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Gill acetylcholinesterase activity and condition index in *Donax rugosus* as candidate biomarkers of coastal water quality in Mauritania

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ABSTRACT

Coastal waters off Nouakchott support fisheries and, more recently, offshore oil activity and iron-ore export, all of which raise the risk of marine contamination. Local biomonitoring tools are still lacking. We asked whether the wedge clam *Donax rugosus*, abundant on Nouakchott's beaches, can serve as a sentinel. Acetylcholinesterase (AChE), an enzyme inhibited by organophosphate and carbamate pesticides and by several trace metals, was measured together with the physiological condition index (CI). Clams were collected monthly from September 2022 to July 2023 at two beaches, Nicola and Hôtel Sabah. AChE was first assayed in four organs (mantle, digestive gland, foot, gill) and then followed monthly in the gills. Activity differed strongly among organs: the mantle was highest (about 16.0 nmol min^{-1} mg^{-1} protein, two-site mean) and the gill lowest (about 2.6), roughly a six-fold range. We retained the gill for the time series because its low, stable baseline is easy to read. Gill AChE and CI both changed with season, each peaking in summer; gill AChE was lowest in autumn and rose with water temperature, while CI fell in spring, the period when *Donax* spawns. Paired tests showed no significant difference between the two beaches for either variable, and the AChE–CI link was weak overall. Gill AChE in *D. rugosus* is a workable, low-baseline biomarker for coastal biomonitoring in Mauritania, on the condition that temperature and reproductive state are accounted for when the data are interpreted.

Keywords: *Donax rugosus*, acetylcholinesterase, biomarkers, biomonitoring, condition index, neurotoxicity, coastal water quality, Mauritania.

INTRODUCTION

Sandy beaches and nearshore waters carry a heavy and growing load from farming, cities and industry, and the discharges that follow degrade habitats and reduce biological production (Smolders et al., 2005; Sarkar et al., 2006). Mauritania depends economically on fisheries, offshore petroleum and iron-ore mining, and each of these adds pressure on the coastal environment. What the country largely lacks is a routine way to detect that pressure early, before it shows up as lost biodiversity or declining catches.

Bivalve molluscs have filled this role elsewhere for half a century, ever since the original "mussel watch" proposal (Goldberg, 1975). They stay in place, so a contamination signal can be tied to a particular beach; they filter large volumes of water and concentrate pollutants in their tissues; and they tolerate exposure well enough to be sampled repeatedly (Boening, 1999; Beyer et al., 2017). Among the biochemical responses measured in these programmes, the inhibition of acetylcholinesterase (AChE) is one of the best established. AChE hydrolyses acetylcholine at nerve synapses, and organophosphate and carbamate pesticides, along with some trace metals, block it; the response is easy to measure, dose-dependent and tied to a clear physiological effect, which is why it has become a standard biomarker of neurotoxic exposure (Lionetto et al., 2013; Sarkar et al., 2006; Tlili et al., 2010).

The wedge clam *Donax* is a good candidate on exposed Atlantic and Mediterranean beaches. In *Donax trunculus*, AChE and companion biomarkers have separated contaminated beaches from cleaner ones and have tracked seasonal and spatial exposure (Tlili et al., 2010; Chahouri et al., 2021, 2022; Secco et al., 2024). *D. rugosus* is just as common and easy to collect on the Mauritanian coast, yet no one has tested it as a monitoring species. We set out to do that near Nouakchott. The study had three aims: to map AChE activity across four organs and pick the best tissue for routine work; to follow gill AChE through a full year; and to measure the condition index alongside it, so that enzyme activity and physiological state could be read together rather than in isolation.

MATERIALS AND METHODS

Study area

Sampling took place along the Atlantic coast of the Nouakchott region (about 17°56′ to 18°14′ N). Two beaches with comparable human footprints were chosen (Figure 1): Nicola (18°06′38″ N, 16°01′25″ W), a coastal tourism area; and Hôtel Sabah (18°13′11″ N, 16°02′10″ W), a coastal tourism area with seasonal hotel and restaurant traffic. Both are exposed sandy shores of the kind *Donax rugosus* lives in. The two stations were selected primarily for their comparable exposed sandy-beach habitat, a prerequisite for the species, and for their accessibility to regular monthly sampling, rather than to contrast a heavily impacted site with a clean one. Both carry a broadly similar, low-to-moderate human footprint dominated by coastal tourism, Hotel Sabah being additionally subject to seasonal hotel and restaurant activity; the design was therefore intended to establish baseline seasonal dynamics under comparable conditions.

