

Supplementary information

eDNA reveals the associated metazoan diversity of Mediterranean seagrass sediments

Marlene Wesselmann¹, Nathan R. Geraldi², Núria Marbà¹, Iris E. Hendriks¹, Rubén Díaz-Rúa², Carlos M. Duarte²

¹Global Change Research Group, IMEDEA (CSIC-UIB), Institut Mediterrani d'Estudis Avançats, Miguel Marques 21, 07190, Esporles, Illes Balears, Spain

²Red Sea Research Centre (RSRC) and Computational Bioscience Research Center (CBRC), King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

*Corresponding author: m.wesselmann@imedea.uib-csic.es

Figure S1: Nonmetric multidimensional scaling plot of temporal changes in metazoan communities within seagrass meadows (*H. stipulacea* in green; *C. nodosa* in orange; *P. oceanica* in blue). Ellipses show 95% standard error around the mean of the four time intervals and dark grey arrows indicate significant relationship ($p < 0.05$) between the NMDS axes and different metazoan phyla. Greater length of arrows indicates greater r^2 value and is relative to the importance of the explanatory variable in the ordination, and the arrow direction indicates positive or negative correlations. Kruskal stress = 0.16.

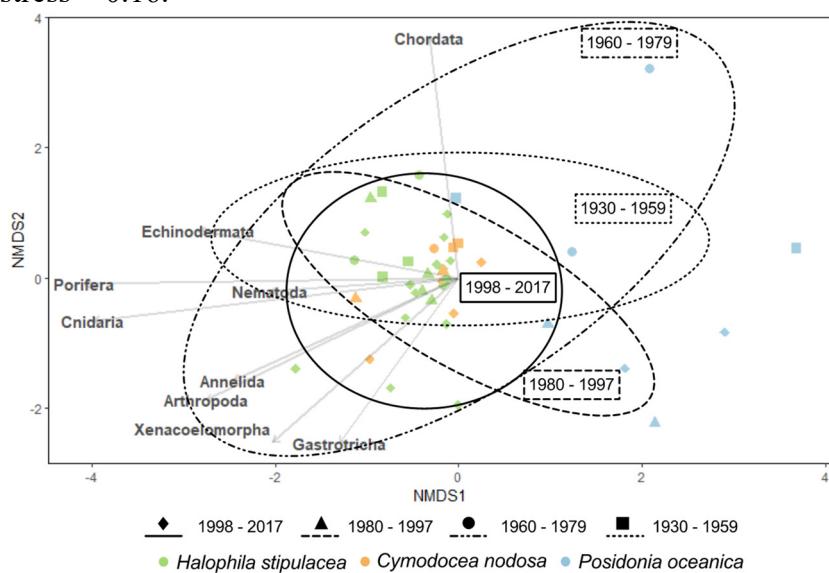


Figure S2: Number of metazoan orders in seagrass sediments identified for each time period (black dots). Columns indicate the number of orders either gained (blue) or lost (red) relative to the period before.

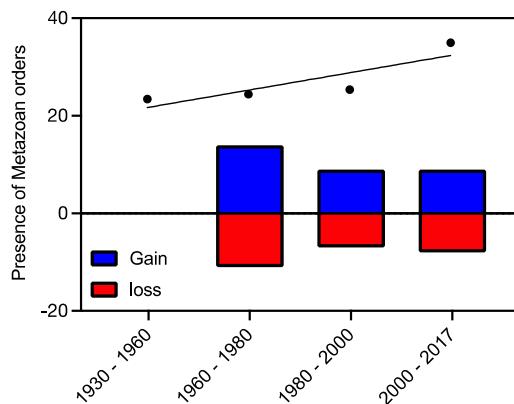


Table S1: Mock sample nucleic acid concentration ($\text{ng } \mu\text{l}^{-1}$ per genus/ $\text{ng } \mu\text{L}^{-1}$ of total sample $\times 100$) and Sequence Variants reads (SV per genus/ SV reads of total sample $\times 100$) after filtering and taxonomy assignment of several marine metazoans.

