil, labelled and transported to the laboratory in an icebox, ensuring they were stored for no longer than one day. Pigments were extracted from the algal material by grinding and suspending it in 90% acetone. After centrifugation, the extract was carefully decanted into a 5 ml cuvette for spectrophotometric analysis of Chlorophyll a absorbance at wavelengths of 665 nm and 750 nm. Total suspended solids (TSS) samples were filtered using Whatman GF/F filters and analysed according to the methodology outlined by Wetzel and Likens (2000).

Sampling of benthic diatoms

Diatoms were scraped from cobbles and macrophytes in the littoral areas of Lake Hawassa at a maximum depth of 1 m. Sampling was conducted by taking cobbles (five stones with upper surface areas of cobble $\sim 25 \text{ cm}^2$) and macrophytes (five random stems of macrophytes with 5 cm length) from the lake shore (King et al. 2005; Martin and Fernandez 2012). Approximately 50 ml of lake water was poured into a tray. The biofilm from the upper surfaces of each cobble and the macrophyte was removed by vigorously scrubbing with a toothbrush, which was regularly rinsed with lake water. Afterwards, the suspension was poured into a labelled 150 ml plastic bottle. Before pouring, the suspension was swirled in the tray to re-suspend any settled particles. Finally, the diatom samples were preserved using 70% ethanol. A 5–10 ml aliquot of each sample was taken from the bottle and homogenised by shaking. Diatom samples were treated with concentrated sulfuric acid and potassium dichromate following the protocol of Patrick and Reimer (1966). A drop of cleaned diatom samples was placed onto a microscopic slide and dried on a hot plate. A permanent slide was prepared by adding Naphrax® (refractive index of 1.73) to the coverslip, which was then positioned over the dried slide.

Table 1: Criteria for Human Disturbance Scores Within 100 m of the Shore Zone of Lake Hawassa (adapted from Wondimagegn et al. 2019))

Sites	VQ	SWI	BSQ	LAHA	UBZ	AWLU	TQS	HQ%	PDS	CB	Clustering	Site status	Selection
KW1	18	19	15	16	10	10	88	73.33	26.67	26–50	C1	Minimally disturbed	Reference sites
KW2	18	19	15	16	11	10	89	74.17	25.83		C1		
WSH	18	16	13	6	11	2	66	55	45		C1		
MK	12	14	4	1	7	1	39	32.5	67.5	51–75	C2	Moderately disturbed	Non-reference sites
TW1	12	4	4	8	11	1	40	33.33	66.67		C2		
TW2	14	5	9	12	11	6	57	47.5	52.5		C2		
RH1	5	2	4	1	7	1	20	16.67	83.33	76–100	C3	Highly disturbed	
WR	1	5	1	1	11	1	20	16.67	83.33		C3		
AG1	3	5	1	1	11	1	22	18.33	81.67		C3		

Note: This table outlines the criteria used to assess human disturbance scores in the specified area around Lake Hawassa, detailing key factors and their abbreviations for clarity.

Abbreviations: VQ = vegetation quality, SWI = stormwater inputs, BSQ = bottom substrate quality, LAHA = lakeside adverse human alterations, UBZ = upland buffer zone, AWLU = adverse watershed land use, TS = total score from 120pts, HQ = habitat quality, PDS = percent disturbance score, CB = class boundary, KW1 = Kuyu Wata-1, KW2 = Kuyu Wata-2, WSH = Wabi-Shebele Hotel, MK = Mekilt, TW1 = Tikur Wuha-1, TW2 = Tikur Wuha-2, WR = Worancha, RH1 = referral hospital, AG1 = Amora Gedel.

Diatom frustules were examined using a Carl Zeiss Axioskop light microscope at 1 000× magnification, employing an oil immersion objective and bright-field illumination with a green filter to enhance contrast. This work was conducted in the laboratory of the Environmental Centre at Lancaster University, UK. Identification of diatom species was conducted using standard identification keys and publications, including those by Van Meel (1954); Gasse (1986); Krammer and Lange-Bertalot (1986; 1988; 1991a and 1991b); Kelly (2000); Taylor et al. (2007a; 2007b); (Taylor and Cocquyt 2016). For each slide, 500 valves were counted and the relative abundance of each species was calculated as a percentage.

Diatom indices/metric calculation

The analysis of diatom indices and metrics listed in Table 2 (Lecointe et al. 1993) was conducted using OMNIDIA software version 6.1. Four reference-based metrics (RATD, RRTD, PRATD and PRRTD) were also calculated. The development of the MMI-D was based on three a priori clusters of sites: C1 included minimally disturbed sites (KW1, KW2 and WSH), C2 consisted of moderately disturbed sites (MK, TW1 and TW2) and C3 were highly disturbed sites (WR, RH1 and AG1).