Sampling

Donax rugosus (Mollusca, Bivalvia, Donacidae) lives buried just under the surface of wave-washed sand. Treading the sand at low tide brings the clams up, and they were then picked by hand. The work fell into two parts. First, AChE was measured in four organs: gill (Br), mantle (Mt), foot (Pd) and digestive gland (Gd), to see how activity is distributed. Second, gill AChE and the condition index were followed monthly from September 2022 to July 2023. For the enzyme

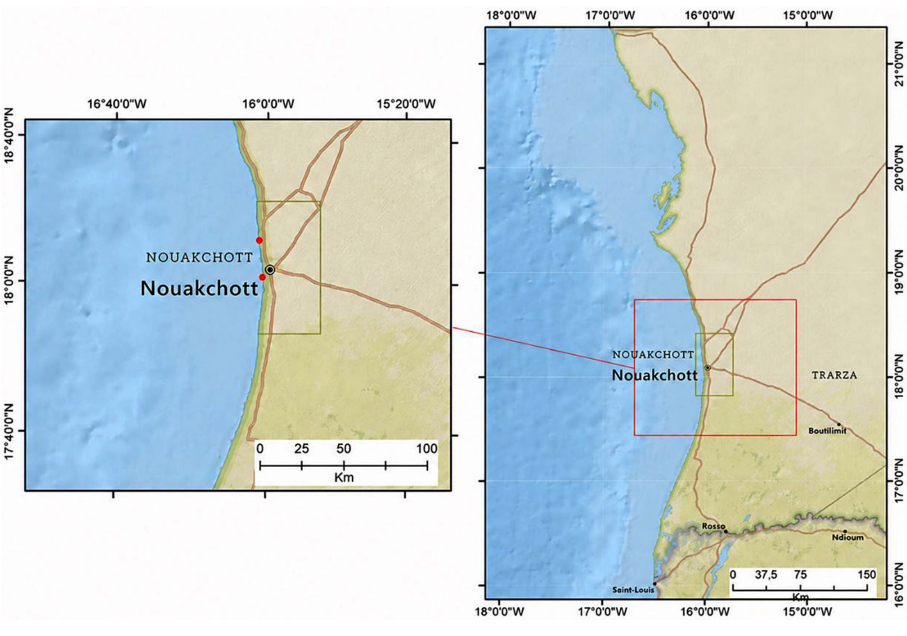

Figure 1. Location of the study area on the Atlantic coast of Mauritania near Nouakchott. The regional map (right) places the Nouakchott area; the enlarged map (left) marks the two sampling sites (red dots), Nicola and Hôtel Sabah. Scale bars in km

work, clams were dissected on the beach, the organs frozen at once in liquid nitrogen, placed in cryotubes and held at −80 °C until analysis. Table 1 gives the sampling design and the number of individuals analysed.

Acetylcholinesterase assay

AChE was assayed by the colorimetric method of Ellman et al. (1961). Tissues were homogenised in buffer (100 mM, pH 7.8) and centrifuged at 9 000 g for 15 min, and the S9 supernatant, which holds the cholinesterases, was used for the assay. The thiocholine released when acetylthiocholine is hydrolysed reacts with 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB, 0.2 mM) to give the yellow 5-thio-2-nitrobenzoate anion, read at 405 nm. Each sample ran against a substrate-free control (for interfering thiols) and an enzyme-free control (for spontaneous hydrolysis). Activity is given in nmol min^{-1} mg^{-1} protein.

Condition index

The condition index (CI) is the ratio of soft-tissue (wet flesh) mass to total mass, as a percentage: CI = (wet soft-tissue mass / total mass) × 100. It is a simple gravimetric measure of a clam's physiological and nutritive state, sensitive to both feeding and the reproductive cycle (Tlili et al., 2011; Benali et al., 2015).

