Upwelling generates a unique refugium from climate change in the collapsing eastern Mediterranean Sea

Supplementary Information 1: additional tables and details on methods

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Additional tables

Table S1. List of sampling stations in Cyprus (Akrotiri: cooler site with upwelling; Cape Greco: warmer site).

Sample	Locality	Latitude [N]	Longitude [E]	Depth [m]	Date spring	Replicates spring	Date autumn	Replicates autumn
***Posidonia oceanica* meadow**								
APos05	Akrotiri	34.5644	33.0125	5	12 May 2022	3	10 Oct 2022	3
APos10	Akrotiri	34.5638	33.0124	10	13 May 2022	3	11 Oct 2022	3
APos15	Akrotiri	34.5596	33.0377	15	10 May 2022	3	14 Oct 2022	3
APos20	Akrotiri	34.5645	33.0470	20	9 May 2022	3	12 Oct 2022	3
APos30	Akrotiri	34.5606	32.9821	30	14 May 2022	2	13 Oct 2022	3
GPos05	Konnos Bay	34.9843	34.0729	5	5 May 2022	3	15 Oct 2022	3
GPos10	Konnos Bay	34.9851	34.0762	10	4 May 2022	3	15 Oct 2022	3
GPos15	Konnos Bay	34.9860	34.0786	15	3 May 2022	3	17 Oct 2022	3
GPos20	Konnos Bay	34.9860	34.0786	20	2 May 2022	3	18 Oct 2022	3
GPos30	Konnos Bay	34.9860	34.0786	30	7 May 2022	3	19 Oct 2022	3
Rocky substrate								
AR05	Akrotiri	34.5644	33.0125	5	12 May 2022	3	10 Oct 2022	3
AR10	Akrotiri	34.5638	33.0124	10	13 May 2022	1	11 Oct 2022	3
AR15	Akrotiri	34.5630	33.0132	15	10 May 2022	3	14 Oct 2022	3
AR20	Akrotiri	34.5584	33.0485	20	9 May 2022	3	12 Oct 2022	2
AR30	Akrotiri	34.6614	33.0792	30	NA	NA	13 Oct 2022	3
GR05	Konnos Bay	34.9843	34.0729	5	5 May 2022	3	15 Oct 2022	3
GR10	Konnos Bay	34.9851	34.0762	10	4 May 2022	3	15 Oct 2022	3
GR15	Konnos Bay	34.9860	34.0786	15	3 May 2022	3	17 Oct 2022	3
GR20	Konnos Bay	34.9860	34.0786	20	2 May 2022	3	18 Oct 2022	3
GR30	Konnos Bay	34.9860	34.0786	30	7 May 2022	3	19 Oct 2022	3

Table S2. Abundance (N, number of individuals) and observed species richness (S) of the living assemblage at both investigated sites and at both sites combined, detailing abundance and richness for the native and non-indigenous (NIS) component.

	Akrotiri (cooler site)	Cape Greco (warmer site)	Both sites combined
N	15,066	87,051	102,117
N_{native}	13,982 (92.8%)	69,670 (80.0%)	83,652 (81.9%)
N_{NIS}	1,084 (7.2%)	17,381 (20.0%)	18,465 (18.1%)
S	178	190	237
S_{native}	165 (92.7%)	159 (83.7%)	204 (86.1%)
S_{NIS}	13 (7.3%)	31 (16.3%)	33 (13.9%)

Table S3. Coverage-standardized native species richness (sample coverage (SC) of 0.907) and ratio between current and historical richness for each sample, with statistical significance (p-value) of its difference from perfect fidelity (i.e. ratio = 1). Sample coding: first letter indicates location: A: Akrotiri (cooler site) and G: Cape Greco (warmer site); the location code is followed by information on habitat type: Pos: *Posidonia oceanica* meadows, R: rocky substrates; the two-digit number indicates the depth in meters; the single number after the underscore is the replicate.

