Caffeine Multispecies : Data Analysis

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1 Statistical Approach

1.1 Data Collection and Curation

The study employed a comprehensive multi-biomarker strategy to evaluate the ecotoxicological effects of caffeine on four marine organisms: Pachygrapsus marmoratus, Magallana gigas, Littorina littorea, and Actinia equina. A dataset was meticulously compiled to focus on these specific species and was categorized by variables such as batch, species, tissue types, and temporal intervals.

1.2 Statistical Software

Data analysis was conducted using R software (version 4.0.5), taking advantage of packages including tidyverse for data manipulation, ggplot2 for data visualization, broom for tidying up statistical outputs, knitr for dynamic report generation, and ggpubr for enhancing ggplot2 plots. Spatial data regarding the sample locations were mapped using QGIS software version 3.0.2.

1.3 Data Treatment

Prior to any statistical inference, tests for normality and homogeneity of variance were performed using the Shapiro-Wilk test and Bartlett's test, respectively. Data were presented as mean ± standard deviation within each stratified group (i.e., batch, species, tissue types, and temporal intervals).

1.4 Normalization

Mean and standard deviation values of each biomarker were normalized between 0 and 1 within species groups to allow for inter-species comparisons.

1.5 Statistical Tests

We used Welch's t-test to assess the significance of differences between concentrations and control groups for each biomarker. This method was chosen for its robustness against unequal variances and sample sizes, which are commonly encountered in ecotoxicological studies.

1.6 Significance Levels

Differences were considered statistically significant when the p-value was less than 0.05. To facilitate interpretation, p-values were converted into asterisks for significance representation on plots. These annotations were positioned above the standard deviation bars.

1.7 Data Presentation

Visual plots were constructed using ggplot2, with asterisks (or 'NS' for non-significant results) placed just above the top of the standard deviation bars to indicate the level of significance.

2 Tables

Table 1: CAT Catalase

Species	Batch	Tissue	Mean Value	SD Value	p-Value
Actinia equina	10 μg/L	Full body	0.6706	0.5052	0.1564
	5 μg/L	Full body	0.8093	0.5563	0.034
Littorina littorea	10 μg/L	Full body	2.1658	1.4764	0.0402
	5 μg/L	Full body	0.8501	0.3113	0.5705
Magallana gigas	10 μg/L	Digestive tract	1.5348	1.2611	0.9512
	10 μg/L	Gills	0.8893	1.8204	0.3571
	5 μg/L	Digestive tract	0.4617	0.4347	0.2106
	5 μg/L	Gills	0.1432	0.2219	0.9328
Pachygrapsus marmoratus	10 μg/L	Digestive tract	0.2217	0.4255	0.4057
	10 μg/L	Gills	0.398	0.2896	0.0633
	5 μg/L	Digestive tract	0.9654	0.22	0.2535
	5 μg/L	Gills	0.0	0.0	0.0808

Table 2: MDA Lipid Peroxidation

Species	Batch	Tissue	Mean Value	SD Value	p-Value
Actinia equina	10 μg/L	Full body	3.7056	0.754	0.0334
	5 μg/L	Full body	2.8305	1.6205	0.7186
Littorina littorea	10 μg/L	Full body	1.1144	0.2778	0.0007
	5 μg/L	Full body	0.6678	0.5723	0.2457
Magallana gigas	10 μg/L	Digestive tract	1.4029	0.1245	0.0886
	10 μg/L	Gills	1.4831	0.3002	0.056
	5 μg/L	Digestive tract	1.6212	0.2907	0.0254
	5 μg/L	Gills	1.7828	0.2732	0.0073
Pachygrapsus marmoratus	10 μg/L	Digestive tract	0.465	0.1903	0.3333
	10 μg/L	Gills	0.1221	0.0491	0.1709
	5 μg/L	Digestive tract	0.2338	0.1166	0.5076
	5 μg/L	Gills	0.2119	0.0867	0.5756

Table 3: Superoxide Dismutase

Species	Batch	Tissue	Mean Value	SD Value	p-Value
Actinia equina	10 μg/L	Full body	0.2677	0.2785	0.8696
	5 μg/L	Full body	0.347	0.3718	0.7637
Littorina littorea	10 μg/L	Full body	0.3852	0.0303	0.0022
	5 μg/L	Full body	0.3754	0.0213	0.001
Magallana gigas	10 μg/L	Digestive tract	0.2189	0.0459	0.192
	10 μg/L	Gills	0.3369	0.0637	0.2737
	5 μg/L	Digestive tract	0.0412	0.0176	0.3972
	5 μg/L	Gills	0.3078	0.0909	0.1525
Pachygrapsus marmoratus	10 μg/L	Digestive tract	0.3345	0.2476	0.1536
	10 μg/L	Gills	0.5088	0.3822	0.5426
	5 μg/L	Digestive tract	0.1423	0.2121	0.9402
	5 μg/L	Gills	0.6591	0.2218	0.0369