Species name	Mock ID	Concentration $\text{ng } \mu\text{l}^{-1}$	Nº sequences
<i>Tursiops truncatus</i>	Mock1	0.0024	16103
<i>Tridacna maxima</i>	Mock1	0.000052	2097
<i>Chelonia mydas</i>	Mock1	0.000216	2446
<i>Rhinoptera bonasus</i>	Mock1	0.000208	3231
<i>Zebrasoma xanthurum</i>	Mock1	0.0134	0
Holothuroidea	Mock1	0.00000086	0
<i>Sebastapistes cyanostigma</i>	Mock1	0.0000066	0
<i>Scylla serrata</i>	Mock1	0.00000399	0
Ophiuroidea	Mock1	4.8E-08	0
<i>Lutjanus kasmira</i>	Mock1	0.00000118	0
Tursiops	Mock1	0	871
Actinopteri	Mock1	0	84288
Chordata	Mock1	0	375
Metazoa	Mock1	0	28
<i>Homo sapiens</i>	Mock1	0	169
<i>Tursiops truncatus</i>	Mock2	0.0000024	0
<i>Chelonia mydas</i>	Mock2	0.00000216	0
<i>Zebrasoma xanthurum</i>	Mock2	0.0134	0
<i>Rhinoptera bonasus</i>	Mock2	0.00208	14537

<i>Lutjanus kasmira</i>	Mock2	0.000118	0
<i>Sebastapistes cyanostigma</i>	Mock2	0.0000066	0
Ophiuroidea	Mock2	4.8E-08	0
Holothuroidea	Mock2	0.000086	0
<i>Scylla serrata</i>	Mock2	0.000399	0
<i>Tridacna maxima</i>	Mock2	0.0000052	0
<i>Sarpa salpa</i>	Mock2	0.00000492	0
<i>Siganus luridus</i>	Mock2	0.0000186	798
<i>Siganus rivulatus</i>	Mock2	0.0026	17977
Chondrichthyes	Mock2	0	175
Actinopteri	Mock2	0	136
Actinopteri	Mock2	0	43284

Table S2: Linear mixed effect model analysis of Metazoan communities richness through time in seagrass meadows (*H.s.*: *H. stipulacea*, *C.n.*: *C.nodosa* and *P.o.*: *P. oceanica*) with Site as a random factor, Core nested in Site and Seagrass and Time as fixed factors.

FIXED EFFECTS:						RANDOM EFFECTS:		
	Estimate	SE	T ratio	df	p value	Group	Parameter	SD
Richness								
<i>P.o.</i>	19	3.45	5.51	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-7.67	4.45	-1.72	35	0.09	SITE	(Intercept)	0
<i>H.s.</i>	-9.13	3.66	-2.49	35	0.02	Residual		4.88
1980 - 1997	2.5	4.88	0.51	35	0.61			
1960 - 1979	6	4.88	1.23	35	0.23			
1930 - 1959	4	4.88	0.82	35	0.42			
<i>C.n.</i> 1980 - 1997	-5.5	6.3	-0.87	35	0.39			
<i>H.s.</i> 1980 - 1997	-4.71	5.41	-0.87	35	0.39			
<i>C.n.</i> 1960 - 1979	-10.33	6.6	-1.56	35	0.13			
<i>H.s.</i> 1960 - 1979	-7.12	5.59	-1.28	35	0.21			
<i>C.n.</i> 1930 - 1959	-9.67	6.3	-1.54	35	0.13			
<i>H.s.</i> 1930 - 1959	-0.87	6.1	-0.14	35	0.89			
Shannon								
<i>P.o.</i>	2.91	0.41	7.08	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-0.49	0.53	-0.92	35	0.36	SITE	(Intercept)	0
<i>H.s.</i>	-0.72	0.44	-1.64	35	0.11	Residual		0.58
1980 - 1997	0.14	0.58	0.24	35	0.81			
1960 - 1979	0.29	0.58	0.5	35	0.62			
1930 - 1959	0.18	0.58	0.31	35	0.76			
<i>C.n.</i> 1980 - 1997	-0.46	0.75	-0.62	35	0.54			
<i>H.s.</i> 1980 - 1997	-0.41	0.64	-0.64	35	0.53			
<i>C.n.</i> 1960 - 1979	-0.81	0.79	-1.03	35	0.31			
<i>H.s.</i> 1960 - 1979	-0.7	0.67	-1.06	35	0.3			
<i>C.n.</i> 1930 - 1959	-1.17	0.75	-1.57	35	0.13			
<i>H.s.</i> 1930 - 1959	0.18	0.73	0.25	35	0.8			
Chordata								
<i>P.o.</i>	1.32	2.12	0.62	27.26	0.54	CORE:SITE	(Intercept)	0
<i>C.n.</i>	0.74	2.71	0.27	32.39	0.79	SITE	(Intercept)	0.58
<i>H.s.</i>	1.55	2.2	0.71	34.99	0.48	Residual		2.9
1980 - 1997	2	2.9	0.69	32.27	0.49			
1960 - 1979	8	2.9	2.76	32.27	0.01			
1930 - 1959	7	2.9	2.42	32.27	0.02			
<i>C.n.</i> 1980 - 1997	-0.24	3.75	-0.06	33.17	0.95			
<i>H.s.</i> 1980 - 1997	-1.98	3.21	-0.62	32.44	0.54			
<i>C.n.</i> 1960 - 1979	-5.85	3.93	-1.49	32.85	0.15			
<i>H.s.</i> 1960 - 1979	-7.56	3.33	-2.27	33.77	0.03			
<i>C.n.</i> 1930 - 1959	-6.03	3.76	-1.6	34.22	0.12			
<i>H.s.</i> 1930 - 1959	-4.11	3.65	-1.13	34.55	0.27			
Mollusca								