Physico-chemical parameters

Water temperature, pH and salinity were read in situ at each visit with a multi-parameter probe held in the surf. Monthly values for both sites are in Table 2.

Statistical analysis

The analytical unit was the monthly site mean. With few monthly means per site and no safe assumption of normality, we compared sites with the paired Wilcoxon signed-rank test (months as matched pairs) and reported the paired Student t-test beside it, with the rank-biserial effect size r for the Wilcoxon test. The gill AChE–CI relationship was examined with the Spearman rank correlation (ρ), overall and within each site. Because the number of monthly means is small, the difference and correlation tests are treated as exploratory. Two-tailed $p < 0.05$ was taken as significant. All analyses were carried out in R (version 4.3.1; R Core Team, 2023).

RESULTS

Physico-chemical parameters

Water temperature ran from 18.9 °C (Nicola, January) to 26.9 °C (Sabah, March) and warmed at both sites from late spring into summer. pH

Table 1. Sampling design and number of individuals analysed for each assay during the monthly monitoring (September 2022–July 2023, two sites)

Assay	Site-months with data	Individuals per site-month	Total individuals analysed
Gill AChE (monthly monitoring)	18	15	270
Condition index (monthly monitoring)	16	15	240

Table 2. Monthly in-situ physico-chemical parameters at the two sites (T, temperature in °C; pH; salinity)

Month	Sabah T	Sabah pH	Sabah sal.	Nicola T	Nicola pH	Nicola sal.
Sep	24.4	7.25	27.73	24.1	7.04	29.74
Oct	25.03	7.41	28.55	23.9	7.15	29.74
Nov	24.11	7.34	22.36	23.2	7.30	25.40
Dec	22.8	7.56	27.40	22.6	7.10	24.70
Jan	19.5	7.43	29.20	18.9	7.35	23.30
Feb	25.7	7.11	29.80	22.4	7.90	25.10
Mar	26.9	7.20	31.20	23.8	7.18	30.20
Jun	26.2	6.56	32.30	25.2	6.76	30.80
Jul	25.6	6.70	31.50	25.1	7.30	31.02

stayed between 6.56 (Sabah, June) and 7.90 (Nicola, February), and salinity between 22.36 (Sabah, November) and 32.30 (Sabah, June). The two beaches behaved much alike through the year (Table 2).

Organotropism of AChE activity

Activity varied widely among organs at both sites (Figure 2, Table 3). The mantle held the most (two-site mean about 16.0 nmol min^{-1} mg^{-1} protein), far above the digestive gland (about 4.2) and foot (about 3.8), with the gill lowest (about 2.6), a ranking of mantle ≫ digestive gland ≈ foot > gill. Tissue-dependent cholinesterase activity of this kind is well documented in other bivalves, where the mantle/gonad complex, digestive gland and gills differ markedly (Dailianis et al., 2003; Kopecka-Pilarczyk, 2010; Oliveira et al., 2018), and AChE in *D. trunculus* falls in the same range (Tlili et al., 2010). The gill is in direct contact with the water and renews its cells quickly; its low, steady baseline makes it convenient to monitor, and short-term work has also found the gill the most practical tissue for AChE in mussels (Kopecka-Pilarczyk, 2010). We therefore used it for the monthly series.

Seasonal and monthly dynamics of gill AChE

Gill AChE changed over the year at both sites (Figures 3 and 4). Seasonal means were lowest in autumn (Sabah 6.9; Nicola 6.4 nmol min^{-1} mg^{-1} protein) and highest in summer (Sabah 12.1; Nicola 16.2), with winter and spring in between. Month by month, activity was low in early autumn, held a fairly flat plateau through winter, then climbed sharply towards summer, peaking in June at Sabah (about 18.0) and in July at Nicola (about 19.5). The series tracks the seasonal warming of the water rather than dropping in summer, which is what a strong neurotoxic-inhibition signal would have produced.