Table 4: Glutathion Peroxydase

Species	Batch	Tissue	Mean Value	SD Value	p-Value
Actinia equina	10 μg/L	Full body	0.5215	0.1294	0.0355
	5 μg/L	Full body	0.5388	0.2733	0.3258
Littorina littorea	10 μg/L	Full body	0.1406	0.0527	0.0088
	5 μg/L	Full body	0.1059	0.0255	0.0122
Magallana gigas	10 μg/L	Digestive tract	0.7349	0.1785	0.4592
	10 μg/L	Gills	0.2723	0.0344	0.049
	5 μg/L	Digestive tract	0.8298	0.1567	0.1477
	5 μg/L	Gills	0.2933	0.0512	0.0116
Pachygrapsus marmoratus	10 μg/L	Digestive tract	0.1398	0.0188	0.4872
	10 μg/L	Gills	0.1672	0.0225	0.5833
	5 μg/L	Digestive tract	0.1788	0.0898	0.8576
	5 μg/L	Gills	0.2252	0.0699	0.2852

Table 5: Acetylcholinesterase

Species	Batch	Tissue	Mean Value	SD Value	p-Value
Actinia equina	10 μg/L	Full Body	0.0384	0.1001	0.0033
	10 μg/L	Full Body	-0.0179	0.0278	0.0002
	10 μg/L	Full Body	-0.0229	0.0382	0.0016
	10 μg/L	Full Body	0.0092	0.0173	0.9171
	5 μg/L	Full Body	0.0578	0.0843	0.0042
	5 μg/L	Full Body	0.0047	0.0181	0.0076
	5 μg/L	Full Body	0.0334	0.0305	0.7461
	5 μg/L	Full Body	0.0016	0.0351	0.7019
Litttorina littorea	10 μg/L	Full Body	1.1056	0.2774	0.0011
	10 μg/L	Full Body	1.2027	0.373	0.0008
	10 μg/L	Full Body	1.1046	0.3071	0.0021
	10 μg/L	Full Body	1.1233	0.3471	0.0016
	5 μg/L	Full Body	1.3591	0.2859	0.0051
	5 μg/L	Full Body	1.4098	0.2215	0.0027
	5 μg/L	Full Body	1.5064	0.2387	0.0296
	5 μg/L	Full Body	1.3829	0.2388	0.0044
Magallana gigas	10 μg/L	Digestive Tract	0.8483	0.1337	0.5408
	10 μg/L	Digestive Tract	0.2492	0.2119	0.1054
	10 μg/L	Digestive Tract	0.0896	0.0801	0.1652
	10 μg/L	Digestive Tract	0.022	0.1209	0.1707
	10 μg/L	Gills	0.6822	0.2334	0.9521
	10 μg/L	Gills	0.3325	0.0705	0.029
	10 μg/L	Gills	0.3029	0.2289	0.5986
	10 μg/L	Gills	0.1064	0.1346	0.0074
	5 μg/L	Digestive Tract	0.3372	0.3897	0.0464
	5 μg/L	Digestive Tract	0.2474	0.2154	0.0987
	5 μg/L	Digestive Tract	0.1009	0.0971	0.1671
	5 μg/L	Digestive Tract	0.057	0.0478	0.2415
	5 μg/L	Gills	0.7697	0.1736	0.5948
	5 μg/L	Gills	0.7534	0.0809	0.1888
	5 μg/L	Gills	0.4569	0.2084	0.568
	5 μg/L	Gills	0.1409	0.1523	0.0782
Pachygrapsus marmoratus	10 μg/L	Digestive Tract	-0.0431	0.233	0.1222
	10 μg/L	Digestive Tract	0.0488	0.0938	0.1385
	10 μg/L	Digestive Tract	0.0251	0.074	0.2282
	10 μg/L	Digestive Tract	-0.0298	0.0885	0.0413
	10 μg/L	Gills	0.2391	0.3095	0.2978
	10 μg/L	Gills	-0.0382	0.1163	0.1054
	10 μg/L	Gills	-0.0012	0.0264	0.0536
	10 μg/L	Gills	0.0191	0.0175	0.0984
	5 μg/L	Digestive Tract	-0.0401	0.0688	0.1024
	5 μg/L	Digestive Tract	-0.0284	0.0888	0.1053
	5 μg/L	Digestive Tract	-0.0491	0.0608	0.1161
	5 μg/L	Digestive Tract	0.0367	0.048	0.1169
	5 μg/L	Gills	0.2014	0.2839	0.3711
	5 μg/L	Gills	-0.021	0.0449	0.071
	5 μg/L	Gills	0.0196	0.0665	0.3076
	5 μg/L	Gills	0.0709	0.0697	0.6576

Table 6: Sampling sites - GPS coordinates

Species	**Site**	**Latitude**	**Longitude**
Pachygrapsus marmoratus	Noirmoutier *(pl. vieil)*	47.02505	-2.2492860
Actinia equina	Donville sur mer	48.85260	-1.5838020
Litttorina littorea	Baie de Seine	49.28931	-0.1788736
Magallana gigas	Oleron	45.95872	-1.2413988

Table 7: Summary of volume measurements.

Species	Mean Volume (cm^3)	Std Dev (cm^3)
Magallana gigas	96.62	27.03
Pachygrapsus marmoratus	6.96	7.76
Littorina littorea	2.36	0.22

Table 8: Actinia equina

Species	Mean Weight (g)	Std Dev (g)
Actinia equina	5.28	3.70