<i>P.o.</i>	0.65	0.64	1.01	28.27	0.32	CORE:SITE	(Intercept)	0
<i>C.n.</i>	0.3	0.8	0.38	34.99	0.71	SITE	(Intercept)	0.36
<i>H.s.</i>	0.05	0.64	0.07	34.11	0.94	Residual		0.82
1980 - 1997	0	0.82	0	31.78	1			
1960 - 1979	0.5	0.82	0.61	31.78	0.55			
1930 - 1959	0.5	0.82	0.61	31.78	0.55			
<i>C.n.</i> 1980 - 1997	-0.16	1.07	-0.15	32.51	0.88			
<i>H.s.</i> 1980 - 1997	0.44	0.91	0.48	31.92	0.64			
<i>C.n.</i> 1960 - 1979	-0.92	1.12	-0.82	32.18	0.42			
<i>H.s.</i> 1960 - 1979	-0.67	0.95	-0.7	32.73	0.49			
<i>C.n.</i> 1930 - 1959	-1.36	1.08	-1.26	33.05	0.22			
<i>H.s.</i> 1930 - 1959	1.1	1.05	1.05	33.31	0.3			
Cnidaria								
<i>P.o.</i>	2.5	0.56	4.49	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-2.17	0.72	-3.02	35	<0.001	SITE	(Intercept)	0
<i>H.s.</i>	-2.13	0.59	-3.6	35	<0.001	Residual		0.79
1980 - 1997	1	0.79	1.27	35	0.21			
1960 - 1979	-1	0.79	-1.27	35	0.21			
1930 - 1959	1	0.79	1.27	35	0.21			
<i>C.n.</i> 1980 - 1997	-1.33	1.02	-1.31	35	0.2			
<i>H.s.</i> 1980 - 1997	-1.37	0.87	-1.58	35	0.12			
<i>C.n.</i> 1960 - 1979	0.67	1.07	0.63	35	0.54			
<i>H.s.</i> 1960 - 1979	0.88	0.9	0.97	35	0.34			
<i>C.n.</i> 1930 - 1959	-1.33	1.02	-1.31	35	0.2			
<i>H.s.</i> 1930 - 1959	-0.87	0.98	-0.89	35	0.38			
Porifera								
<i>P.o.</i>	2.5	0.23	10.8	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-2.5	0.3	-8.37	35	<0.001	SITE	(Intercept)	0
<i>H.s.</i>	-2.5	0.25	-	35	<0.001	Residual		0.33
			10.18					
1980 - 1997	-1	0.33	-3.06	35	<0.001			
1960 - 1979	-1.5	0.33	-4.58	35	<0.001			
1930 - 1959	-1.5	0.33	-4.58	35	<0.001			
<i>C.n.</i> 1980 - 1997	1	0.42	2.37	35	0.02			
<i>H.s.</i> 1980 - 1997	1	0.36	2.76	35	0.01			
<i>C.n.</i> 1960 - 1979	1.5	0.44	3.38	35	<0.001			
<i>H.s.</i> 1960 - 1979	1.75	0.38	4.67	35	<0.001			
<i>C.n.</i> 1930 - 1959	1.5	0.42	3.55	35	<0.001			
<i>H.s.</i> 1930 - 1959	1.5	0.41	3.67	35	<0.001			
Echinodermata								
<i>P.o.</i>	1.5	0.39	3.86	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-1.5	0.5	-2.99	35	0.01	SITE	(Intercept)	0
<i>H.s.</i>	-1.38	0.41	-3.33	35	<0.001	Residual		0.55
1980 - 1997	-0.5	0.55	-0.91	35	0.37			
1960 - 1979	0	0.55	0	35	1			
1930 - 1959	-1	0.55	-1.82	35	0.08			