Condition index

The condition index followed a similar yearly course at both sites (Figures 5 and 6), high in summer (Sabah 22.7; Nicola 23.1) and low in spring (Sabah 16.2; Nicola 17.0), with a sharp October

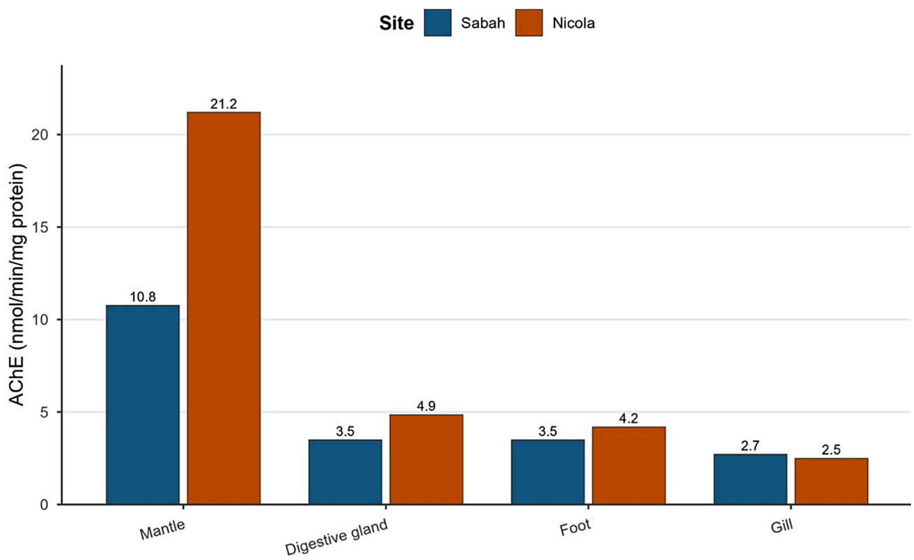

Figure 2. Organotropism of AChE activity in *D. rugosus* measured in mantle, digestive gland, foot and gill at the two sites. Values are mean activities (nmol min^{-1} mg^{-1} protein)

Table 3. Mean AChE activity (nmol min^{-1} mg^{-1} protein) per organ at each site and two-site mean

Organ	Nicola	Sabah	Mean (2 sites)
Mantle	21.21	10.77	15.99
Digestive gland	4.85	3.50	4.17
Foot	4.19	3.50	3.85
Gill	2.49	2.72	2.60

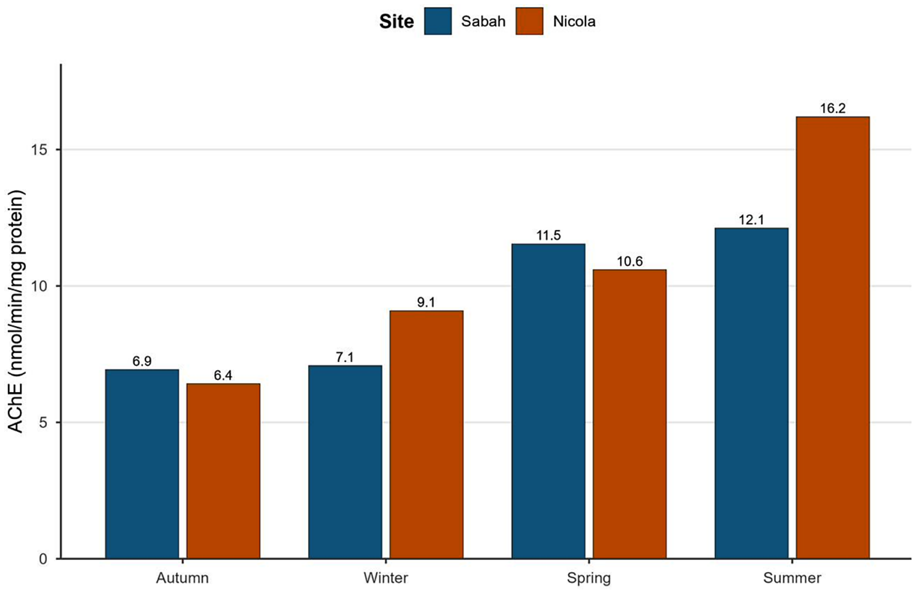

Figure 3. Seasonal variation of gill AChE activity in *D. rugosus* at the two sites (mean nmol min^{-1} mg^{-1} protein)