Selection and removal of redundant metrics

A total of 24 diatom candidate metrics (17 of them published in Wondmagegn et al. 2019; Table 2) represented various aspects of the diatom communities related to family richness, taxonomic composition, tolerance measures, biotic indices and other metrics were compiled (Kelly and Whitton 1995; Rott et al. 1999; Lecointe et al. 2003). The listed candidate metrics and indices were used to select the

core metrics for developing the MMI-D of biotic integrity for Lake Hawassa. The procedure for selecting the potential metrics was performed according to Barbour et al. (1999), with some modification. The selection was done based on the metrics' response to physicochemical parameters, their interrelationships and their effectiveness in characterising both reference and non-reference sites. Metrics which significantly correlated with physicochemical variables were selected for the subsequent analysis. Redundancy analysis was employed to identify pairs of metrics with a significant correlation (i.e., correlation coefficient ≥ 0.7) (Ofenböck et al. 2004; Hering et al. 2006). From the redundant metrics, the one most strongly correlated with the physicochemical variables was considered for further analysis, while the others were rejected. The percentage of discriminatory efficiency (%DE) for each metric was calculated to identify the most suitable ones, with a focus on those achieving a % DE of 50% or higher, as noted by Wondmagegn et al. (2019) and Wondmagegn and Mengistou (2023). In addition, the inclusion percentage of diatom species in index calculations was considered an important criterion for selecting diatom indices, with any indices showing less than 50% inclusion being excluded from further analysis (Wondmagegn et al. 2019). To visualise the distribution of each metric between reference and non-reference sites, box and whisker plots were used. The sensitivity of the metrics was assessed based on the degree of interquartile overlap, according to the methodology outlined by Barbour et al. (1996).

Scoring of metrics

Using the discrete type of scoring system, calculated metric values were converted (normalised) to metric scores of 5, 3 or 1 depending on their proximity to the optimal values used

Table 2: List of candidate indices/metrics of macroinvertebrate and diatoms tested in this study

	Metric	*Response
CEE	Commission for Economical Community metrics (Descy and Coste, 1991)	Decrease
DES	Descy's pollution metric (Descy, 1979)	Decrease
EPID	pollution metric based on diatoms (Dell'Uomo, 2004)	Decrease
IBD	biological diatom index (Monnier et al. 2009)	Decrease
IDG	generic diatom index (Coste and Ayphassorho 1991)	Decrease
IDAP	indice diatomique Artois Pircardie (Prygiel et al., 1996)	Decrease
IPS	specific pollution sensitivity index (CEMAGREF 1982)	Decrease
ROTT	trophic metric (Rott et al. 1999)	Decrease
SHE	Steinberg & Schiefele trophic metric (Steinberg and Schiefele 1988)	Decrease
PSI	indice saprobique (Rott et al. 1999)	Decrease
TID	indicetrophique (Rott et al. 1999)	Decrease
SLA	Sládeček's pollution index (Sládeček 1986)	Decrease
LOBO	Lobo and Bresil (Lobo et al. 2004)	Decrease
PDI	Pampean diatom index (Gómez and Licursi 2001)	Decrease
WAT	Watanabe pollution metric (Lecointe et al. 2003)	Decrease
DI-CH	Swiss diatom index (Lecointe et al. 2003)	Decrease
TDI	Trophic diatom index (Kelly and Whitton 1995)	Increase
LDTI 2	Phytobenthos – diatoms for assessing river and lake ecological quality (WFD-UKTAG 2014)	Decrease
T.PI	New trophic index (Pfister et al. 2016)	
TDIL	trophic diatom index for lakes (Stenger-Kovács et al. 2007)	Decrease
RATD	Reference abundance taxa of diatoms (present study)	Decrease
RRTD	Richness of reference taxa of diatoms (present study)	Decrease
PRATD	Percent reference abundance taxa of diatoms (present study)	Decrease
PRRTD	Percent richness of reference taxa of diatoms (present study)	Decrease

Notes: (*Expected response with increasing disturbance)

by Wondmagegn and Mengistou (2023). Metrics whose values decreased with the increase of disturbance (positive metric) 5, 3 and 1 scoring was used, and the reverse scoring for negative metrics. For instance, positive metric values above the 75th percentile were scored as five. Metric values between and including the 75th and 25th percentiles were scored as 3, and all metric values below the 25th percentile were scored as 1 (Barbour et al. 1996; Wang et al. 2005).

Development of multimetric index of biotic integrity for Lake Hawassa

The scored values of each potential metric value were combined into one index, the MMI-D, by summing up the score of each individual metric. The possible maximum and minimum MMI-D values were calculated (maximum value = total number of selected metrics multiplied by 5, and minimum value = total number of selected metrics multiplied by 1) and divided into quartile ranges to have four quality classes. Thus, the highest score indicated very good quality and the lowest score, poor quality. Box and whisker plots were also used to visualise MMI-D's distribution between the reference and non-reference sites. The plots also tested the MMI-D's potential to discriminate between the minimally disturbed sites and the moderately and highly disturbed sites of Lake Hawassa.

Ecological quality ratio (EQR)

The ecological quality ratio (EQR) was employed to classify the ecological status of each sampling station (Wondmagegn and Mengistou 2023). The EQR was calculated by dividing MMI-D values of each site by the median MMI-D values of reference sites, resulting in a numerical value typically ranging from zero to one. To categorise the ecological status, the 90th percentile of the EQR values from the reference sites was used, leading to five ecological classes. Generally, an EQR value close to one indicates a high ecological status, while a value approaching zero is considered to have a poor ecological status (EQR 2007; Delgado et al. 2010).