<i>P.o.</i>	0.89	1.52	0.59	28.18	0.56	CORE:SITE	(Intercept)	0
<i>C.n.</i>	0.98	1.88	0.52	35	0.61	SITE	(Intercept)	0.88
<i>H.s.</i>	0.83	1.5	0.56	34.02	0.58	Residual		1.94
1980 - 1997	1.5	1.94	0.77	31.78	0.44			
1960 - 1979	6	1.94	3.1	31.78	<0.001			
1930 - 1959	4.5	1.94	2.32	31.78	0.03			
<i>C.n.</i> 1980 - 1997	-0.85	2.52	-0.34	32.48	0.74			
<i>H.s.</i> 1980 - 1997	-0.92	2.15	-0.43	31.92	0.67			
<i>C.n.</i> 1960 - 1979	-4.8	2.63	-1.82	32.17	0.08			
<i>H.s.</i> 1960 - 1979	-5.63	2.24	-2.51	32.69	0.02			
<i>C.n.</i> 1930 - 1959	-3.63	2.54	-1.43	32.99	0.16			
<i>H.s.</i> 1930 - 1959	-3.26	2.47	-1.32	33.23	0.2			
Bivalvia								
<i>P.o.</i>	0.57	0.6	0.95	27.46	0.35	CORE:SITE	(Intercept)	0
<i>C.n.</i>	0.08	0.75	0.1	34.64	0.92	SITE	(Intercept)	0.28
<i>H.s.</i>	0.05	0.6	0.08	34.4	0.93	Residual		0.78
1980 - 1997	0	0.78	0	31.34	1			
1960 - 1979	0	0.78	0	31.34	1			
1930 - 1959	0.5	0.78	0.64	31.34	0.53			
<i>C.n.</i> 1980 - 1997	-0.2	1.02	-0.2	32.28	0.85			
<i>H.s.</i> 1980 - 1997	0.34	0.87	0.39	31.52	0.7			
<i>C.n.</i> 1960 - 1979	-0.1	1.06	-0.09	31.88	0.93			
<i>H.s.</i> 1960 - 1979	-0.02	0.91	-0.02	32.68	0.98			
<i>C.n.</i> 1930 - 1959	-0.95	1.02	-0.93	33.11	0.36			
<i>H.s.</i> 1930 - 1959	1.22	0.99	1.22	33.47	0.23			
Anthozoa								
<i>P.o.</i>	0	0.22	0	35	1	CORE:SITE	(Intercept)	0
<i>C.n.</i>	0	0.28	0	35	1	SITE	(Intercept)	0
<i>H.s.</i>	0	0.23	0	35	1	Residual		0.3
1980 - 1997	0	0.3	0	35	1			
1960 - 1979	0	0.3	0	35	1			
1930 - 1959	1	0.3	3.28	35	<0.001			
<i>C.n.</i> 1980 - 1997	0	0.39	0	35	1			
<i>H.s.</i> 1980 - 1997	0	0.34	0	35	1			
<i>C.n.</i> 1960 - 1979	0	0.41	0	35	1			
<i>H.s.</i> 1960 - 1979	0.25	0.35	0.72	35	0.48			
<i>C.n.</i> 1930 - 1959	-1	0.39	-2.54	35	0.02			
<i>H.s.</i> 1930 - 1959	-0.5	0.38	-1.31	35	0.2			
Staurozoa								
<i>P.o.</i>	0	0.08	0	35	1	CORE:SITE	(Intercept)	0
<i>C.n.</i>	0	0.11	0	35	1	SITE	(Intercept)	0
<i>H.s.</i>	0	0.09	0	35	1	Residual		0.12
1980 - 1997	1	0.12	8.37	35	<0.001			
1960 - 1979	0	0.12	0	35	1			
1930 - 1959	0.5	0.12	4.18	35	<0.001			
<i>C.n.</i> 1980 - 1997	-1	0.15	-6.48	35	<0.001			