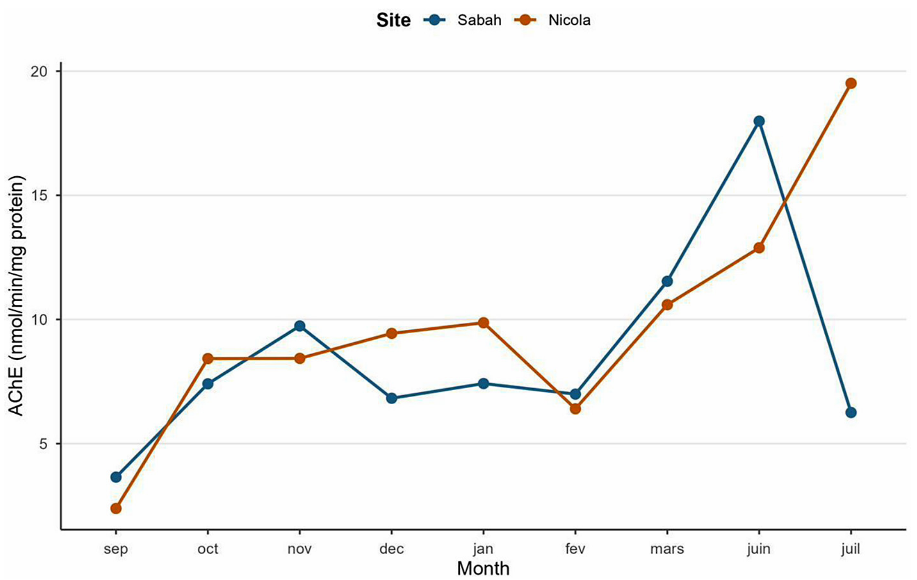

Figure 4. Monthly dynamics of gill AChE activity in *D. rugosus* (September 2022–July 2023)

drop and a partial winter recovery. The spring low matches the cost of reproduction, when *Donax* draws on body reserves for gametogenesis and spawning (Tlili et al., 2011). From November to March, CI ran a little higher at Sabah than at Nicola, which may point to slightly better feeding or environmental conditions at Sabah over that stretch.

Site comparison and AChE–condition index relationship

Neither variable differed significantly between the two beaches (Table 4): gill AChE (medians 7.41 vs 9.44; Wilcoxon V = 20, p = 0.82; paired-t p = 0.53; r = −0.11) and CI (medians 20.12 vs 17.70; Wilcoxon V = 26, p = 0.31; paired-t p = 0.37; r = 0.44). Pooling all monthly means, gill AChE and CI were only weakly related (Spearman ρ = 0.16, p = 0.55; Figure 8). Within sites the relationship was weakly negative at Sabah (ρ = −0.14, p = 0.75) and moderately positive at Nicola (ρ = 0.60, p = 0.13); neither was significant with so few monthly means (Figure 7, Table 5), so these correlations are best read as exploratory.