Validation of the MMI-D

The validation of the MMI-D was conducted using independent data sets which were not incorporated in the MMI-D development for Lake Hawassa. One of the independent data sets was taken from the reference and non-reference sites of Lake Hawassa. Moreover, it was validated using the independent data set from Lake Ziway with box and whisker plot of Sigma Software version 10.0. The independent data set of Lake Ziway was obtained from unpublished data of Abnet Woldeesenbet. Two-stage Least Squares (TSLS) regression analysis was also employed to test the relationship between the MMI-D and percent disturbance score in the SPSS package version 20. Principal component analysis (PCA) was also used to visualise the capability of MMI-D's distribution between the reference and non-reference sites of Lake Hawassa.

Data analysis

OMNIDIA software version 6.1 was employed to calculate the diatom indices/metric. Spearman rank correlation was used to check relationships of candidate metrics with

themselves and with environmental parameters, to select the core metrics. Two-stage least squares (TSLS) regression analysis was also employed to test relationship between the MMI-D and percent disturbance score. The above analyses were done with Statistical Package for Social Science Students (SPSS Inc., software version 20.0). Box and whisker plot and PCA were the techniques employed to show the discriminatory potential of the MMI-D across the reference and test sites of Lake Hawassa using Sigma-plot version 15.0 and PAST 3.15 software programmes, respectively. The validation of the potential of the MMI-D with an independent data set of Lake Ziway was checked with box and whisker figures using Sigma-plot version 10.0.

Results

Metric selection

Metric selection was based on percent inclusion of the diatom species in the index calculation (the greater the number of species included in the index calculation, the more efficient the index is to explain the ecology). In addition, their discrimination efficiency, correlation, and response to environmental parameters were checked (Tables 2 and 3). As a result, the indices IDAP, P.SI, WAT, DES, LOBO, PDI, and P.TI indices were excluded from selection due to their inclusion of less than 50% of diatom species in the index calculations using OMNIDIA software. Moreover, these indices did not exhibit a significant correlation with ecologically important physicochemical variables such as total phosphorus (TP), soluble reactive phosphorus (SRP) and nitrate (Table 3). Furthermore, IBD, IPS, and CEE were also excluded from the metrics selection due to their high correlation with SHE, IDG and TDI. Therefore, indices with greater than 50% discrimination efficiency, at least 50% species inclusion, minimal redundancy and significant correlations with most of the physicochemical variables (EPID, SHE, SLA, IDG, TDI, ROTT, TDIL and LDTI2) were included in the metrics selection (Table 3). Among the four reference taxa-based metrics, we selected RATD and PRATD due to their high discriminatory efficiency for the development of the MMI-D (Tables 2 and 3). These metrics did not exhibit a complete overlap in their interquartile ranges, demonstrating their potential to effectively characterise both reference sites and non-reference sites as illustrated in Figure 2.

Development of a multimetric index of Lake Hawassa

From the list of 24 diatom candidate metrics, ten diatom metrics were selected, based on their response to the different level of disturbances, their discriminatory power between the reference and test sites and their correlation with physicochemical parameters. The selected metrics were used for the development of a MMI-D for Lake Hawassa (Table 4). The total score for each site was summed to calculate the MMI-D value, which ranges from a minimum of 10 to a maximum of 50 (Table 5). These MMI-D values were then divided into quartiles: 41–50 was classified as “very good”, 31–40 as “good”, 21–30 as “fair” and 10–20 as “poor quality”.

Therefore, the current study showed that the MMI-D had the potential to discriminate clearly between the

Table 3: Spearman correlation between diatom indices/metrics and selected physicochemical variables, along with discrimination efficiency percentage (%DE) and average percent inclusion (%AI) of diatom species used in the calculation of these indices and metrics in the OMNIDIA software

Metric	T	EC	TP	SRP	NO ₃	Chl a	TUR	%DE	%AI
CEE	-0.483	-0.677*	0.250	0.528*	0.610*	-0.367	-0.383*	59.1	64.48
IBD	-0.527*	-0.142	-0.477	-0.412*	-0.366	-0.218	-0.335	13.4	77.11
IPS	-0.833**	-0.233	-0.467	-0.234	-0.402*	-0.483	-0.533	59.1	94.92
IDG	-0.851**	-0.678*	-0.802**	0.692*	0.675*	-0.705*	-0.877**	89.4	100
DES	-0.681*	-0.192	0.059	-0.214	0.210	-0.126	-0.426*	40.5	37.5
P.SI	-0.595	-0.132	-0.108	-0.155	-0.17	-0.158	-0.466**	12.3	47.53
SLA	-0.777**	-0.201	0.726*	-0.655*	0.722*	-0.635*	-0.835**	80.1	52.91
IDAP	-0.287	-0.675*	-0.236	0.093	0.292	-0.354	-0.673*	28.1	19.23
EPID	-0.800**	-0.671*	-0.833**	0.679*	0.390	-0.667*	-0.833**	60.1	64.28
LOBO	0.563*	0.101	-0.092	0.11	-0.085	0.395	0.521*	23.3	32.6
DICH	-0.717*	-0.433	-0.183	-0.134	0.186	-0.650	-0.800**	11.1	54.56
ROTT	-0.686*	-0.861**	-0.442	-0.671*	-0.879**	0.803**	-0.703*	83.3	52.48
WAT	-0.083	0.627*	0.133	0.059	0.237	-0.35*	-0.383**	47.2	37.43
TDI	-0.674*	-0.667*	0.837**	0.962**	0.659*	-0.683*	-0.693*	71.1	79.8
PDI	-0.686*	-0.485	0.025	0.118	0.281	-0.268	-0.351	87.7	43.38
SHE	-0.752*	0.109	-0.694*	0.726*	0.802**	-0.435	-0.602*	67.2	59.91
P.TI	-0.644	-0.685*	0.059	-0.203	0.377	0.08	-0.619	83.3	49.04
TDIL	-0.633*	-0.767**	-0.670*	0.759*	0.825**	-0.683*	-0.667**	63.9	51.47
TDI4	-0.672*	-0.185	0.084	-0.181	0.111	-0.269	-0.437*	17.1	52.48
LDTI2	-0.679*	-0.673*	-0.705*	-0.826**	-0.675*	-0.694*	-0.956**	81.6	59.1
RATD	-0.150	-0.832**	-0.677*	-0.851**	0.620*	-0.741*	-0.853**	77.4	NA
RRTD	0.755*	0.155	-0.627*	-0.424*	-0.414	-0.377	-0.309	47.7	NA
PRATD	-0.150	-0.832**	-0.677*	-0.851**	0.620*	-0.741*	-0.853**	77.4	NA
PRRTD	-0.706*	0.417	-0.333	-0.335	-0.220	-0.400	-0.680*	23.3	NA