Sample	Coverage-standardized native species richness	Ratio between current and historical species richness	p-value
APos05_1	17	0.292	0
APos05_2	25	0.403	0
APos05_3	12	0.222	0
APos10_1	21	0.254	0
APos10_2	19	0.21	0
APos10_3	20	0.246	0
APos15_1	32	0.327	0
APos15_2	26	0.257	0
APos15_3	29	0.297	0
APos20_1	37	0.528	0
APos20_2	33	0.407	0
APos20_3	31	0.398	0
APos30_1	34	1	0.52
APos30_2	31	0.968	0.37
APos30_3	22	0.692	0
GPos05_1	2	0.041	0
GPos05_2	2	0.027	0
GPos05_3	1	0.027	0
GPos10_1	2	0.093	0
GPos10_2	4	0.241	0
GPos10_3	2	0.13	0
GPos15_1	3	0.108	0
GPos15_2	3	0.068	0
GPos15_3	2	0.054	0
GPos20_1	8	0.266	0
GPos20_2	17	0.392	0
GPos20_3	2	0.114	0
GPos30_1	16	0.228	0
GPos30_2	13	0.207	0
GPos30_3	12	0.163	0
AR05_1	23	0.325	0
AR05_2	23	0.325	0
AR05_3	27	0.397	0
AR10_1	22	0.261	0
AR10_2	NA	NA	NA
AR10_3	NA	NA	NA
AR15_1	20	0.272	0
AR15_2	16	0.228	0
AR15_3	17	0.217	0
AR20_1	17	0.303	0
AR20_2	15	0.288	0
AR20_3	NA	NA	NA
AR30_1	21	0.325	0

Sample	Coverage-standardized native species richness	Ratio between current and historical species richness	p-value
AR30_2	24	0.351	0
AR30_3	18	0.273	0
GR05_1	2	0.043	0
GR05_2	5	0.149	0
GR05_3	2	0.043	0
GR10_1	3	0.2	0
GR10_2	3	0.1	0
GR10_3	4	0.233	0
GR15_1	3	0.207	0
GR15_2	3	0.172	0
GR15_3	3	0.276	0
GR20_1	4	0.783	0.18
GR20_2	4	0.435	0.03
GR20_3	3	0.435	0
GR30_1	6	0.282	0
GR30_2	12	0.408	0
GR30_3	6	0.282	0

Table S4. Holm-Bonferroni-corrected significance (p-values) of the pairwise comparisons of ratios between current and historical species richness at different depths, using Wilcoxon rank sum test.

	5 m	10 m	15 m	20 m
10 m	1.000	-	-	-
15 m	1.000	1.000	-	-
20 m	0.074	0.011	0.021	-
30 m	0.230	0.050	0.099	1.000

Table S5. Summary statistics of the age-frequency distributions of shells in death assemblages at the investigated sites. Calibrated radiocarbon ages are expressed in years before 2022 AD, the year of collection.

	Minimum	5^{th} quantile	25^{th} quantile	Median	75^{th} quantile	95^{th} quantile	Maximum	Total range	95% range	Inter-quartile range
Akrotiri Posidonia - 5 m	9	15	39	55	201	2883	3554	3545	2868	162
Akrotiri Posidonia - 20 m	6	7	13	55	168	205	214	208	197	155
Cape Greco Posidonia - 5 m	92	117	219	261	1430	3949	4579	4487	3832	1211
Cape Greco Posidonia - 20 m	6	6	6	7	7	7	7	1	1	1
Akrotiri rocks -5 m	6	7	11	15	163	179	183	177	172	152
Akrotiri rocks -20 m	6	6	7	7	9	13	14	8	7	2

	Minimum	5th quantile	25th quantile	Median	75th quantile	95th quantile	Maximum	Total range	95% range	Inter-quartile range
Cape Greco rocks -5 m	6	6	6	7	10	11	11	5	5	4
Cape Greco rocks -20 m	8	9	12	13	23	25	25	17	16	11

Detailed methodology for radiocarbon dating

To quantify the age of death assemblages, we radiocarbon dated five valves at each habitat and site at 5 and 20 m depth of the bivalves *Striarca lactea* (Linnaeus, 1758) and *Glans trapezia* (Linnaeus, 1767), common species living byssally attached to hard substrates and *Posidonia* rhizomes in the shallow subtidal. Bivalves are particularly suitable targets for dating because of their abundance and because of their shape that limits the retention of sediments and contaminants. In addition, most bivalves are suspension feeders, whose ^{14}C content reflect that of seawater's dissolved organic carbon, and are therefore considered as the most reliable target for radiocarbon dating. *Glans trapezia* was present also in the living assemblage, and its choice thus avoids the truncation of the young end of the age frequency distribution. Gastropods, equally abundant, have spires that are often filled with carbonate particles that either require a more time-consuming preparation or may bias the dating results.