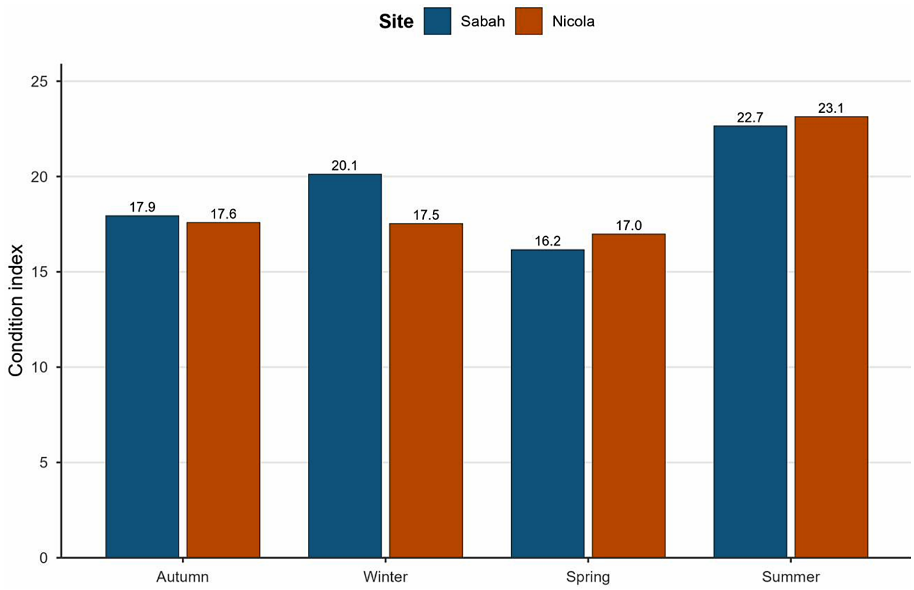

Figure 5. Seasonal variation of the condition index in *D. rugosus* at the two sites

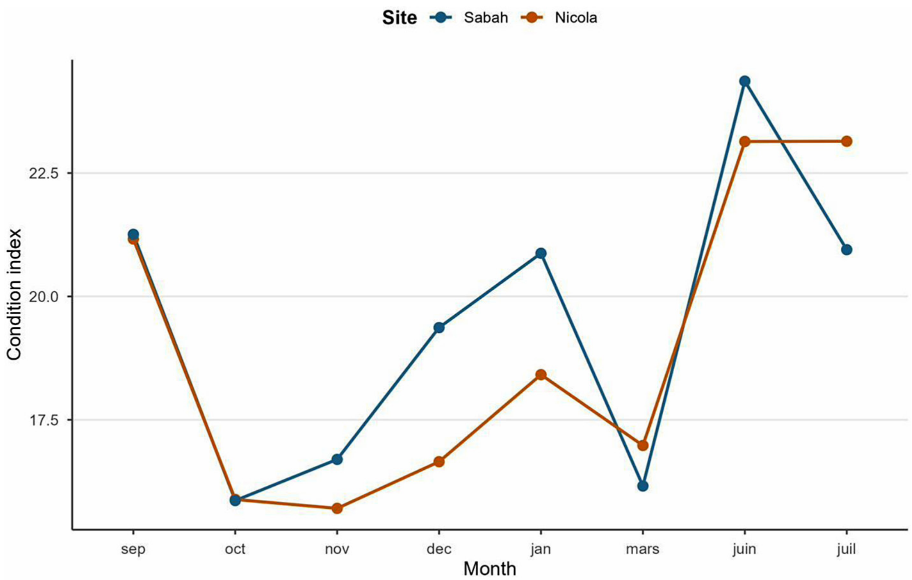

Figure 6. Monthly dynamics of the condition index in *D. rugosus* at the two sites

Table 4. Paired site comparison (Wilcoxon signed-rank test on monthly means; paired t-test and effect size r for reference)

Variable	n pairs	Median Sabah	Median Nicola	Wilcoxon V	Wilcoxon p	t p
AChE	9	7.41	9.44	20	0.820	0.527
CI	8	20.12	17.70	26	0.313	0.371

Table 5. Spearman rank correlation between gill AChE and condition index (monthly means), globally and per site