* & ** Correlation is significant at the 0.05 & 0.01 level, respectively (2-tailed)

Abbreviations: T = temperature, EC = water conductivity, TP = total phosphorus, SRP = soluble reactive phosphate, Chl a = chlorophyll a and Tur = turbidity

Note: NA indicates not applicable; additional abbreviations can be found in Table 2

reference and the non-reference sites and between the three-disturbance levels (minimal, moderate and high disturbance level) of the clustered sampling stations of the lake (Figure 3). It classified the minimally disturbed sites (C1) as “very good” to “good” categories (KW1, KW2 and WSH), the moderately disturbed sites (C2) as fair (MK, TW1 and TW2) and the highly disturbed sites (C3) as “poor” categories (Table 6).

The discriminatory capacity of the MMI-D was evaluated using box and whisker plots to compare reference and non-reference sites, based on the previous classification of sampling stations in Lake Hawassa. Therefore, the MMI-D effectively distinguished between reference sites and non-reference (test) sites (Figure 3a). It also demonstrated a clear separation among the three clusters: C1 (minimally disturbed), C2 (moderately disturbed) and C3 (highly disturbed sites) in the lake (Figure 3b).

Ecological quality ratio (EQR)

The EQR ratio was calculated at a range between one and zero. Values greater than one were considered as one. The 90th percentile of the reference site of the EQR values was one. The sampling site's ecological status was classified based on the range of the EQR values (Table 7).

The EQR effectively distinguished between reference and non-reference sites. The minimally disturbed sites (C1) were classified into high quality and good quality or reference conditions (KW1, KW2, and WSH). The

moderately disturbed sites were categorised as moderate quality, while the highly disturbed sites were identified as poor-quality sites (Table 8). The box and whisker plots demonstrated that the EQR effectively distinguished between reference and non-reference sites and the three clustered sites (C1, C2 and C3; Figure 4).

Validation of the multimetric index

The effectiveness of the MMI-D in distinguishing between reference and non-reference sites, as well as in classifying sampling stations based on disturbance levels (minimal, moderate and high) was evaluated by using box and whisker plots in the previous section. Furthermore, to ensure the suitability and robustness of the newly developed MMI-D, it underwent a validation process. This validation was conducted using independent data sets that were not involved in the index's development. The performance of the MMI-D was specifically tested with new data sets from Lake Hawassa and Lake Ziway. Three sites from Lake Hawassa were subjected to validation using the MMI-D. The results classified one site as “very good” with an MMI-D value of 34, while the other two sites were categorised as “fair”, both with MMI-D values of 28. These findings coincided with the previous classification of these sites. The developed MMI-D also discriminated between reference and test sites in Lake Ziway as shown in Figure 5. It showed strong potential for characterising the lake's clustered sites. Specifically, all three reference sites (C1) were classified as very good