<i>H.s.</i> 1980 - 1997	-1	0.13	-7.55	35	<0.001			
<i>C.n.</i> 1960 - 1979	0	0.16	0	35	1			
<i>H.s.</i> 1960 - 1979	0	0.14	0	35	1			
<i>C.n.</i> 1930 - 1959	-0.5	0.15	-3.24	35	<0.001			
<i>H.s.</i> 1930 - 1959	-0.5	0.15	-3.35	35	<0.001			
Hydrozoa								
<i>P.o.</i>	1.5	0.24	6.32	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-1.5	0.31	-4.9	35	<0.001	SITE	(Intercept)	0
<i>H.s.</i>	-1.5	0.34	-4.47	35	<0.001	Residual		0.34
1980 - 1997	-0.5	0.34	-1.49	35	0.14			
1960 - 1979	1	0.34	2.98	35	0.01			
1930 - 1959	0	0.34	0	35	1			
<i>C.n.</i> 1980 - 1997	0.5	0.45	1.1	35	0.28			
<i>H.s.</i> 1980 - 1997	0.5	0.44	1.13	35	0.27			
<i>C.n.</i> 1960 - 1979	-1	0.43	-2.31	35	0.03			
<i>H.s.</i> 1960 - 1979	-1	0.43	-2.31	35	0.03			
<i>C.n.</i> 1930 - 1959	0	0.43	0	35	1			
<i>H.s.</i> 1930 - 1959	0.19	0.42	0.45	35	0.66			
Hexanauplia								
<i>P.o.</i>	2.24	0.67	3.37	18.49	<0.001	CORE:SITE	(Intercept)	0.08
<i>C.n.</i>	-0.99	0.84	-1.17	24.07	0.25	SITE	(Intercept)	0.26
<i>H.s.</i>	-1.68	0.68	-2.47	14.38	0.03	Residual		0.88
1980 - 1997	-0.5	0.88	-0.57	27.84	0.57			
1960 - 1979	-1.5	0.88	-1.7	27.84	0.1			
1930 - 1959	-0.5	0.88	-0.57	27.84	0.57			
<i>C.n.</i> 1980 - 1997	-0.84	1.14	-0.74	29.65	0.47			
<i>H.s.</i> 1980 - 1997	0.35	0.98	0.36	28.44	0.72			
<i>C.n.</i> 1960 - 1979	0.66	1.2	0.55	29.13	0.59			
<i>H.s.</i> 1960 - 1979	1.6	1.02	1.57	31.11	0.13			
<i>C.n.</i> 1930 - 1959	-0.85	1.15	-0.74	32	0.46			
<i>H.s.</i> 1930 - 1959	0.86	1.12	0.77	30.51	0.45			
Polychaeta								
<i>P.o.</i>	1.5	0.55	2.74	35	0.01	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-0.5	0.71	-0.71	35	0.48	SITE	(Intercept)	0
<i>H.s.</i>	-0.94	0.58	-1.61	35	0.12	Residual		0.77
1980 - 1997	1	0.77	1.29	35	0.21			
1960 - 1979	1	0.77	1.29	35	0.21			
1930 - 1959	0.5	0.77	0.65	35	0.52			
<i>C.n.</i> 1980 - 1997	-2	1	-2	35	0.05			
<i>H.s.</i> 1980 - 1997	-1.4	0.86	-1.62	35	0.11			
<i>C.n.</i> 1960 - 1979	-2	1.05	-1.91	35	0.06			
<i>H.s.</i> 1960 - 1979	-1.31	0.89	-1.48	35	0.15			
<i>C.n.</i> 1930 - 1959	-1.5	1	-1.5	35	0.14			
<i>H.s.</i> 1930 - 1959	-1.06	0.97	-1.1	35	0.28			
Demospongiae								
<i>P.o.</i>	2	0.27	7.3	35	<0.001	CORE:SITE	(Intercept)	0