Dataset	Spearman ρ	p-value
Global	0.16	0.549
Sabah	−0.14	0.752
Nicola	0.60	0.132

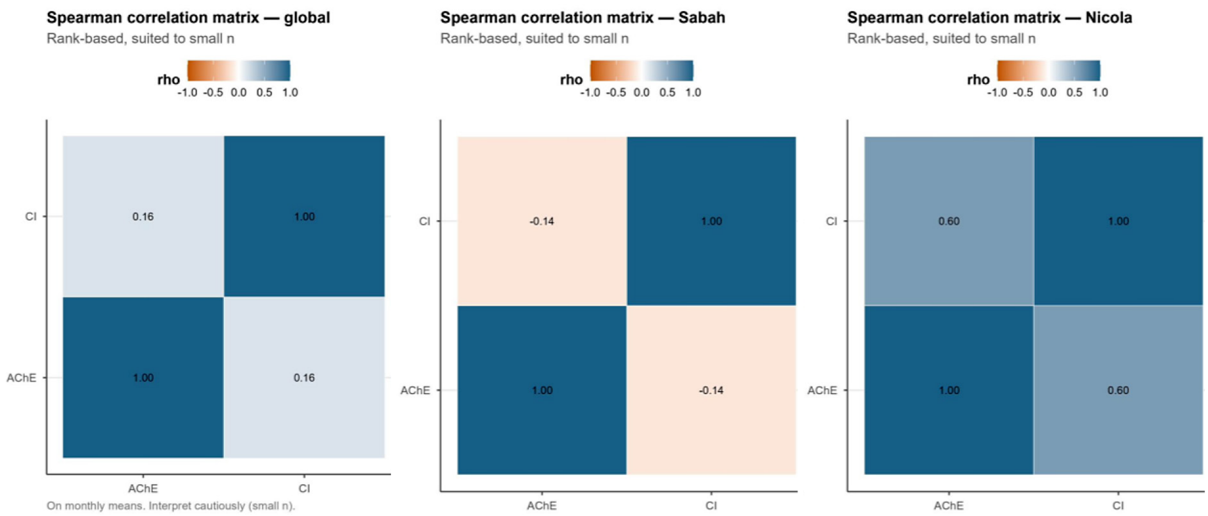

Figure 7. Spearman correlation matrices (AChE vs CI) for the global dataset (left), Sabah (centre) and Nicola (right). To be read with caution given the small sample size

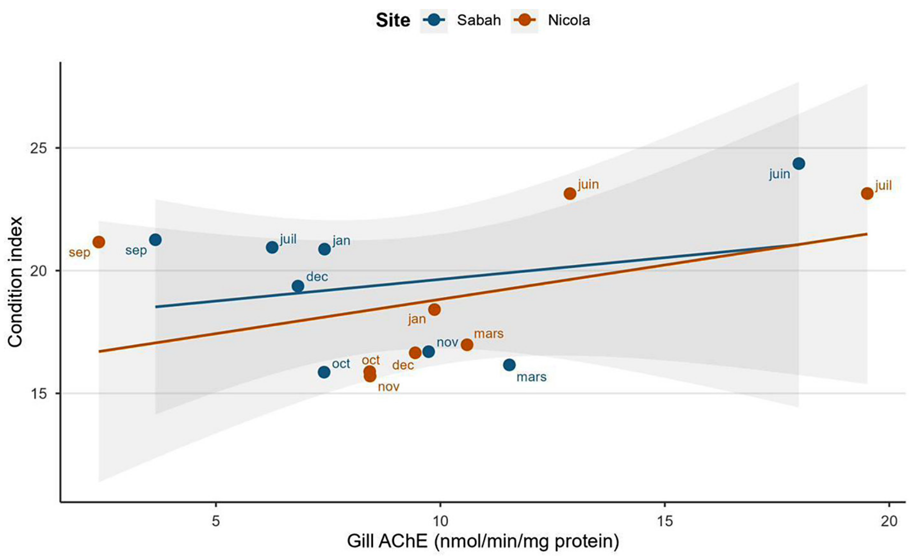

Figure 8. Condition index against gill AChE (monthly means) at the two sites. Trend lines are indicative; the formal test is the Spearman correlation (Table 5)

DISCUSSION

The clearest result here is how unevenly AChE is distributed among the organs of *D. rugosus*, with the mantle about six times the gill. Other bivalves show the same kind of tissue ranking (Dailianis et al., 2003; Oliveira et al., 2018). A low, steady gill baseline is convenient for routine work because it keeps individual-to-individual scatter down, though very low cholinesterase activity in clam gills can also limit sensitivity, which is why some authors turn to complementary esterases (Solé et al., 2018). On balance, the gill is a sensible compromise: it is the tissue in closest contact with the water and the one that short-term studies have found most practical for AChE (Kopecka-Pilarczyk, 2010). Importantly, sensitivity for biomonitoring depends on the signal-to-noise ratio rather than on the absolute level of activity: the low but stable gill baseline limits inter-individual variability, and inhibition is best expressed as a percentage relative to local reference baselines, with season-matched comparisons and, ideally, dose-response validation alongside a panel of complementary esterases (carboxylesterases) and oxidative-stress enzymes.