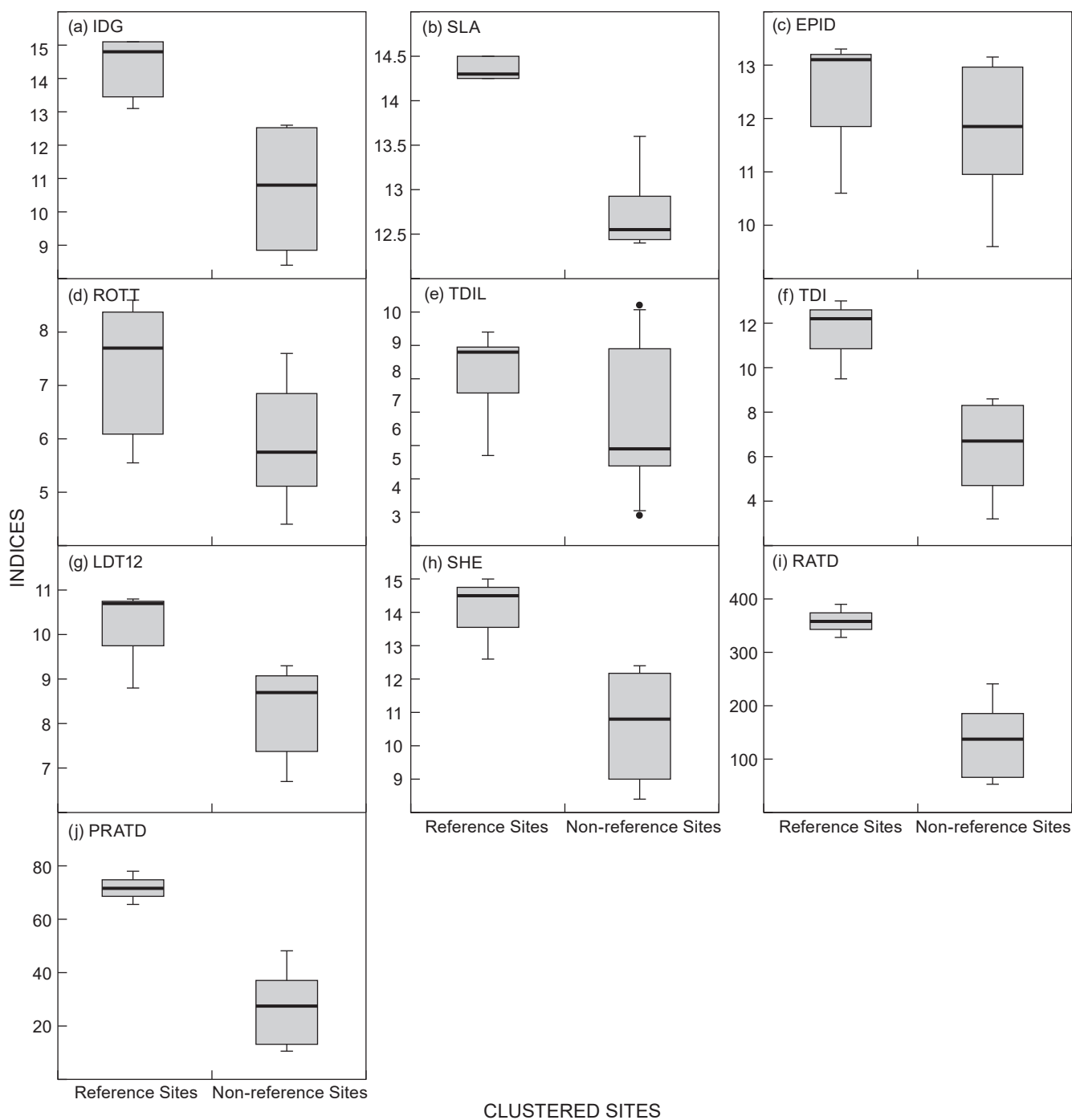


Figure 2: Boxplots illustrating the distribution of selected indices across the clustered sites of Lake Hawassa. The line in each box indicates the median, while the boxes represent the interquartile range (25th to 75th percentiles). The range bars show the maximum and minimum values. Note that except for RATD and PRATD, all values were standardised with a quality level of 20 (for abbreviations, refer to Table 2)

quality, while the three moderately disturbed sites (C2) were rated as fair. The highly disturbed sites (C3) fell into the poor category. The PCA accounted for 96.85% of the variation between reference site and non-reference sites in Lake Hawassa, highlighting the significance of both the first and second axes (Figure 6). This confirms the effectiveness of the MMI-D in differentiating between these site types. The TSLS regression analysis of the MMI-D showed a strong

inverse relationship with the percent disturbance score which demonstrates that the MMI-D is highly responsive to habitat quality degradation in Lake Hawassa ($n = 9$, $R^2 = 0.921$, $p = 0.000$; Figure 7). The MMI-D also showed significant positive correlation with DO and significant negative correlations with temperature, nutrient levels, TSS and turbidity. The index also demonstrated a high percent discriminatory efficiency (%DE = 95.1; Table 9).

Table 4: Distribution of selected metric values across clustered sites in Lake Hawassa

Metrics	C1				C2			C3	
	KW1	KW2	WSH	MK	TW1	TW2	WR	RH1	AG1
IDG	15.1	15.1	13.1	12.1	12.5	12.6	9.5	9	8.4
SLA	14.5	14.3	14.25	13.6	12.7	12.5	12.45	12.4	12.6
EPID	13.3	13.1	10.6	12.2	12.9	13.15	11.4	11.5	9.6
ROTT	7.7	8.6	5.55	6	6.6	7.6	5.35	5.5	4.4
TDIL	8.8	9.4	5.7	5.9	8.9	10.2	5.4	5.35	3.9
TDI	12.2	13	9.5	8.1	8.6	8.2	5.2	5.3	3.2
LDTI 2	10.7	10.8	8.8	8.8	9.3	9	8	7.6	6.7
SHE	14.5	15	12.6	12.1	12.4	12.1	9.5	9.2	8.4
RATD	328.0	390	358	241	53	127	167	148	70
PRATD	65.6	78	71.6	48.2	10.6	25.4	33.4	29.6	14

Note: except for RATD and PRATD, all values of diatom metrics used were standardised with quality 20)

Table 5: Frequency distribution statistics for final metrics and scoring criteria