<i>C.n.</i>	-2	0.35	-5.66	35	<0.001	SITE	(Intercept)	0
<i>H.s.</i>	-2	0.29	-6.89	35	<0.001	Residual		0.39
1980 - 1997	-0.5	0.39	-1.29	35	0.21			
1960 - 1979	-1	0.39	-2.58	35	0.01			
1930 - 1959	-1	0.39	-2.58	35	0.01			
<i>C.n.</i> 1980 - 1997	0.5	0.5	1	35	0.32			
<i>H.s.</i> 1980 - 1997	0.5	0.43	1.16	35	0.25			
<i>C.n.</i> 1960 - 1979	1	0.52	1.91	35	0.06			
<i>H.s.</i> 1960 - 1979	1.25	0.44	2.82	35	0.01			
<i>C.n.</i> 1930 - 1959	1	0.5	2	35	0.05			
<i>H.s.</i> 1930 - 1959	1	0.48	2.07	35	0.05			
Ophiuroidea								
<i>P.o.</i>	1	0.22	4.64	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-1	0.28	-3.59	35	<0.001	SITE	(Intercept)	0
<i>H.s.</i>	-1	0.23	-4.38	35	<0.001	Residual		0.3
1980 - 1997	0	0.3	0	35	1			
1960 - 1979	0	0.3	0	35	1			
1930 - 1959	-0.5	0.3	-1.64	35	0.11			
<i>C.n.</i> 1980 - 1997	0	0.39	0	35	1			
<i>H.s.</i> 1980 - 1997	0	0.34	0	35	1			
<i>C.n.</i> 1960 - 1979	0	0.41	0	35	1			
<i>H.s.</i> 1960 - 1979	0.25	0.35	0.72	35	0.48			
<i>C.n.</i> 1930 - 1959	0.5	0.39	1.27	35	0.21			
<i>H.s.</i> 1930 - 1959	0.5	0.38	1.31	35	0.2			
Gastropoda								
<i>P.o.</i>	0	0.23	0	35	1	CORE:SITE	(Intercept)	0
<i>C.n.</i>	0.33	0.29	1.14	35	0.26	SITE	(Intercept)	0
<i>H.s.</i>	0.06	0.24	0.26	35	0.8	Residual		0.32
1980 - 1997	0	0.32	0	35	1			
1960 - 1979	0.5	0.32	1.56	35	0.13			
1930 - 1959	0	0.32	0	35	1			
<i>C.n.</i> 1980 - 1997	0	0.41	0	35	1			
<i>H.s.</i> 1980 - 1997	0.1	0.36	0.29	35	0.77			
<i>C.n.</i> 1960 - 1979	-0.83	0.43	-1.92	35	0.06			
<i>H.s.</i> 1960 - 1979	-0.56	0.37	-1.53	35	0.13			
<i>C.n.</i> 1930 - 1959	-0.33	0.41	-0.8	35	0.43			
<i>H.s.</i> 1930 - 1959	-0.06	0.4	-0.16	35	0.88			
Echinoidea								
<i>P.o.</i>	0.5	0.08	5.92	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-0.5	0.11	-4.58	35	<0.001	SITE	(Intercept)	0
<i>H.s.</i>	-0.5	0.09	-5.58	35	<0.001	Residual		0.12
1980 - 1997	-0.5	0.12	-4.18	35	<0.001			
1960 - 1979	-0.5	0.12	-4.18	35	<0.001			
1930 - 1959	-0.5	0.12	-4.18	35	<0.001			
<i>C.n.</i> 1980 - 1997	0.5	0.15	3.24	35	<0.001			
<i>H.s.</i> 1980 - 1997	0.5	0.13	3.77	35	<0.001			

<i>C.n.</i> 1960 - 1979	0.5	0.16	3.09	35	<0.001			
<i>H.s.</i> 1960 - 1979	0.5	0.14	3.65	35	<0.001			
<i>C.n.</i> 1930 - 1959	0.5	0.15	3.24	35	<0.001			
<i>H.s.</i> 1930 - 1959	0.5	0.15	3.35	35	<0.001			
Homoscleromorpha								
<i>P.o.</i>	0.5	0.08	5.92	35	<0.001	CORE:SITE	(Intercept)	0
<i>C.n.</i>	-0.5	0.11	-4.58	35	<0.001	SITE	(Intercept)	0
<i>H.s.</i>	-0.5	0.09	-5.58	35	<0.001	Residual		0.12
1980 - 1997	-0.5	0.12	-4.18	35	<0.001			
1960 - 1979	-0.5	0.12	-4.18	35	<0.001			
1930 - 1959	-0.5	0.12	-4.18	35	<0.001			
<i>C.n.</i> 1980 - 1997	0.5	0.15	3.24	35	<0.001			
<i>H.s.</i> 1980 - 1997	0.5	0.13	3.77	35	<0.001			
<i>C.n.</i> 1960 - 1979	0.5	0.16	3.09	35	<0.001			
<i>H.s.</i> 1960 - 1979	0.5	0.14	3.65	35	<0.001			
<i>C.n.</i> 1930 - 1959	0.5	0.15	3.24	35	<0.001			
<i>H.s.</i> 1930 - 1959	0.5	0.15	3.35	35	<0.001			