Gill AChE and CI moved together through the year and both peaked in summer. Instead of the summer dip that a heavy neurotoxic load would cause, gill AChE rose with water temperature. That points away from any strong, persistent inhibition at either beach during the study, and towards the ordinary modulation of enzyme activity by temperature and physiological state, both known to shape cholinesterase readings in bivalves (Kopecka-Pilarczyk, 2010; Leiniö and Lehtonen, 2005; Farcy et al., 2012). To use AChE in *D. rugosus* as a pollution signal, these natural drivers have to be controlled, ideally with local reference baselines and season-matched comparisons (Beyer et al., 2017). Because contaminant concentrations were not measured directly in this study, any assessment of environmental quality remains indirect and inferential; confirming it will require coupling the gill AChE response with direct chemical analysis of metals and pesticides.

The spring CI minimum fits the reproductive biology of *Donax*: in *D. trunculus*, spawning starts in spring and peaks around May, with a measurable drop in energy reserves and in the condition index (Tlili et al., 2011). The summer rebound most likely reflects recovery of tissue and reserves after spawning. The condition index is itself increasingly read as a sub-lethal, integrative measure of bivalve health in contamination work (Karray et al., 2023; Benali et al., 2015), and measuring it next to AChE puts the enzyme data in physiological context. The two responses tracking together, and both with temperature and reproduction, is consistent with the seasonal biomarker patterns reported for mussels in other temperate and Mediterranean settings (Leiniö and Lehtonen, 2005; Benali et al., 2015).

We found no significant gill-AChE or CI difference between Nicola and Hôtel Sabah, and only a weak overall AChE–CI link, with a moderate but non-significant positive trend at Nicola. Either the two beaches were under broadly similar pressure during the year, or the handful of monthly means left too little power to resolve a modest gap. The gill AChE values are, in any case, of the same order as those reported for *D. trunculus* on the nearby Moroccan Atlantic coast (Chahouri et al., 2021), which supports treating *D. rugosus* as comparable to the established Donacid sentinels. In *D. trunculus*, AChE has separated polluted from reference sites and has been tied to organophosphate, carbamate and metal exposure (Tlili et al., 2010; Secco et al., 2024), and AChE remains among the core biomarkers recommended for marine pollution monitoring (Lionetto et al., 2013; Sarkar et al., 2006). The two comparable beaches were chosen deliberately for this baseline year; demonstrating the discriminating power of the species will require, in future work, contrasting a clearly impacted station (for example near a port or an industrial discharge) with a low-impact reference site.

The study has limits worth stating plainly. The analyses rest on monthly site means with little temporal replication, so the difference and correlation tests are exploratory; one spring month and the February CI are missing; and AChE was not paired with chemical measurements of contaminants. The next step should combine season-matched, replicated sampling with direct contaminant analysis and a small panel of complementary biomarkers (oxidative-stress enzymes and carboxylesterases, for instance) to separate pollution effects from natural variation, as integrated studies on related clams have done (Chahouri et al., 2021, 2022; Benali et al., 2015). In particular, as long as the contaminants themselves are not quantified, any statement about the environmental quality of the two sites remains indirect.

CONCLUSIONS

AChE activity in *D. rugosus* is strongly organ-dependent, highest in the mantle and lowest in the gill. The low, steady gill baseline, together with the gill's direct contact with the water, makes it the right tissue for routine monitoring. Over a full year, gill AChE and the condition index both varied with season and both peaked in summer, following temperature and reproduction more closely than any pollution signal, and the two beaches did not differ. *D. rugosus* is therefore a promising and locally abundant sentinel for coastal monitoring in Mauritania, as long as the natural drivers are controlled and the enzyme data are backed by direct contaminant analysis in future surveys of the Nicola and Hôtel Sabah stations.

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