Metric	Frequency distribution		Score		
	25th Percentile	75th Percentile	5	3	1
IDG	9.25	14.10	>14.10	9.25–14.10	<9.25
SLA	12.55	14.15	>14.15	12.55–14.15	<12.55
EPID	11.00	13.20	>13.20	11.00–13.20	<11.00
ROTT	5.45	7.65	>7.65	5.45–7.65	<5.45
TDIL	5.45	9.15	>9.15	5.45–9.15	<5.45
TDI	5.25	10.85	<5.25	5.25–10.85	>10.85
LDTI2	8.10	10.00	>10.00	8.10–10.00	<8.10
SHE	9.60	13.55	>13.55	9.60–13.55	<9.60
RATD	98.50	343.00	>343.00	98.50–343.00	<53.00
PRATD	19.70	68.60	>68.60	19.70–68.60	<19.70

Table 6: The MMI-D and its ecological interpretation

Clustered sites		Sites	Ecological interpretation of the MMI-D		
			MMI-D	WQC	Ecological status
C1	Minimally disturbed reference	KW1	44	I	Very good
		KW2	48	I	Very good
		WSH	34	II	Good
C2	Moderately disturbed	MK	30	III	Fair
		TW1	26	III	Fair
		TW2	30	III	Fair
C3	Highly disturbed	WR	18	III	Poor
		RH1	20	IV	Poor
		AG1	12	IV	Poor

Abbreviations: WQC= water quality class; C1, C2 and C3= Cluster 1, Cluster 2 and Cluster 3, respectively

Table 7: The classification of the ecological status of Lake Hawassa based on Ecological Quality Ratio (EQR) values

No.	Values of ecological quality ratio (EQR)	Ecological status
1	≥ 1	High quality (reference site) (I)
2	$1 < \text{EQR} \leq 0.75$	Good quality (II)
3	$0.75 < \text{EQR} \leq 0.5$	Moderate quality (III)
4	$0.5 < \text{EQR} \leq 0.25$	Poor (IV)
5	$0.25 < \text{EQR} \leq 0$	Bad (V)

Table 8: The Ecological Quality Ratio (EQR) and its interpretations

Clustered sites		Sites	Ecological interpretation of the EQR		
			EQR	WQC	Ecological Status
C1	Minimally disturbed reference	KW1	1	I	High quality
		KW2	1.09	I	High quality
		WSH	0.77	II	Good quality
C2	Moderately disturbed	MK	0.68	III	Moderate quality
		TW1	0.59	III	Moderate quality
		TW2	0.68	III	Moderate quality
C3	Highly disturbed	WR	0.41	IV	Poor quality
		RH1	0.45	IV	Poor quality
		AG1	0.27	IV	Poor quality

Abbreviations: WQC= water quality class; C1, C2, C3= Cluster 1, Cluster 2 and Cluster 3, respectively.

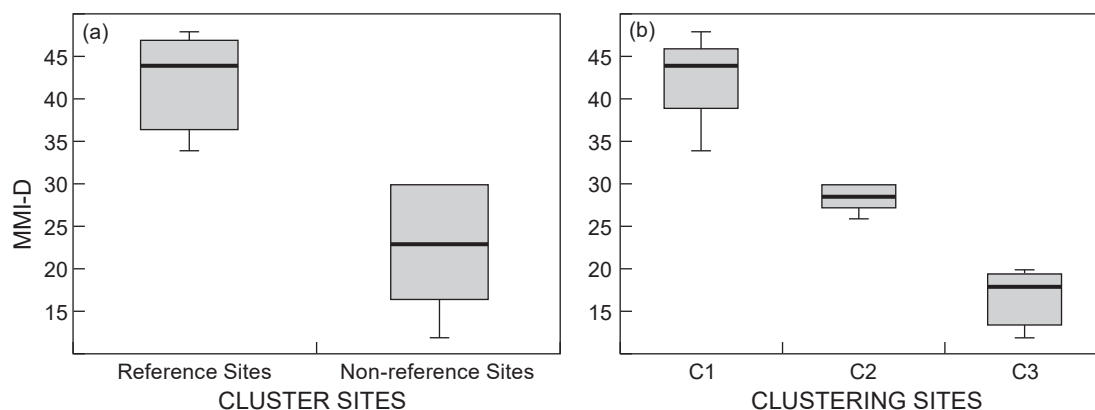


Figure 3: Boxplots of the multimetric index (MMI-D) across different clustered sites. (a) compares reference and non-reference sites, (b) highlights three clusters: C1(minimally disturbed sites), C2 (moderately disturbed sites) and C3 (highly disturbed sites). The line in each box indicates the median, the boxes represent the interquartile range (25th to 75th percentiles) and the range bars show maximum and minimum values