Valves were dated by accelerator mass spectrometry at the Arizona Climate and Ecosystems (ACE) Isotope Laboratory at Northern Arizona University using a MIni CArbon DAting System (MICADAS) (Synal et al. 2007). Mollusk shells were subsampled by gently breaking and selecting a small fragment, then cleaning by sonicating and rinsing in deionized distilled water (DDI; 16.3 Mohm·cm) up to three times. The surface of each sample was dissolved in 2 M HCl to remove the outer biocarbonate, the portion most esposed to environmental exchange. The extent of dissolution depended on sample mass: samples larger than 1 mg were leached to remove about 30% by mass and those between ca 1 and 0.5 mg were leached to remove about 15%. Samples were rinsed three times with DDI water, dried in a 50 °C oven, then sealed in a sterilized glass vial. A Carbonate Handling System (CHS) was used to acidify the inorganic carbon and inject the evolved CO_2 directly into the Gas Ion Source (GIS) of the MICADAS (Wacker et al. 2013). The analytical precision of the CHS-GIS-MICADAS is typically better than 0.8% (± 1σ Fm). Details about performance of this system in the ACE Lab and how it compares with other methods for measuring radiocarbon in biocarbonates is documented by Bright et al. (2024).

Radiocarbon ages were converted to calendar years using OxCal v. 4.4.2 (Bronk Ramsey, 2009), Marine20 data (Heaton et al. 2020), and a constant regional marine reservoir correction (ΔR) of -142 ± 66 yrs, which is the weighted mean of eight published pre-bomb ΔR values from Israel and Lebanon (Reimer & McCormac 2002; Boaretto et al. 2010) (Table S6). For samples younger than 1950 AD, the fraction of modern carbon ($F^{14}C$) was converted to calendar ages using a regional marine calibration curve and the calibration software OxCal v4.4.2. The post-1950 regional marine curve was constructed using 10 live-collected *Corbula gibba* shells collected along the coast of Israel (Albano et al. 2020).

Table S6. Regional pre-bomb ΔR values for our study sites. These pre-bomb ΔR values, listed in the Online Marine Reservoir Correction Database (http://calib.org/marine/), were used for the calculation of a weighted mean ΔR value of -142 ± 66 ^{14}C yr (n=8).

	Location	Latitude	Longitude	Year of collection	ΔR ± 1σ (14C yr)	References
1	Netanya, Israel	32.17	34.83	AD 1937	-93 ± 40	Reimer & McCormac 2002
2	Beirut, Lebanon	33.87	35.5	AD 1929	-114 ± 40	Reimer & McCormac, 2002
3	Beirut, Lebanon	33.87	35.5	AD 1929	-204 ± 50	Reimer & McCormac, 2002
4	Israel	32.3384	34.8482	AD 1937	-93 ± 40	Boaretto et al. 2010
5	Israel	32.3384	34.8482	AD 1937	-213 ± 50	Boaretto et al., 2010
6	Israel	32.6432	34.9227	AD 1937	-163 ± 50	Boaretto et al., 2010
7	Israel	32.6432	34.9227	AD 1937	-115 ± 50	Boaretto et al., 2010
8	Israel	32.8431	35.0138	AD 1937	-258 ± 50	Boaretto et al., 2010

References

Albano PG, Hua Q, Kaufman DS, Tomašových A, Zuschin M, Agiadi K. 2020. Radiocarbon dating supports bivalve-fish age coupling along a bathymetric gradient in high-resolution paleoenvironmental studies. Geology **48**:589–593.

Boaretto E, Mienis HK, Sivan D. 2010. Reservoir age based on pre-bomb shells from the intertidal zone along the coast of Israel. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms **268**:966–968.

Bright J, Ebert C, Flores C, Harnik PG, Huntley JW, Kowalewski M, Portell RW, Retelle M, Schuur EAG, Kaufman DS. 2024. Comparing MICADAS gas source, direct carbonate, and standard graphite ^{14}C determinations of biogenic carbonate. Radiocarbon **66**:295–305.

Heaton TJ et al. 2020. Marine20—The marine radiocarbon age calibration curve (0–55,000 cal BP). Radiocarbon **62**:779–820. Cambridge University Press.

Reimer PJ, McCormac FG. 2002. Marine radiocarbon reservoir corrections for the Mediterranean and Aegean seas. Radiocarbon **44**:159–166.

Synal H-A, Stocker M, Suter M. 2007. MICADAS: A new compact radiocarbon AMS system. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms **259**:7–13.

Wacker L, Fahrni SM, Hajdas I, Molnar M, Synal H-A, Szidat S, Zhang YL. 2013. A versatile gas interface for routine radiocarbon analysis with a gas ion source. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms **294**:315–319.