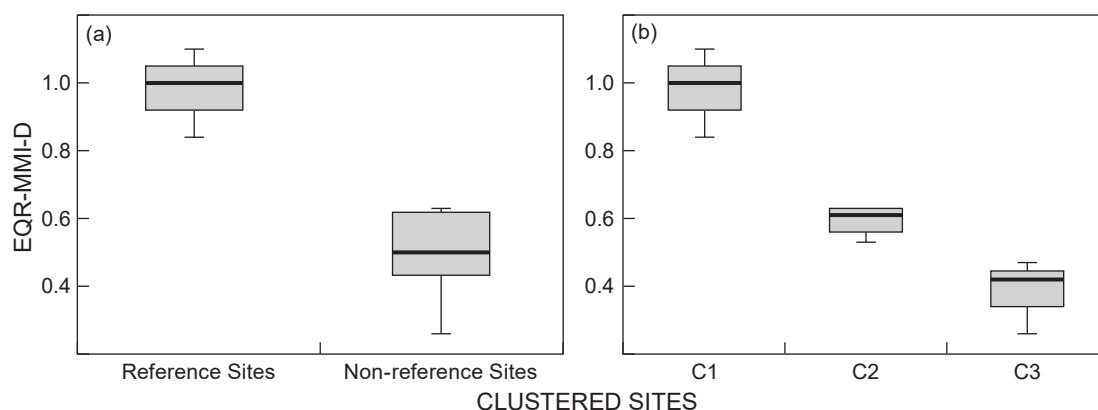


Figure 4: Box plots of the EQR across clustered sites. The central line in each box represents the median, while the boxes represent the interquartile ranges (25th to 75th percentiles). The range bars show the maximum and minimum values. The clusters are categorised as follows: C1= minimally disturbed sites; C2= moderately disturbed sites and C3 = highly disturbed sites

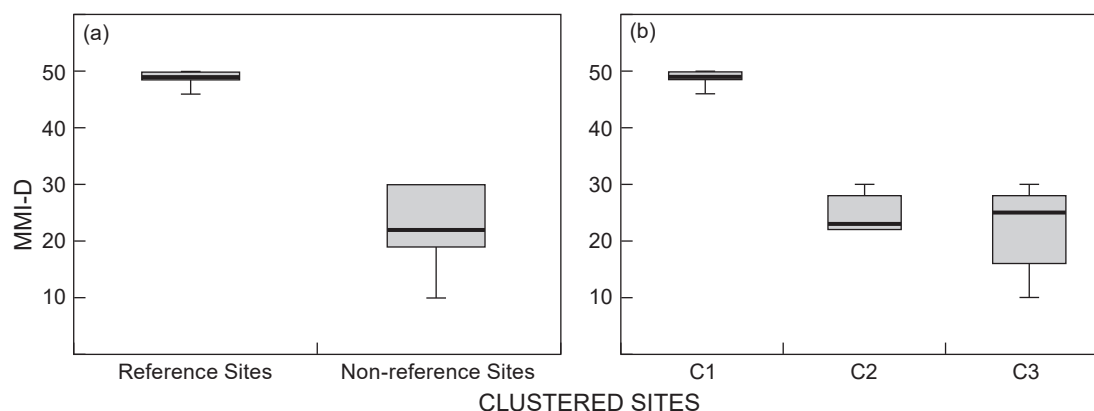


Figure 5: Validation of the MMI-D using box and whiskers plots for clustered sites: (a) reference and non-reference sites, and (b) C1, C2 and C3 in Lake Ziway. In these plots, the line in each box represents the median, the boxes indicate the interquartile range (25th to 75th percentiles) and the whiskers depict the maximum and minimum values

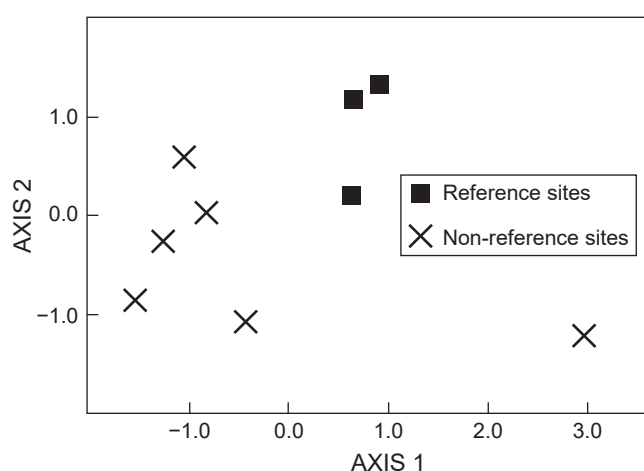


Figure 6: Principal component analysis (PCA) effectively distinguishes between reference and non-reference sites based on the efficiency of MMI-D

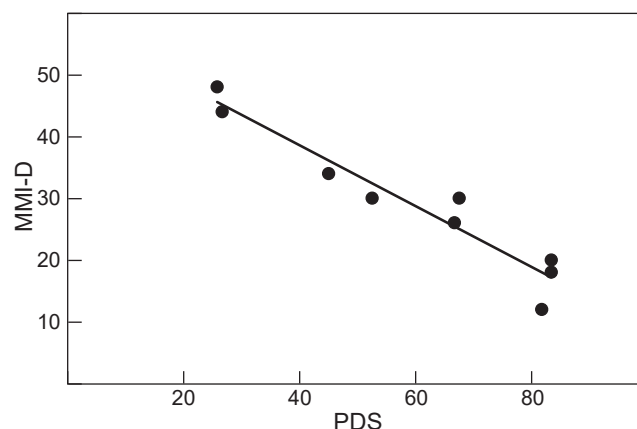


Figure 7: Two-stage least squares (TSLS) regression analysis of MMI-D values in relation to the PDS, based on a sample size of nine. The model shows a high explanatory power ($R^2 = 0.921$) and a statistically significant relationship ($p < 0.001$)

Table 9. Spearman correlation between the MMI-D and selected physicochemical variables

	T	DO	TP	SRP	Nitrate	TSS	Tur	%DE
MMI-D	-0.610*	0.667*	-0.851**	-0.651*	-0.675*	-0.680*	-0.777**	95.1

Note: Refer to Table 3 for abbreviations.

Discussion

Lake Hawassa's ecological health has been severely impacted by various stressors which come from different sources—primarily non-point sources. The development of a multimetric index is essential for assessing the ecological status of different sampling stations affected by human induced disturbances and to quantify the ecological integrity of Lake Hawassa, enabling better management and conservation efforts.

The MMI-D for Lake Hawassa showed a clear demarcation between reference and non-reference/test sites shown in Table 6 and Figure 3. It also placed the

minimally disturbed sites (C1) in the very good to good categories (KW1, KW2 and WSH), while moderately disturbed sites (MK, TW1 and TW2) were rated as fair and highly disturbed sites (WR, RH1 and AG1) as poor quality (Table 6). The MMI-D, therefore, effectively discriminated among the three clusters of sampling stations of the lake as previously classified. In addition, the EQR provided insight into the ecological condition of the sampling sites (Table 8). The EQR is an ecological expression of the MMI-D as noted by Lepistö et al. (2004); Wells et al. (2007) and Gabriels et al. (2010). Generally, the MMI-D and EQR showed robust results that clearly distinguish the ecological quality levels of sampling stations as very

good, fair, or poor. Therefore, it is crucial to conserve the very good quality sites, while also focusing on the rehabilitation of those classified as fair or poor. The suitability and robustness of the newly developed MMI-D requires validation to effectively assess lake conditions and recommend future applications, restoration and conservation methods and to identify research gaps (Jun et al. 2012). The use of independent data sets to validate MMI-D is a valuable approach as it demonstrates the multimetric index's expected performance across various sampling sites and offers good insight for broader application in other lakes in the ecoregion (Lunde and Resh 2012; Villamarín et al. 2013). The potential use of MMI-D was confirmed by the discrimination efficiency of the independent data set taken from Lake Hawassa and Lake Ziway, which coincided with previous classification of these sites. Consequently, the validation of MMI-D showed its potential for biomonitoring both Lake Hawassa and other lakes in the East African Rift system (Thieme et al. 2005).

The MMI-D was further validated through the analysis of physicochemical parameters and PCA, highlighting its responsiveness to different levels of ecological degradation observed at both reference and non-reference sites. A significant inverse correlation was found between MMI-D and the percent disturbance score (PDS) representing the index's strong responsiveness to lake habitat quality (PDS: $n = 9$, $R^2 = 0.921$, $p < 0.01$, Figure 7). A similar phenomenon was observed in studies on multimetric indices in Lake Kariba, Africa (Phiri et al. 2007), lakes in the USA (Stevenson et al. 2013) and Lake Dongting (Wang et al. 2015). The MMI-D, considered a positive metric, demonstrated a decrease in value in relation to habitat disturbance (Figure 7), similar to findings from the wetlands in California (Lunde and Resh 2012). This wetland report showed that higher multimetric index scores signalled a less disturbed environment, whereas lower scores reflected greater habitat disturbance. The MMI-D out-performed individual diatom metrics tested in the study by Wondmagegn et al. (2019). Similar effectiveness was observed in Alaska's biomonitoring practices, which exhibited high precision and discrimination efficiency in their multimetric developments (Bouchard et al. 2004). In addition, the MMI-D showed a significant positive response to DO, while exhibiting a significant negative correlation with temperature, nutrient levels, TSS and turbidity (Table 9). This pattern aligns with findings from lakes in the USA (Stevenson et al. 2013), Lithuanian lakes (Šidagytė et al. 2013) and lakes in Flanders, Belgium (Gabriels et al. 2010). The negative correlation of the MMI-D with nutrients, TSS and turbidity is consistent with expectations for stress-related variables (Gabriels et al. 2010). These results suggest that the MMI-D is a suitable tool for assessing the ecological quality of Lake Hawassa.

The overall findings of the current research highlight the significant advantages of a multimetric approach when assessing a water body exposed to various stressors, as noted by Everard et al. (2011). The index can also reduce the independent metric prediction problem by capturing disturbances in lake ecosystem segments that are not visually apparent or easily measured. According to Schoolmaster et al. (2012), the multimetric approach is most effective in situations where the exact cause of water quality degradation is not clear. The discriminatory power of

the MMI-D between different levels of human disturbance, further demonstrates the effectiveness of this multimetric approach. In addition, the development of the MMI-D for specific lakes enhances its performance by reducing natural variability in factors, such as geology, soils, landscapes, climate and water chemistry, across different sites. It can be challenging to distinguish natural variability from human induced variability when assessments are conducted over large spatial scales (Stevenson et al. 2013). Furthermore, the MMI-D clearly discriminated between reference and non-reference sites in Lake Hawassa and demonstrated similar effectiveness in Lake Ziway (both located in the same ecoregion). Therefore, it can be recommended for application in biomonitoring activities in other lakes in this ecoregion.

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